

M

© Macmillan Journals Ltd 1977

Published weekly
ISSN 0028-0836Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs	Judy Redfearn
*Sandy Grimwade	Miranda Robertson
Eleanor Lawrence	Stuart Sharrock
Mary Lindley	Chris Sherwell
Sara Nash	Hilary Taphouse
Peter Newmark	Robert Vickers
Colin Norman	Mary Wade

Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.
1901 West 8th StLos Angeles, California 90057
Telephone (213) 483-8530

also at

57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794

and

Henry G. Dale Co and
Hudson-Wright Associates235 East 50th St
New York NY 10022

Telephone: (212) 421 0142

and

Mary Wade in Nature's
Washington Office

Classified advertisement enquiries to:

Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	Surface
	Airmail
Rest of world	Surface
	Airmail

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045Second-class postage paid at New York, NY
US mailing agent is:Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

Nucleosome cores crystallising. For
the first X-ray analysis of single
crystals see page 29.

nature

Volume 269

1 September 1977

South Africa's nuclear intentions

Technik: the relevance of a missing concept

The French 'nuclear war'

USA/In brief/Jukes on wilderness areas

Correspondence

NEWS AND VIEWS

Nucleosome structure/Metal surfaces/Protein folding/mRNA precursors/

Neptune's weather/Myosin gene/Tibet survey/Crops under stress/

Earthquake prediction

REVIEW ARTICLE

Decision making in animals

D. J. McFarland

ARTICLES

On the optical identifications of five
X-ray sourcesH. V. Bradt, K. M. V. Apparao,
G. W. Clark, R. Dower,
R. Doxsey, D. R. Hearn,
J. G. Jernigan, P. C. Joss,
W. Mayer, J. McClintock
and F. WalterGeneration of Icelandic rhyolites
by melting of plagiogranites
in the oceanic layer

H. Sigurdsson

Structure of nucleosome core
particles of chromatinJ. T. Finch, L. C. Lutter
D. Rhodes, R. S. Brown, B. Rushton,
M. Levitt and A. KlugImmunological selection of tumour cells
which have lost SV40 antigen expressionP. T. Mora, C. Chang,
L. Couvillion, J. M. Kuster
and V. W. McFarland

LETTERS TO NATURE

Upper limits for the radio pulse
emission rate from exploding black holes

W. P. S. Meikle

Neutrino-induced
nucleosynthesis and deuterium

S. E. Woosley

Support for the astronomical
theory of climatic change

A. L. Berger

LaNi_{5-x}Al_x is a versatile alloy
system for metal hydride applicationsM. H. Mendelsohn, D. M. Gruen
and E. DwightNi-Sn interaction in temper embrittled
steel detected by Mössbauer spectroscopyB. C. Edwards, B. L. Eyre
and T. E. Cranshaw

Water content of Russian tektites

E. A. King and J. Arndt

Source models to account for

Lake Mungo palaeomagnetic
excursion and their implications

R. S. Coe

Trace elements in
zooplankton particulate products

S. W. Fowler

Weis-Fogh clap and
flying mechanism in *Locusta*

R. J. Cooter and P. S. Baker

Post-metamorphic eye
migration in *Rana* and *Xenopus*

P. Grobstein and C. Comer

Optimal mate selection in the
toad *Bufo bufo*

N. B. Davies and T. R. Halliday

Use of Ficoll-sodium metrizoate
density gradient to separateP. R. Shastry, U. C. Hegde
and S. S. Rao

Antigen expression on early mouse trophoblast

M. H. Sellens

Ly-6 is a T-cell differentiation antigen

J. N. Woody

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely.

Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Regulation of requirements for anchorage-independent growth of Syrian hamster fibroblasts by somatic mutation	J. C. Leavitt, B. D. Crawford, J. C. Barrett and P. O. P. Ts'o	63
Rapid induction of amphotericin B sensitivity in L1210 leukaemia cells by liposomes containing ergosterol	F. J. Schiffman and I. Klein	65
Prostaglandin endoperoxides and thromboxane A ₂ can induce platelet aggregation in the absence of secretion	I. F. Charo, R. D. Feinman, T. C. Detwiler, J. B. Smith, C. M. Ingberman and M. J. Silver	66
New polymorphism of platelet membrane glycoproteins	R. B. Bolin, T. Okumura and G. A. Jamieson	69
Interaction of colchicine with phosphatidylcholine membranes	L. D. Altstiel and F. R. Landsberger	70
Are benzodiazepines GABA antagonists?	S. N. Kozhechkin and R. U. Ostrovskaya	72
Agonist-antagonist properties of N-allyl-[D-Ala ²]-Met-enkephalin	C. B. Pert, D. L. Bowie, A. Pert, J. L. Morell and E. Gross	73
Subcellular localisation of leucine-enkephalin-hydrolysing activity in rat brain	A. C. Lane, M. J. Rance and D. S. Walter	75
Unconventional serotonergic excitation in <i>Aplysia</i>	T. C. Pellmar and W. A. Wilson	76
Light-induced fluctuations in membrane current of single toad rod outer segments	K.-W. Yau, T. D. Lamb and D. A. Baylor	78
Transport mechanism operating between blood supply and osteocytes in long bones	K. Piekarski and M. Munro	80
Unstable protein-mediated ultraviolet light resistance in <i>Anacystis nidulans</i>	S. K. Bhattacharjee	82
Detection of casein messenger RNA in hormone-dependent mammary cancer by molecular hybridisation	J. M. Rosen and S. H. Socher	83
A theory for genetic regulation by chromosome folding	S. Vaughan	86
Water binding by antifreeze glycoproteins from Antarctic fish	A. E. V. Haschemeyer, W. Guschlbauer and A. L. DeVries	87

BOOK REVIEWS

The Human Quality (Aurelio Peccei)	Eric Ashby	89
Fundamentals of Integrated GC-MS (B. J. Gudwinowicz, M. J. Gudwinowicz and H. F. Martin)	James A. Ballantine	90
Statistical Mechanics (J. E. Mayer and M. G. Meyer)	Peter Landsberg	90
Stereochemistry of Heterocyclic compounds (W. L. F. Armarego)	M. J. T. Robinson	91
Weak Interactions (David Bailin)	G. Karl	91
Vector Fields: Vector Analysis Developed Through its Applications to Engineering and Physics (J. A. Shercliff)	David J. Miller	92
Chemical Evolution of the Giant Planets (Cyril Ponnampertuma, editor)	C. T. Pillinger	92

Obituaries

Benjamin Lee	Mary K. Gaillard	93
Fred Hardy	E. W. Russell	93
William Parker	James S. Roberts	94

Newly on the Market

xiii

M

© Macmillan Journals Ltd 1977

Published weekly
ISSN 0028-0836Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs	Judy Redfearn
*Sandy Grimwade	Miranda Robertson
Eleanor Lawrence	Stuart Sharrock
Mary Lindley	Chris Sherwell
Sara Nash	Hilary Taphouse
Peter Newmark	Robert Vickers
Colin Norman	Mary Wade

Charles Wenz
*Washington officePublishing Director
Jenny HughesAdvertisement Manager
Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.
1901 West 8th StLos Angeles, California 90057
Telephone (213) 483-8530
also at57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794and
Henry G. Dale Co and
Hudson-Wright Associates
235 East 50th St
New York NY 10022Telephone: (212) 421 0142
andMary Wade in Nature's
Washington OfficeClassified advertisement enquiries to:
Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	{ Surface US\$145
	{ Airmail US\$145
Rest of world	{ Air/surface US\$98
	{ Airmail US\$145
	{ Surface US\$94
	{ Airmail US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045Second-class postage paid at New York, NY
US mailing agent is:Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

A crystalline array of ribosomes in
the lizard oocyte. The configuration
of the ribosomes at the oocyte mem-
brane is discussed on page 118.

Orthopositronium lifetime—page 109.

Vol. 269 No. 5624 8 September 1977

nature

3474

Volume 269

8 September 1977



When science turns up the unpalatable	95
La Soufrière, volcanology and forecasting	96
Voyage to the outer planets	98
USA/USSR/New Zealand/In brief/Mellanby on energy and fertility	99

Correspondence	102
----------------	-----

NEWS AND VIEWS

Predator switching/Two-faced neurones/Giant molecular clouds/ Virus synthesis <i>in vitro</i> /Actin cables/Meteor streams/Calcium measurement	103
--	-----

REVIEW ARTICLE

The mean lifetime of orthopositronium in vacuum	T. C. Griffith and G. R. Heyland	109
---	-------------------------------------	-----

ARTICLES

Positions of galactic X-ray sources: $20^\circ < l^\text{II} < 55^\circ$	R. E. Doxsey, K. M. V. Apparao, H. V. Bradt, R. G. Dower and J. G. Jernigan	112
Supernovae, grains and the formation of the Solar System	J. M. Lattimer, D. N. Schramm and L. Grossman	116
Three-dimensional model of membrane-bound ribosomes obtained by electron microscopy	P. N. T. Unwin	118
<i>In vitro</i> synthesis of infectious DNA of murine leukaemia virus	E. Rothenberg, D. Smotkin, D. Baltimore and R. A. Weinberg	122

LETTERS TO NATURE

Pulsar interulses and other off-pulse emission	E. B. Cady and R. T. Ritchings	126
Observable gravitational effects on polarised radiation coming from near a black hole	P. A. Connors and R. F. Stark	128
Quantum uncertainty in the final state of gravitational collapse	J. V. Narlikar	129
Origin of diffuse interstellar lines	A. E. Douglas	130
Carbonaceous compounds in interstellar dust	R. F. Knacke	132
Lateral variation of phenocryst assemblages in volcanic rocks of the Japanese islands	M. Sakuyama	134
Rare earth element mobility and geochemical characterisation of spilitic rocks	P. A. Floyd	134
Transuranic depositional history in South Greenland firn layers	M. Koide, E. D. Goldberg, M. M. Herron and C. C. Langway, Jr	137
Angiosperm fossils from latitude 70°S	M. R. A. Thomson and R. W. Burn	139
Megafaunal biomass in the deep sea	R. L. Haedrich and G. T. Rowe	141
Ultradian rhythms in urine flow in waking humans	P. Lavie and D. F. Kripke	142
Natural selection at the α -GDH locus in <i>Drosophila</i>	S. Alahiotis, S. Müller and E. Berger	144
Genetic control of cell division in yeast cultured at different growth rates	M. N. Jagadish and B. L. A. Carter	145
Blood flukes have a double outer membrane	D. J. McLaren and D. J. Hockley	147

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (a) is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

- (i) only one reference number need be used if the reference is to several papers by identical authors,
- (ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Macrophages synthesise and release prostaglandins in response to inflammatory stimuli	J. L. Humes, R. J. Bonney, L. Pelus, M. E. Dahlgren, S. J. Sadowski, F. A. Kuehl, Jr and P. Davies	149
Primary <i>in vitro</i> sensitisation of human T cells	W. Newman, G. L. Stoner, and B. R. Bloom	151
Genetic non-responsiveness of murine fibroblasts to bacterial endotoxin	J. L. Ryan and K. P. W. J. McAdam	153
Density-dependent inhibition of fibroblast growth is overcome by pure mitogenic factors	K. Mierzejewski and E. Rozengurt	155
Cultured human breast cancer cells lose selectivity in direct intercellular communication	I. S. Fentiman and J. Taylor-Papadimitriou	156
Growing central axons deprived of normal target neurones by neonatal X-ray irradiation still terminate in a precisely laminated fashion	S. Laurberg and A. Hjorth-Simonsen	158
Corollary discharge to cockroach giant interneurons	F. Delcomyn	160
Regulation of adenylate cyclase activity in glial-adrenal hybrid cells	B. P. Schimmer, J. Tsao and N. H. Cheung	162
Tricyclic antidepressant drugs block histamine H_2 receptor in brain	J. P. Green and S. Maayani	163
Reversal of the Na-K pump and apparent affinity for intracellular potassium	J. D. Robinson, E. S. Hall and P. B. Dunham	165
Analgesic activity of lipotropin C fragment depends on carboxyl terminal tetrapeptide	M. J. Geisow, J. F. W. Deakin, J. O. Dostrovsky and D. G. Smyth	167
Proinsulin conversion to desalanyl insulin by α_2 -macroglobin-bound trypsin	C. Largman, J. H. Johnson, J. W. Brodrick and M. C. Geokas	168
Structural analysis of purified platelet-activating factor by lipases	J. Benveniste, J. P. Le Couedic, J. Polonsky and M. Tence	170
New oncogenic papova virus from primate cells	W. Waldeck and G. Sauer	171
Type C RNA virus production and cell competence for normal differentiation in myeloid leukaemic cells	D. Liebermann and L. Sachs	173
Post-transcriptional control of avian oncornavirus-transforming gene sequences in mammalian cells	R. A. Krzyzek, A. F. Lau, A. J. Faras and D. H. Spector	175
<i>Cis-trans</i> isomerisation in rhodopsin occurs in picoseconds	B. H. Green, T. G. Monger, R. R. Alfano, B. Aton and R. H. Callender	179

BOOK REVIEWS

The Collected Papers of Charles Darwin (P. H. Barrett, editor)	Frederick Burkhardt	181
The Foundations of Cybernetics (F. H. George)	V. Zakian	181
Stereochemistry of Heterocyclic Compounds (W. L. F. Armarego)	J. E. Saxton	182
Introduction to the Spectroscopy of Biological Polymers (D. W. Jones, editor)	Michael T. Flanagan	182
Asymptotic Structure of Space-Time (F. P. Esposito and L. Witten, editors)	Paul Davies	183
Minamata Disease: Methylmercury Poisoning in Minamata and Niigata (T. Tsubaki and K. Irukayama, editors)	Laszlo Magos	183
Chemistry of Functional Groups (S. Patai, editor)	D. I. Davies	184
Climate, Water and Agriculture in the Tropics (I. J. Jackson)	J. G. Lockwood	184

Author index

M

© Macmillan Journals Ltd 1977

Published weekly

ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF

Telephone: (01) 836 6633 Telex: 262024

Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045

Telephone: (202) 737 2355

Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs Judy Redfearn
 *Sandy Grimwade Miranda Robertson
 Eleanor Lawrence Stuart Sharrock
 Mary Lindley Chris Sherwell
 Sara Nash Hilary Taphouse
 Peter Newmark Robert Vickers
 Colin Norman Mary Wade

Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.

1901 West 8th St

Los Angeles, California 90057

Telephone (213) 483-8530

also at

57 Post Street, Suite 712-715,

San Francisco, California 94104

Telephone: (415) 392 6794

and

Henry G. Dale Co and
Hudson-Wright Associates

235 East 50th St

New York NY 10022

Telephone: (212) 421 0142

and

Mary Wade in Nature's

Washington Office

Classified advertisement enquiries to:

Classified Department, London Office,

Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	Surface
	Airmail
Rest of world	Air/surface
	Airmail
	US\$145
	US\$98
	US\$145
	US\$94
	US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,

Basingstoke, Hants. RG21 2XS

Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature.

711 National Press Building, Washington DC 20045

Second-class postage paid at New York, NY

US mailing agent is:

Expeditors of the Printed Word Ltd.,

527 Madison Avenue,

New York, NY 10022

Cover picture

A photograph of the quasi-stellar object 3C 273. One of the nearest and the brightest of the quasars, 3C 273 has a unique jet extending from its otherwise star-like image. Superimposed is a graph of the ultraviolet spectrum of the quasar obtained with a telescope launched aboard a sounding rocket. The strongest emission feature is the Lyman α line of hydrogen. See page 203.

Hale Observatories and The Johns Hopkins University

Vol. 269 No. 5625 15 September 1977



Volume 269

15 September 1977

Destination without route-map	185
A good time to raise standards	186
Research funding: the new trichotomy	186
From détente to development	187
Geos/USA/USSR/In brief/Mellanby on nutrition	189
Correspondence	194

NEWS AND VIEWS

QSO ultraviolet spectrum/HSV transformation/Free electron laser/ Planetary craters/Superconductors/Lithosphere/Communal nesting/ Carbon fixation pathways/Gravitation/Oocyte microinjection	195
---	-----

ARTICLES

Ultraviolet spectrum of quasi-stellar object 3C273	A. F. Davidsen, G. F. Hartig and W. G. Fastie	203
Future sea-level changes due to West Antarctic ice sheet fluctuations	J. A. Clark and C. S. Lingle	206
Isotopic evidence for source of diagenetic carbonates formed during burial of organic-rich sediments	H. Irwin, C. Curtis and M. Coleman	209
X-ray structures of two oxidation states of a flavin-nicotinamide biscoenzyme and models for flavin-nicotinamide interactions	D. J. T. Porter, H. J. Bright and D. Voet	213
Possible regulatory function of acetylcholine receptor in maintenance of retinotectal synapses	J. A. Freeman	218

LETTERS TO NATURE

Observations of OE323(4C34.13), MO758 + 120 and OS210(4C28.40) with the 5-km telescope	M. A. C. Perryman and M. Ryle	223
Reducing greenhouses and the temperature history of Earth and Mars	C. Sagan	224
Stratospheric CH ₄ , HCl and ClO and the chlorine-ozone cycle	M. Ackerman, D. Frimout and C. Muller	226
Defects in natural type IB diamond	P. R. W. Hudson and P. P. Phakey	227
Correlation between dielectric constant and defect structure of non-stoichiometric solids	R. J. D. Tilley	229
Crystal structure of rustumite	R. A. Howie and V. V. Ilyukhin	231
Imaging practical surfaces in a field ion microscope	P. Vijendran, D. Ramanathan and S. Dass	232
Archaeological evidence for Subrecent seismic activity along the Dead Sea-Jordan Rift	I. Karcz, U. Kafri and Z. Meshel	234
Bacterial sulphate reduction and calcite precipitation in hypersaline deposition of bituminous shales	B. Spiro and Z. Aizenshtat	235
Inducer of pectic acid lyase in <i>Erwinia carotovora</i>	S. Tsuyumu	237
Steroids stimulate secretion by insect Malpighian tubules	J. D. Gee, D. L. Whitehead and J. Koolman	238
Meiotic crossing-over in lily and mouse	Y. Hotta, A. C. Chandley and H. Stern	240
Evidence for two active X chromosomes in germ cells of female before meiotic entry	B. R. Migeon and K. Jelalian	242

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note: (i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v.11 Nov. (1976).

Transcriptional controls and dosage compensation in <i>Drosophila melanogaster</i>	R. L. Roehrdanz and J. C. Lucchesi	243
Endocytosis of red blood cells or haemoglobin by activated macrophages inhibits their tumoricidal effect	J. B. Weinberg and J. B. Hibbs, Jr	245
Inhibition of adipose conversion of 3T3 fibroblasts by tumour promoters	L. Diamond, T. G. O'Brien and G. Rovera	247
Effect of microtubular antagonists on lymphocyte mitogenesis	S. A. Rasmussen and R. P. Davis	249
Immunoglobulin binding to herpes virus-induced Fc receptors inhibits virus growth	J. Costa, A. S. Rabson, C. Yee and T. S. Tralka	251
Excretion of feline leukaemia virus by naturally infected pet cats	D. P. Francis, M. Essex and W. D. Hardy, Jr	252
Blood group-like activity released by human mammary carcinoma cells in culture	J. H. Anglin, Jr, M. P. Lerner and R. E. Nordquist	254
A-specific autoantigenic ovarian glycolipids inducing production of 'natural' anti-A antibody	P. Arend and J. Nijssen	255
Reconstruction of photoreceptor membrane potentials from simultaneous intracellular and extracellular recordings	P. B. Pynsent and G. Duncan	257
Immunological identification of rat neuropsychin precursors	M. J. Brownstein, A. G. Robinson and H. Gainer	259
Several of the adenosine deaminase isozymes are glycoproteins	D. M. Swallow, L. Evans and D. A. Hopkinson	261
Translation of human immunoglobulin heavy chain mRNA <i>in vitro</i>	C. K. Klukas, F. Cramer and H. Gould	262
Identification of chromosomal location of yeast DNA from hybrid plasmid <i>pYeleu 10</i>	J. Hicks and G. R. Fink	265
Deuterium NMR study of lipid organisation in <i>Acholeplasma laidlawii</i> membranes	G. W. Stockton, K. G. Johnson, K. W. Butler, A. P. Tulloch, Y. Boulanger, I. C. P. Smith, J. H. Davis and M. Bloom	267

BOOK REVIEWS

Energy: The Continuing Crisis (Norman Metzger)	John Chesshire	269
The Phylogeny of the Vertebrata (Søren Løvtrup)	R. S. Miles	269
Ellipsometry and Polarized Light (R. M. A. Azzam and N. M. Bahsara)	P. H. Lissberger	270
Ethological Dictionary (Armin Heymer)	John Krebs	270
A Nature Conservation Review (D. A. Ratcliffe, editor)	J. H. Andrews	271
The Mammals of Pakistan (T. J. Roberts)	I. R. Bishop	271
Grasshoppers and Locusts (Boris Uvarov)	J. S. Kennedy	272
Semiempirical Methods of Electronic Structure Calculation (G. A. Segal, editor)	Graham Richards	272

Announcements and Person to Person	273
------------------------------------	-----

Ocean Sciences Supplement

Reprints of this supplement (*Nature*, 30 June 1977) are now available. For details and order form see page xviii.

M

© Macmillan Journals Ltd 1977

Published weekly

ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF

Telephone: (01) 836 6633 Telex: 262024

Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045

Telephone: (202) 737 2355

Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs Judy Redfearn
 *Sandy Grimwade Miranda Robertson
 Eleanor Lawrence Stuart Sharrock
 Mary Lindley Chris Sherwell
 Sara Nash Hilary Taphouse
 Peter Newmark Robert Vickers
 Colin Norman Mary Wade

Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United
StatesJobson/Jordan/Harrison and Schulz Inc.
1901 West 8th St

Los Angeles, California 90057

Telephone (213) 483-8530

also at

57 Post Street, Suite 712-715,

San Francisco, California 94104

Telephone: (415) 392 6794

and

Henry G. Dale Co and

Hudson-Wright Associates

235 East 50th St

New York NY 10022

Telephone: (212) 421 0142

and

Mary Wade in Nature's

Washington Office

Classified advertisement enquiries to:

Classified Department, London Office,

Telephone (01) 240 1101

Annual subscription

UK		£37.50
Europe		US\$98
Japan	Surface	US\$98
	Airmail	US\$145
USA & Canada	Air/surface	US\$98
	Airmail	US\$145
Rest of world	Surface	US\$94
	Airmail	US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of

World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,

Basingstoke, Hants. RG21 2XS

Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature,

711 National Press Building, Washington DC 20045

Second-class postage paid at New York, NY

US mailing agent is:

Expeditors of the Printed Word Ltd.,

527 Madison Avenue

New York, NY 10022

Cover picture

Artist's impression of the molecular
 structure of the plasma membrane
 (lipid bilayer, inserted proteins and
 underlying microtubules).

[Eleanor Walsh]

See page 307.

Vol. 269 No. 5626 22 September 1977

nature



Volume 269

22 September 1977

Room at the top—for whom?	275
Refusnik scientists: keeping science alive	276
Tethering the tides	277
OTS/Britain/USA/Pakistan/China/In brief/Jukes on Laetrile	279
Correspondence	284

NEWS AND VIEWS

Intercalation of DNA/Elementary particles/Sarcoma virus gene product/	
Lysosomal enzymes/Diffusion in polymers	285

REVIEW ARTICLE

Mathematical models in applied ecology	G. R. Conway	291
--	--------------	-----

ARTICLES

Ocean-floor magnetotelluric sounding over North Central Pacific	J. H. Filloux	297
Deep-sea carbonate and the deglaciation preservation spike in pteropods and foraminifera	W. H. Berger	301
Structure of a dinucleoside phosphate-drug complex as model for nucleic acid-drug interaction	S. Neidle, A. Achari, G. L. Taylor, H. M. Berman, H. L. Carrell, J. P. Glusker and W. C. Stallings	304
Orientation of cell-surface antigens in the lipid bilayer of lymphocyte plasma membrane	F. S. Walsh and M. J. Crumpton	307

LETTERS TO NATURE

Possible fast variability of the nucleus of Cen A at 13.5 mm	P. Kaufmann, P. M. dos Santos, J. C. Raffaelli and E. Scalise, Jr	311
Pi 1-2 magnetic field pulsations on dayside cleft field lines	R. R. Heacock and R. D. Hunsucker	313
Linear dichroism probes to study internal electric fields	B. Norden	314
Quasi-Fermi level measurement in an illuminated GaP photoelectrolysis cell	W. E. Pinson	316
The first cubane-type fluoro-bridged cluster	J. C. Jansen, H. van Koningsveld and J. Reedijk	318
Hydrothermal manganese in the Galapagos Rift	G. Klinkhammer, M. Bender and R. F. Weiss	319
Solar influence on North Atlantic mean sea level pressure	P. M. Kelly	320
Copper exclusion as a mechanism of heavy metal tolerance in a green alga	P. L. Foster	322
Genetic variation in wild populations of rain-forest trees	Y.-Y. Gan, F. W. Robertson, P. S. Ashton, E. Soepadmo and D. W. Lee	323
Freezing avoidance by deep supercooling in hydrated lettuce seeds	O. Junttila and C. Stushnoff	325
Guard cell malic acid metabolism during stomatal movements	C. M. Willmer and J. C. Rutter	327
Orientation columns in macaque monkey visual cortex demonstrated by the 2-deoxyglucose autoradiographic technique	D. H. Hubel, T. N. Wiesel and M. P. Stryker	328
Instability of the eye in the dark and proprioception	A. Fiorentini and L. Maffei	330

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note: (i) only one reference number need be used if the reference is to several papers by identical authors, (ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely.

Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Leukocytes are consistently associated with degenerating embryos in IUD-bearing rhesus monkeys	P. R. Hurst, K. Jefferies, P. Eckstein, K. Dawson and A. G. Wheeler	331
Active thymus derived suppressor lymphocytes in human cord blood	M. B. A. Oldstone, A. Tishon and L. Moretta	333
Evidence for a common evolutionary origin of gastrin and cholecystokinin	L.-I. Larsson and J. F. Rehfeld	335
Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism	B. M. Cattanach, C. A. Iddon, H. M. Charlton, S. A. Chiappa and G. Fink	338
Nigral and striatal dopamine release under sensory stimuli	A. Nieoullon, A. Cheramy and J. Glowinski	340
Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures	D. W. Choi, D. H. Farb and G. D. Fischbach	342
Phosphorylation of chloroplast membrane polypeptides	J. Bennett	344
Identification of a transformation-specific antigen induced by an avian sarcoma virus	J. S. Brugge and R. L. Erikson	346
Structures of benzo(a)pyrene-nucleic acid adducts formed in human and bovine bronchial explants	A. M. Jeffrey, I. B. Weinstein, K. W. Jennette, K. Grzeskowiak, K. Nakanishi, R. G. Harvey, H. Autrup and C. Harris	348
Nucleotide sequence of <i>Bombyx mori</i> L. tRNA ₁ ^{Gly}	J. P. Garel and G. Keith	350
Melting fine structure of DNA fragments of known base sequence from Φ X174	A. Wada, H. Tachibana, S. Ueno, Y. Husimi and Y. Machida	352

MATTERS ARISING

Generation length and rates of hominoid molecular evolution	V. M. Sarich and J. E. Cronin	354
Reply	G. J. Todaro and R. E. Benveniste	354
Complementation of immune response genes for (T, G)-A-L	A. J. Munro and M. J. Taussig	355
High explosive analogue of the Tunguska Event	B. W. Augenstein	355
Reply	G. H. S. Jones	355
β -Globin mRNA in Ferrara β^0 -thalassaemia	B. G. Forget and D. G. Hillman	355
Reply	S. Ottolenghi, P. Comi, B. Giglioni, R. Williamson, G. Vullo, L. del Senno and F. Conconi	356

BOOK REVIEWS

Essential Relativity: Special, General and Cosmological (W. Rindler)	Paul Davies	357
Comprehensive Immunology (T. Makinodan, E. Yunis, N. K. Day and R. A. Good, editors)	H. S. Micklem	357
Anatomy of Seed Plants (Katherine Esau)	A. W. Robards	358
Biology of Diatoms (Dietrich Werner, editor)	F. E. Round	358
Analytical Physiology of Cells and Developing Organisms (B. C. Goodwin)	Lewis Wolpert	359
Qualitative Methods in Quantum Theory (A. B. Migdal)	Norman Dombey	359
Frontiers in Laser Spectroscopy (R. Balian, S. Haroche and S. Liberman, editors)	Peter Knight	360
Myelin (P. Morell, editor)	A. N. Davison	360

Announcements and Person to Person

364

M

© Macmillan Journals Ltd 1977

Published weekly

ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs	Miranda Robertson
*Sandy Grimwade	Stuart Sharrock
Eleanor Lawrence	Chris Sherwell
Mary Lindley	Hilary Taphouse
Sara Nash	Robert Vickers
Peter Newmark	Mary Wade*
Judy Redfearn	Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United
StatesJobson/Jordan/Harrison and Schulz Inc.
1901 West 8th St
Los Angeles, California 90057
Telephone (213) 483-8530

also at

57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794

and

Henry G. Dale Co and
Hudson-Wright Associates
235 East 50th St
New York NY 10022
Telephone: (212) 421 0142

and

Mary Wade in Nature's
Washington OfficeClassified advertisement enquiries to:
Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	Surface US\$145
	Airmail US\$145
Rest of world	Surface US\$145
	Airmail US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045Second-class postage paid at New York, NY
US mailing agent is.Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

Three micrographs showing etch pits
in large single crystals of stearic acid.
On page 399 K. Sato and M. Okada
describe this method of studying
dislocations in fatty acid crystals.

Vol. 269 No. 5627 29 September 1977

nature

Volume 269

29 September 1977

Political will now the issue	365
BA address: Huxley responds	366
Desertification: another mirage?	367
Britain/Comecon/In brief/Mellanby on Mauritius	369

Correspondence	372
----------------	-----

NEWS AND VIEWS

Fly photoreceptors/Interferon action/High-energy physics/Disturbed environments/Earth's lower crust/Nucleolus at Salamanca/ Cancer in China	373
---	-----

REVIEW ARTICLE

Recent developments in phase transitions and critical phenomena	D. R. Nelson	379
--	--------------	-----

ARTICLES

The Messinian salinity crisis and evidence of late Miocene eustatic changes in the world ocean	C. G. Adams, R. H. Benson, R. B. Kidd, W. B. F. Ryan and R. C. Wright	383
Evidence for a sensitising pigment in fly photoreceptors	K. Kirschfeld, N. Franceschini and B. Minke	386
Nucleotide sequences of 5'-terminal ribosome-protected initiation regions from two reovirus messages	M. Kozak	390

LETTERS TO NATURE

Identification of cosmic γ -ray sources CG135+1 and CG189+1 with HII regions	A. W. Strong	394
Tidal interactions and the massive halo hypothesis	S. D. M. White and N. A. Sharp	395
Surface area and secondary nucleation theory	P. H. Lindenmeyer	396
Crystal structure of alinitite	V. V. Ilyukhin, N. N. Nevsky, M. J. Bickbau and R. A. Howie	397
The nature of metastable phases of carbon	B. V. Derjaguin, D. V. Fedoseev, V. P. Varnin and S. P. Vnukov	398
Etching on large single crystals of stearic acid	K. Sato and M. Okada	399
Can sunspots influence our weather?	H. Volland	400
Equatorial solar rotation and its relation to climatic changes	K. Sakurai	401
Reproductive effort and life history strategy of the Aldabran giant tortoise	I. R. Swingland	402
Behaviour of adult and immature male baboons during inter-group encounters	D. L. Cheney and R. M. Seyfarth	404
Nitrate reductase-deficient mutants in barley	R. L. Warner, C. J. Lin and A. Kleinhofs	406
Possible <i>in vitro</i> test for screening drugs for activity against <i>Babesia</i> and other blood protozoa	A. D. Irvin and E. R. Young	407
Synergistic interaction of testosterone and oestradiol inhibits spermatogenesis in rats	L. L. Ewing, C. Desjardins, D. C. Irby and B. Robaire	409
Anti-Müllerian hormone is a functional marker of foetal Sertoli cells	D. Tran, N. Meusy-Dessolle and N. Jossso	411
<i>In vitro</i> duplication and 'cure' of haemopoietic defects in genetically anaemic mice	T. M. Dexter and M. A. S. Moore	412
HLA 'help' for human B2-microglobulin across species barriers	A. R. Sanderson	414

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (*a*) is preferred to e^a if '*a*' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely.

Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

In a radiation chimaera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells	M. J. Bevan	417
Cytotoxic T lymphocytes induced in mice by inactivated influenza virus vaccine	F. A. Ennis, W. J. Martin and M. W. Verbonitz	418
Induction of plaque-forming cells in cultured human lymphocytes by combined action of antigen and EB virus	A. L. Luzzati, H. Hengartner and M. H. Schreier	419
EB virus-induced B lymphocyte cell lines producing specific antibody	M. Steinitz, G. Klein, S. Koskimies and O. Makel	420
Interferon-induced transfer of viral resistance between animal cells	J. E. Blalock and S. Baron	422
Hyperacute autoimmune encephalomyelitis induced by a synthetic autoantigen	F. C. Westall, M. Thompson and V. A. Lennon	425
Increased endoneurial fluid pressure in experimental lead neuropathy	P. A. Low and P. J. Dyck	427
Development-related changes of triiodothyronine binding to brain cytosol receptors	S. E. Geel	428
Aconal accessibility and adaptation to osmotic stress in an extreme osmoconformer	J. E. Treherne, J. A. Benson and H. le B. Skaer	430
Modification of synaptic input following unilateral labyrinthectomy	N. Dieringer and W. Precht	431
Enkephalin effects on release of brain acetylcholine	K. Jhamandas, J. Sawynok and M. Sutak	433
Polymersing ability of C ₆ glial cell microtubule protein decays much faster than its colchicine-binding activity	G. Wiche, L. S. Honig and R. D. Cole	435
Translocation of intracellularly stored calcium during the contraction-relaxation cycle in guinea pig taenia coli	H. Sugi and T. Daimon	436
Tumour-specific transplantation antigen from SV40 transformed cells binds to DNA	C. Chang, S. W. Luborsky and P. T. Mora	438
Productive T7 infection of <i>Escherichia coli</i> F ⁺ cells and anucleate minicells	H. Ponta, J. N. Reeve, M. Pfennig-Yeh, H. Hirsch-Kauffman, M. Schweiger and P. Herrlich	440
Ratios of α - to β -globin mRNA and regulation of globin synthesis in reticulocytes	J. A. Phillips III, P. G. Snyder and H. H. Kazanian, Jr	442
Evidence for alkali-sensitive linkers in DNA of African green monkey kidney cells	E. Filippidis and R. Meneghini	445
Chloramphenicol-sensitive labelling of protein in microsomes of <i>Neurospora crassa</i>	W. B. Macklin, D. J. Meyer, D. O. Woodward and S. K. Erickson	447
A cholesterol-isopropanol gel	W. E. Acree, Jr and G. L. Bertrand	450

BOOK REVIEWS

The Process of Spermatogenesis in Animals (E. C. Roosen-Runge)	B. P. Setchell	451
Population Structure and Human Variation (G. A. Harrison, editor)	Alan E. H. Emery	451
Three-Dimensional Nets and Polyhedra (A. F. Wells)	A. L. Mackay	452
Scientific Results of the Oman Flora and Fauna Society 1975	Sarah Bunney	452
The Theory of Binocular Vision (E. Hering)	J. D. Mollon	453
Inhomogeneous Optical Waveguides (M. S. Sodha and A. K. Ghatak)	L. Solymar	453
Proteinases in Mammalian Cells and Tissues (A. J. Barrett, editor)	A. H. Gordon	454
Stereo-Differentiating Reactions (Y. Izumi and A. Tai)	T. L. V. Ulbricht	454

Announcements and Person to Person	455
------------------------------------	-----

M

© Macmillan Journals Ltd 1977

Published weekly

ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF

Telephone: (01) 836 6633 Telex: 262024

Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045

Telephone: (202) 737 2355

Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs Miranda Robertson
 *Sandy Grimwade Stuart Sharrock
 Eleanor Lawrence Chris Sherwell
 Mary Lindley Hilary Taphouse
 Sara Nash Robert Vickers
 Peter Newmark Mary Wade*
 Judy Redfearn Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United
States

Jobson/Jordan/Harrison and Schulz Inc.

1901 West 8th St

Los Angeles, California 90057

Telephone (213) 483-8530

also at

57 Post Street, Suite 712-715,

San Francisco, California 94104

Telephone: (415) 392 6794

and

Henry G. Dale Co and

Hudson-Wright Associates

235 East 50th St

New York NY 10022

Telephone: (212) 421 0142

and

Mary Wade in Nature's

Washington Office

Classified advertisement enquiries to:
Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	Surface US\$145
	Airmail US\$98
Rest of world	Surface US\$145
	Airmail US\$94
	US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS

Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045Second-class postage paid at New York, NY
US mailing agent is:Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

Grazing sheep in Australia. There
 may be more than one stable state in
 the relationship between animals and
 the vegetation they graze on, as well
 as in other ecosystems. R. M. May
 reviews ecosystems with a multiplicity
 of stable states on page 471.

[Popperfoto]

Vol. 269 No. 5628

6 October 1977

nature

Volume 269

23/2/78

6 October 1977

Facing up to demography	457
The long journey into the end of the artificial night	458
Two decades of Soviet space	459
Canada/USA/In brief/Jukes on evolution	461

NEWS AND VIEWS

Plant tumours/Galactic γ rays/Quasars/Hypothalamus/Ecotoxicology/ Degradative plasmids	465
--	-----

REVIEW ARTICLE

Thresholds and breakpoints in ecosystems with a multiplicity of stable states	R. M. May	471
--	-----------	-----

ARTICLES

Hypervelocity cratering and impact magnetisation of basalt	G. Martelli and G. Newton	478
Thermodynamic theory of size dependence of melting temperature in metals	P. R. Couchman and W. A. Jesser	481
Corrected age of the Pliocene/Pleistocene boundary	B. U. Haq, W. A. Berggren and J. A. Van Couvering	483
Cultured epithelial cells of cornea, conjunctiva and skin: absence of marked intrinsic divergence of their differentiated states	T.-T. Sun and H. Green	489

LETTERS TO NATURE

Rapid fluctuations of radio flux and polarisation in quasar 3C273	V. A. Efanov, I. G. Moiseev, N. S. Nesterov and N. M. Shakhovskoy	493
New high energy γ -ray sources observed by COS B	W. Hermsen, B. N. Swanenburg, G. F. Bignami, G. Boella, R. Buccheri, L. Scarsi, G. Kanbach, H. A. Mayer-Hasselwander, J. L. Masnou, J. A. Paul, K. Bennett, J. C. Higdon, G. G. Lichti, B. G. Taylor and R. D. Wills	494
Positions of galactic X-ray sources Cir X-1, TrA X-1 and 3U162-67	H. V. Bradt, K. M. V. Apparao, R. Dower, R. E. Doxsey, J. G. Jernigan and T. H. Markert	496
Atmospheric waves in the ionosphere due to total solar eclipse	R. N. E. Baulch and E. C. Butcher	497
Charge states of the vacancy in diamond	G. Davies	498
Alkali cations and direct reduction of wustite	T. Szendrei and P. C. van Berge	500
Pacific sea-surface temperature related to rain in California	C. G. Markham and D. R. McLain	501
Direct measurements of secondary currents in river bends	J. C. Bathurst, C. R. Thorne and R. D. Hey	504
Prehistoric dental calculus gives evidence for coca in early coastal Ecuador	L. L. Klepinger, J. K. Kuhn and J. Thomas, Jr	506
High levels of cadmium in Atlantic seabirds and sea-skaters	K. R. Bull, R. K. Murton, D. Osborn, P. Ward and L. Cheng	507
Pectolytic anaerobic bacteria cause symptoms of cavity spot in carrots	D. A. Perry and J. G. Harrison	509
Carcinogenic activity of hexachlorobenzene in hamsters	J. R. P. Cabral, P. Shubik, T. Mollner and F. Raitano	510
Effect of aspirin administration on retinoic acid toxicity in mice	S. D. Harrison, Jr, E. J. Hixon, J. A. Burdeshaw and E. P. Denine	511
L-Dopa methyl ester as a new antitumour agent	M. M. Wick	512

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Dopamine agonists induce recovery from surgically-induced septal rage	R. F. Marotta, N. Logan, M. Potegal, M. Glusman and E. L. Gardner	513
Viable chimaeras produced from normal and parthenogenetic mouse embryos	L. C. Stevens, D. S. Varnum and E. M. Eicher	515
Ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division	J. J. Eppig, L. P. Kozak, E. M. Eicher and L. C. Stevens	517
Extreme sensitivity of some intestinal crypt cells to X and γ irradiation	C. S. Potten	518
Cellular serine proteinase induces chemotaxis by complement activation	C. A. Thomas, F. J. Yost, Jr, R. Snyderman, V. B. Hatcher and G. S. Lazarus	521
The ECF-A tetrapeptides and histamine selectively enhance human eosinophil complement receptors	A. R. E. Anwar and A. B. Kay	522
Molecular basis for acquired haemoglobin H disease	J. Old, J. Longley, W. G. Wood, J. B. Clegg and D. J. Weatherall	524
Polymerisation of haemoglobin SA hybrid tetramers	R. M. Bookchin, T. Balazs, R. L. Nagel and I. Tellez	526
The mechanism by which actinomycin D inhibits protein synthesis in animal cells	H. L. Cooper and R. Braverman	527
Regulation of maternal mRNA translation in developing embryos of the surf clam <i>Spisula solidissima</i>	F. Gabrielli and C. Baglioni	529
Variability of DNA content of murine fibrosarcoma cells	N. Suzuki, H. R. Withers and L. Y. Lee	531
Use of coupled transcription and translation to study mRNA production by vaccinia cores	H. R. B. Pelham	532
Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumours	M. H. Drummond, M. P. Gordon, E. W. Nester and M-D. Chilton	535
Decreased initiation factor activity in mouse L cells treated with interferon	K. Ohtsuki, F. Dianzani and S. Baron	536

BOOK REVIEWS

A Unified Theory of the Nucleus (K. Wildermuth and Y. C. Tang)	D. F. Jackson	539
Biosynthetic Products for Cancer Chemotherapy (G. R. Pettit)	J. A. Stock	539
Energetic Materials (H. D. Fair and R. F. Walker, editors)	J. H. Turnbull	540
Smoke, Dust and Haze: Fundamentals of Aerosol Behaviour (S. K. Friedlander)	P. Fabian	540
Molar Mass Measurements in Polymer Science (N. C. Billingham)	A. Ledwith	541
Introduction to Liquid State Chemistry (Y. Marcus)	J. S. Rowlinson	541
Precambrian of the Northern Hemisphere (L. J. Salop)	B. Windley	542
Cyclic Nucleotides in the Nervous System (John Daly)	R. Rodnight	542

OBITUARY

Lord Adrian	A. L. Hodgkin	543
-------------	---------------	-----

M

© Macmillan Journals Ltd 1977

Published weekly

ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs	Miranda Robertson
*Sandy Grimwade	Stuart Sharrock
Eleanor Lawrence	Chris Sherwell
Mary Lindley	Hilary Taphouse
Sara Nash	Robert Vickers
Peter Newmark	Mary Wade*
Judy Redfearn	Charles Wenz

*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.
1901 West 8th St.
Los Angeles, California 90057
Telephone (213) 483-8530also at
57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794and
Henry G. Dale Co and
Hudson-Wright Associates
235 East 50th St
New York NY 10022
Telephone: (212) 421 0142and
Mary Wade in Nature's
Washington Office

Advertising Representatives in Japan

Douglas Kenrick (Far East) Ltd,
Kowa Daisan Bldg, 4th Floor,
11-45 Akasaka, 1-Chome
Minato-Ku, Tokyo, Japan
Telephone: (582) 0951-5Classified advertisement enquiries to:
Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	Surface US\$145
	Air/surface US\$98
Rest of world	Air/surface US\$145
	Surface US\$94
	Airmail US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50. Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045Second-class postage paid at New York, NY
US mailing agent is:Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

Three-dimensional representation of a
surge on Barnes Ice Cap, Baffin Island,
Canada. See page 588.

Vol. 269 No. 5629

13 October 1977

nature

Volume 269

13 October 1977

Scientists in public	547
GDR's state of science	548
IMS at the crossroads	549
WHO's show on the road	550
Britain/USSR/USA/Canada/ West Germany/Mellanby on models	552
Correspondence	556

NEWS AND VIEWS

Non-synaptic axonal boutons/Fertilisation/Ciliar dyneins/Calculating energy levels in diamond defects/Primaevial heavy leptons/Antarctic geology/ <i>Nuée ardente</i>	549
---	-----

ARTICLES

Upper limits on the Faraday rotation in variable radio sources	J. F. C. Wardle	563
Uranium anomaly in Black Sea sediments	E. T. Degens, F. Khoo and W. Michaelis	566
Ozone concentrations in South-East England during the summer of 1976	A. J. Apling, E. J. Sullivan, M. L. Williams, D. J. Ball, R. E. Bernard, R. G. Derwent, A. E. J. Eggleton, L. Hampton and R. E. Waller	569
Palaeoclimates of Central Sahara during the early Holocene	J. Maley	573
Dispersal in stable habitats	W. D. Hamilton and R. M. May	578

LETTERS TO NATURE

Radio emission from a normal HD26676 star	R. G. Strom and D. E. Harris	581
Enhanced metal depletions and interstellar H ₂ abundances	R. G. Tabak	582
Interstellar grains as possible cold seeds of life	V. I. Goldanskii	583
Muon catalysis of hot fusion	E. P. Hincks, M. K. Sundaresan and P. J. S. Watson	584
Water-promoted oxidation of carbon monoxide over tin (iv) oxide-supported palladium	G. Croft and M. J. Fuller	585
Windscale effluent in the waters and sediments of the Minch	H. D. Livingston and V. T. Bowen	586
Surge activity on the Barnes Ice Cap	G. Holdsworth	588
The rise and fall of intracellular pH of sea urchin eggs after fertilisation	A. Lopo and V. D. Vacquier	590
Calcium uptake during mitosis in the myxomycete <i>Physarum polycephalum</i>	R. P. Holmes and P. R. Stewart	592
Killed <i>Listeria monocytogenes</i> vaccine becomes protective on addition of polyanions	C. Van Der Meer, F. M. A. Hofhuis and J. M. N. Willers	594
Non-infectious virus induces cytotoxic T lymphocytes and binds to target cells to permit their lysis	J. C. Palmer, L. J. Lewandowski and D. Waters	595
Effects of a nonapeptide FTS on lymphocyte differentiations <i>in vitro</i>	A. Brand, D. G. Gilmour and G. Goldstein	597
Expression of MuLV GP71-like antigen in normal mouse spleen cells induced by antigenic stimulation	E. Wecker, A. Schimpl and T. Hünig	598
Are endogenous C-type viruses involved in the immune system?	C. Moroni and G. Schumann	600

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (*a*) is preferred to e^a if '*a*' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Development of improved cholera vaccine based on subunit toxoid	J. Holmgren, A.-M. Svennerholm, I. Lönnroth, M. Fall-Persson, B. Markman and H. Lundbeck	602
Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man	G. Uterman, M. Hees and A. Steinmetz	604
Human serum lipoproteins activate adipocyte plasma membrane adenylate cyclase	J. Pairault, J. Leveilliers and M. J. Chapman	607
Interaction of a fluorescent probe with acetylcholine-activated synaptic membrane	P. R. Adams and A. Feltz	609
Role of intracellular calcium in the transient outward current of calf Purkinje fibres	S. A. Siegelbaum, R. W. Tsien and R. S. Kass	611
Voltage noise from hair cells during mechanical stimulation	L. J. DeFelice and D. L. Alkon	613
Stimulation of the sympathetic nervous system during sucrose feeding	J. B. Young and L. Landsberg	615
Occurrence of a new class of tetrahydroisoquinoline alkaloids in L-dopa-treated parkinsonian patients	C. J. Coscia, W. Burke, G. Jamroz, J. M. Lasala, J. McFarlane, J. Mitchell, M. M. O'Toole and M. L. Wilson	617
γ -Endorphin, α -endorphin and Met-enkephalin are formed extracellularly from lipotropin C fragment	B. M. Austen, D. G. Smyth and C. R. Snell	619
Identification of the herpes simplex virus DNA polymerase gene	D. J. M. Purifoy, R. B. Lewis and K. L. Powell	621
Sister chromatid exchange and chromatid interchange as possible manifestation of different DNA repair processes	M. S. Sasaki	623
Long-term persistence of O^6 -methylguanine in rat brain DNA	P. Kleihues and J. Bucheler	625
Daunorubicin and adriamycin facilitate actinomycin D binding to poly(dA-dT)·poly(dA-dT)	T. R. Krugh and M. A. Young	627

BOOK REVIEWS

B and T Cells in Immune Recognition (F. Loor and G. E. Roelants, editors)	Maria de Sousa	629
Quark Confinement and Field Theory (D. R. Stump and D. H. Weingarten, editors)	Larry McLerran	629
The Biology of Symbiotic Fungi (Roderic Cooke)	P. W. Brian	630
Cryobiochemistry: An Introduction (Pierre Douzou)	C. C. F. Blake	630
The Hartree-Fock Method for Atoms: A Numerical Approach (C. Froese Fischer)	M. J. Seaton	631
Dielectric Spectroscopy of Polymers (Peter Hedvig)	Shimon Reich	631
The Year in Metabolism, 1975-1976 (Norbert Freinkel, editor)	R. W. E. Watts	631

OBITUARY

Wernher von Braun	Bernard Lovell	633
D. W. Holder	D. L. Schultz	635
Yves Guitton	Michel Delseny	636

M

© Macmillan Journals Ltd 1977
Published weekly
ISSN 0028-0836

Registered as a newspaper at the
British Post Office

London

4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington

711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor

David Davies

Editorial Staff

Marcus Dobbs Miranda Robertson
*Sandy Grimwade Stuart Sharrock
Eleanor Lawrence Chris Sherwell
Mary Lindley Hilary Taphouse
Sara Nash Robert Vickers
Peter Newmark Mary Wade*
Judy Redfearn Charles Wenz
*Washington office

Publishing Director

Jenny Hughes

Advertisement Manager

Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.
1901 West 8th St
Los Angeles, California 90057
Telephone (213) 483-8530
also at
57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794
and
Henry G. Dale Co and
Hudson-Wright Associates
235 East 50th St
New York NY 10022
Telephone: (212) 421 0142
and
Mary Wade in Nature's
Washington Office

Advertising Representatives in Japan

Douglas Kenrick (Far East) Ltd,
Kowa Daisan Bldg, 4th Floor,
11-45 Akasaka, 1-Chome
Minato-Ku, Tokyo, Japan
Telephone: (582) 0951-5

Classified advertisement enquiries to:

Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK £37.50
Europe US\$98
Japan { Surface US\$98
 Airmail US\$145
USA & Canada { Air/surface US\$98
 Airmail US\$145
Rest of world { Surface US\$94
 Airmail US\$145

Single copy (post-paid)

UK 80p Rest of world US\$2.50

Binders (post-paid)

UK £3.00 each, three for £8.00

Rest of world US\$6, three for US\$16

1976 Index (post-paid) U.K. £2.50, Rest of
World US\$5.00

Orders (with remittance) to:

Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045

Second-class postage paid at New York, NY
US mailing agent is.

Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

View from a cavern in a stranded
iceberg, Terra Nova in the distance,
8 January 1911. See page 639.

[from the Ponting Collection, Popperfoto]

Vol. 269 No. 5630 20 October 1977

nature

Volume 269

20 October 1977

Protection for the non-academic student	637
Read all about something like it	637
Of time and the energy wars	638
Planning Antarctica's future	639
Windscale: Ninety days and more	640
SRC's new head, old hand	643
Britain/USSR/In brief/Jukes on energy for crops	644

Correspondence	646
----------------	-----

NEWS AND VIEWS

Photon antibunching/Immunogenetics/Parity violation/Plant-insect adaptation	
Rio Grande rise/Dendrochronology/Ethology/Cosmic rays/Population regulation	647

REVIEW ARTICLE

Recent excitement in the DNA replication problem	B. Alberts and R. Sternglanz	655
--	------------------------------	-----

ARTICLES

'Unmixing' of the deep-sea record and the deglacial meltwater spike	W. H. Berger, R. F. Johnson and J. S. Killingley	661
Plate boundary within Tjörnes Fracture Zone on Northern Iceland's insular margin	R. L. McMaster, J-G. E. Schilling and P. R. Pinet	663
Amino-terminal fragments of <i>Escherichia coli</i> lac repressor bind to DNA	T. M. Jovin, N. Geisler and K. Weber	668

LETTERS TO NATURE

Electron scattering in X ray-emitting galaxies	A. C. Fabian	672
Absorption lines in the optical spectrum of quasar AO0827+24	M.-H. Ulrich and F. N. Owen	673
Prebiotic polymers and infrared spectra of galactic sources	N. C. Wickramasinghe, F. Hoyle, J. Brookes and G. Shaw	674
Atmospheric absorption between 4 and 30 cm ⁻¹ measured above Mauna Kea	P. H. Moffat, R. A. Bohlander, W. R. Macrae and H. A. Gebbie	676
Carbonate mineral detection by variable atmosphere differential thermal analysis	S. St J. Warne	678
Δ^2 -Sterenes as diagenetic intermediates in sediments	M. Dastillung and P. Albrecht	678
High shear velocity layer in the upper mantle of the Western Pacific	H. Shimamura, T. Asada and M. Kumazawa	680
Uranium series ages and late Quaternary uplift in the New Hebrides	G. Neef and H. H. Veeh	682
New evidence and possible origin of native iron in ophiolites of eastern Canada	E. R. Deutsch, K. V. Rao, R. Laurent and M. K. Seguin	684
Mesozoic sea floor off Dronning Maud Land, Antarctica	H. W. Bergh	686
Unusual diatom off the coast of south-west England and its effect on fishing	G. T. Boalch and D. S. Harbour	687
Pattern regulation and transdetermination in <i>Drosophila</i> imaginal leg disk reagggregates	S. Strub	688
Haemopoietic stem cells of rats but not of mice express Th-1.1 alloantigen	S. Thierfelder	691
Catecholamine hormone receptors are reduced on chronic lymphocytic leukaemic lymphocytes	J. R. Sheppard, R. Gormus and C. F. Moldow	693
Actin co-caps with concanavalin A receptors	B. H. Toh and G. C. Hard	695
Actin and tubulin co-cap with surface immunoglobulins in mouse B lymphocytes	G. Gabbiani, C. Chaponnier, A. Zumbé and P. Vassalli	697
Intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane	I. D. Goldfine, R. Vigneri, D. Cohen, N. B. Plam and C. R. Kahn	698

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; exp (*a*) is preferred to e^a if '*a*' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note: (i) only one reference number need be used if the reference is to several papers by identical authors, (ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely.

Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Inhibition of high-affinity choline transport in peripheral cholinergic endings by presynaptic snake venom neurotoxins	M. J. Dowdall, J. P. Fohlman and D. Eaker	700
High densities of benzodiazepine receptors in human cortical areas	C. Braestrup, R. Albrechtsen and R. F. Squires	702
Altered exploratory behaviour after 6-OHDA lesion to the dorsal noradrenergic bundle	S. T. Mason and H. C. Fibiger	704
Cerebral lateralisation effects in visual half-field experiments	C. Hardyck, O. J. L. Tzeng and W. S-Y. Wang	705
Calcium-dependent regenerative responses in rods	G. L. Fain, F. N. Quandt and H. M. Gerschenfeld	707
Ionic interactions in the potassium channel of squid giant axons	T. Begenisich and P. De Weer	710
Permeant cations alter endplate channel characteristics	D. Van Helden, O. P. Hamill and P. W. Gage	711
Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung	M. R. Boyd	713
Identification of a membrane-embedded segment of the large polypeptide chain of (Na ⁺ , K ⁺)ATPase	S. J. D. Karlish, P. L. Jorgensen and C. Gitler	715
Synthesis of haemoglobin Wayne in erythroid cells	S. M. Hanash, W. P. Winter and D. L. Rucknagel	717
Red cell charge is not a function of cell age	S. J. Luner, D. Szklarek, R. J. Knox, G. V. F. Seaman, J. Y. Josefowicz and B. R. Ware	719
Ion-induced changes in head group conformation of lecithin bilayers	M. F. Brown and J. Seelig	721
Replication of linear adenovirus DNA is not hairpin-primed	B. W. Stillman, A. J. D. Bellett and A. J. Robinson	723
Benzo[a]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA	T. Meehan, K. Straub and M. Calvin	725

MATTERS ARISING

General relativistic incompressibility	K. Brecher and I. Wasserman	728
Reply	F. I. Cooperstock and R. S. Sarracino	728
The nature of the fine structure of DNA melting curves	M. D. Frank-Kamenetskii and A. V. Vologodskii	729
Crustal and <i>in-situ</i> origin of Norwegian eclogites	M. A. Lappin	730
Reply	E. J. Krogh	730

BOOK REVIEWS

Perpetual Motion: The History of an Obsession (A. W. J. G. Ord-Hume)	N. Feather	731
Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture (J. Reinhart and Y. P. S. Bajaj, editors)	Peter S. Carlson	731
Potential Scattering in Atomic Physics (P. G. Burke)	B. H. Bransden	732
The Biology of Crabs (G. F. Warner)	Richard G. Hartnoll	733
The Virus: A History of the Concept (Sally Smith Hughes)	A. P. Waterson	733
The Historical Supernovae (D. H. Clark and F. R. Stephenson)	David W. Hughes	734
Structure Determination by X-Ray Crystallography (M. C. F. Ladd and R. A. Palmer)	P. G. Jones	734

OBITUARY

W. W. Bishop	H. B. S. Cooke	735
J. A. V. Butler	E. W. Johns	735
F. H. Ludlam	John Green	736

What's new—spectrometers

		737
--	--	-----

Announcements

		741
--	--	-----

M

© Macmillan Journals Ltd 1977
Published weekly
ISSN 0028-0836
Registered as a newspaper at the
British Post Office

London
4 Little Essex Street, WC2R 3LF
Telephone: (01) 836 6633 Telex: 262024
Telegrams: Phusis London WC2R 3LF

Washington
711 National Press Building, DC 20045
Telephone: (202) 737 2355
Telex: 64280

Editor
David Davies

Editorial Staff

Marcus Dobbs Miranda Robertson
*Sandy Grimwade Stuart Sharrock
Eleanor Lawrence Chris Sherwell
Mary Lindley Hilary Taphouse
Sara Nash Robert Vickers
Peter Newmark Mary Wade*
Judy Redfearn Charles Wenz
*Washington office

Publishing Director
Jenny Hughes

Advertisement Manager
Richard Webb

Advertising Representatives in the United States

Jobson/Jordan/Harrison and Schulz Inc.
1901 West 8th St
Los Angeles, California 90057
Telephone (213) 483-8530
also at
57 Post Street, Suite 712-715,
San Francisco, California 94104
Telephone: (415) 392 6794
and
Henry G. Dale Co and
Hudson-Wright Associates
235 East 50th St
New York NY 10022
Telephone: (212) 421 0142
and
Mary Wade in Nature's
Washington Office

Advertising Representatives in Japan

Douglas Kenrick (Far East) Ltd,
Kowa Daisan Bldg, 4th Floor,
11-45 Akasaka, 1-Chome
Minato-Ku, Tokyo, Japan
Telephone: (582) 0951-5

Classified advertisement enquiries to:
Classified Department, London Office,
Telephone (01) 240 1101

Annual subscription

UK	£37.50
Europe	US\$98
Japan	US\$98
USA & Canada	US\$145
Rest of world	US\$145

Single copy (post-paid)
UK 80p Rest of world US\$2.50
Binders (post-paid)
UK £3.00 each, three for £8.00
Rest of world US\$6, three for US\$16
1976 Index (post-paid) U.K. £2.50. Rest of World US\$5.00

Orders (with remittance) to:
Macmillan Journals Ltd, Brunel Road,
Basingstoke, Hants. RG21 2XS
Telephone: Basingstoke (0256) 29242

US Postmaster, please send form 3579 to Nature,
711 National Press Building, Washington DC 20045

Second-class postage paid at New York, NY
US mailing agent is:

Expeditors of the Printed Word Ltd.,
527 Madison Avenue,
New York, NY 10022

Cover picture

Spermatophore of the pogonophoran
Siboglinum ekmani, showing sperma-
tozoa cut at the head (at the peri-
phery of the picture) and tail region
(in the centre). See page 800.
(× 10,000)

Vol. 269 No. 5631 27 October 1977

nature

Volume 269

27 October 1977

How much further can the pendulum swing?

The Nobel prizes:

- (1) Rewarding solid state
 - (2) Thermodynamicist
 - (3) Starting a revolution
- Inadvertent collaboration

JET/Dioxin/Backgrounder/In brief/Mellanby on direct drilling

743

744

745

746

747

749

NEWS AND VIEWS

Gene derepression in tumours?/Quarks/RNA sequencing/Building science/
Nucleon optical potential/Hormone receptors/Pollen analysis

REVIEW ARTICLE

Claims and accomplishments of applied
catastrophe theory

R. S. Zahler and
H. J. Sussmann

759

ARTICLES

Radio structure of 3C147 determined by
multi-element very long baseline interferometry

P. N. Wilkinson,
A. C. S. Readhead,
G. H. Purcell and B. Anderson

764

Secular changes in marine inundation of USSR
and North America through the Phanerozoic

A. Hallam

769

Location and bond type of intermolecular contacts
in the polymerisation of haemoglobin S

R. E. Benesch, S. Kwong,
R. Benesch and R. Edalji

772

Synchronised transmembrane insertion and
glycosylation of a nascent membrane protein

J. E. Rothman and
H. F. Lodish

775

LETTERS TO NATURE

Radio haloes around BL Lacertae
objects AO0235 + 164 and 4C0.59

G.-Krishna

780

A constraint on the universal baryon
density from the abundance of ⁷Li

S. M. Austin and
C. H. King

782

Quantitative analysis of the
Dermott-Gold theory for Uranus's rings

K. Aksnes

783

Revenge of tiny Miranda

P. Goldreich and
P. Nicholson

783

Identification of water frost on Callisto

L. A. Lebofsky

785

Isotopic composition of
uranium in chondritic meteorites

J. W. Arden

788

Stacking faults in dolomite

D. J. Barber, H. C. Heard,
M. S. Paterson and H. R. Wenk

789

Acoustic imaging of the New
England shelf-slope water mass interfaces

F. C. Newman, J. R. Proni,
D. J. Walter and H. M. Byrne

790

Effect of climate on
chemical composition of fossil bones

Cs. M. Buczeko and
L. Vas

792

Physiological exclusion of
magnesium from *Mytilus edulis* calcite

R. B. Lorens and
M. L. Bender

793

Polonium and plutonium
in an intertidal food chain

L. Cheng, V. F. Hodge
and R. A. Lewin

795

Free-running activity rhythm
in the natural environment

J. E. Treherne, W. A. Foster,
P. D. Evans and C. N. E. Ruscoe

796

Sexual dimorphism, sociomonic
sex ratio and body weight in primates

T. H. Clutton-Brock,
P. H. Harvey and B. Rudder

797

Ultrastructure of the spermatophores of
Siboglinum ekmani Jägersten (Pogonophora)

H. Flügel

800

Blood-sucking flies and
primate polyspecific associations

W. J. Freeland

801

Physiological correlates
of an enzyme polymorphism

A. J. Leigh Brown
P. J. Polverini, R. S. Cotran,
M. A. Gimbrone, Jr and
E. R. Unanue

803

Activated macrophages
induce vascular proliferation

T. Tursz, W. H. Fridman,
A. Senik, A. Tsapsis
and M. Fellous

804

Human virus-infected target
cells lacking HLA antigens
resist specific T-lymphocyte cytolysis

806

Guide to authors

● Review articles should be aimed at a relatively wide readership. Many reviews are invited, but submitted articles may also be accepted; it is advisable to consult us before writing a review article.

● Articles may be up to 3,000 words long with at most six displayed items (figures and tables); they are reports of major research developments.

● Letters are brief reports of original research of unusual and wide interest, not in general longer than 1,000 words; they have at most three or four displayed items.

● 'Matters Arising' permits short discussion (up to 300 words) of papers that have recently appeared in *Nature*.

Articles should be accompanied by an abstract of not more than fifty words. Letters should begin with a paragraph giving the background and main conclusions in terms intelligible to as wide a readership as possible.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible; $\exp(a)$ is preferred to e^a if 'a' is more than one character.

References are indicated by superscripts in the text. See any contemporary *Nature* for style, but note:

(i) only one reference number need be used if the reference is to several papers by identical authors,

(ii) first and last pages of references should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished' should be incorporated in text.

Artwork should be sent with the manuscript and clearly marked with author's name and the figure number. Line drawings should be either photographic prints or in Indian ink on heavy cartridge paper, tracing paper or similar materials. Most figures are reduced to one column width so originals should be about as wide as a page of *Nature*. To enable figures, particularly maps, to be edited in the same style as the text, they should contain only essential material. Ideally, an unlettered original and three lettered copies should be provided; labelling on half-tones should, if possible, be avoided entirely. Magnifications quoted should be for the figures as submitted. We are always glad to see artwork for possible use on the cover, but cannot guarantee its return.

In order to save on postal expenses we return only the top copy and artwork of manuscripts that we cannot publish.

Nature's publishing policy is outlined in 258, 1 (1975) and 264, v, 11 Nov. (1976).

Evidence for an <i>H-2</i> /viral protein complex on the cell surface as the basis for the <i>H-2</i> restriction of cytotoxicity	K. J. Blank and F. Lilly	808
Effect of protease inhibitors and substrates on deoxycorticosterone binding to its receptor in dog MDCK kidney cells	M. E. Baker and D. D. Fanestil	810
Non-sequential expression of multiple immunoglobulin classes by isolated B-cell clones	P. J. Gearhart	812
Immunoprotection by embryonal carcinoma cells for methyl-cholanthrene-induced murine sarcomas	K. Sikora, P. Stern and E. Lennox	813
Chemical mutagenesis of mammalian cells can be quantified	J. P. O'Neill and A. W. Hsie	815
Increasing ovulation rate in sheep by active immunisation against an ovarian steroid androstenedione	R. J. Scaramuzzi, W. G. Davidson and P. F. A. Van Look	817
Is α MSH a trophic hormone to adrenal function in the foetus?	J. R. G. Challis and J. D. Torosis	818
Local anaesthetic benzyl alcohol increases membrane thickness	R. G. Ashcroft, H. G. L. Coster and J. R. Smith	819
Quantum amplitude distributions point to functional unity of the synaptic 'active zone'	A. Wernig and H. Stirner	820
Light-activated GTPase in vertebrate photoreceptors	G. L. Wheeler, Y. Matuo and M. W. Bitensky	822
Salt-sensitive <i>in vitro</i> protein synthesis by a moderately halophilic bacterium	R. M. Wydro, W. Madira, T. Hiramatsu, M. Kogut and D. J. Kushner	824
Carbon dioxide governs the oxygen affinity of crocodile blood	C. Bauer and W. Jelkmann	825
Hapten-induced allosteric transition in the light chain dimer of an immunoglobulin	D. Lancet, A. Licht, I. Schechter and I. Pecht	827
Two enzymes are required for strand incision in repair of alkylated DNA	J. Laval	829
Mass spectrometric determination of molecular formulas for membrane-modifying antibiotics	K. L. Rinehart, Jr, J. C. Cook, Jr, H. Meng, K. L. Olson and R. C. Pandey	832
New rapid gel sequencing method for RNA	A. Simoncsits, G. G. Brownlee, R. S. Brown, J. R. Rubin and H. Guillely	833

BOOK REVIEWS

Doing Better and Feeling Worse (J. H. Knowles, editor)	W. W. Holland	837
The Differentiation of Cells (N. Maclean)	C. C. Ford	837
The Moon Book: Exploring the Mysteries of the Lunar World (B. M. French)	Neil R. L. Goult	838
Low Density Lipoproteins (C. E. Day and R. S. Levy, editors)	Angelo M. Scanu	838
Modern Physics in Chemistry (E. Fluck and V. I. Goldanskii, editors)	D. W. Turner	839
Modern Practice of Gas Chromatography (R. L. Grob, editor)	A. T. James	839
Organic Chemistry of Sulfur (S. Oae, editor)	A. E. Tipping	839
Morphogenetics of Karst Regions (L. Jakucs)	M. M. Sweeting	840

How Britain Runs Its Science

A completely revised edition of this highly successful wall-chart (*Nature*, 28 August, 1975), showing the organisations and people running British science, is now available. For details and order form, see page xii.

nature*1 September 1977*

South Africa's nuclear intentions

WORLDWIDE alarm over reports that South Africa is on the verge of joining the nuclear weapons club were temporarily stilled last week when Prime Minister John Vorster privately assured the United States that South Africa is not developing nuclear weapons. The assurance is welcome, indeed, but it would be even more comforting if South Africa were to back up its protestations of innocence by signing the nuclear non-proliferation treaty and by placing its nuclear facilities under international inspection, steps which Mr Vorster's government has consistently refuse to take. The flap over South Africa's intentions does, however, have some positive aspects.

The alarm was first raised on 9 August by the Soviet press agency, Tass, which warned that South Africa was about to explode a nuclear device. A few days later, Tass charged more specifically that plutonium for the country's nuclear weapons would come from two 1,000MW reactors that France recently agreed to supply to South Africa. Since the reactors will not be in operation until 1982, and the agreement requires spent fuel from them to be reprocessed in France with the extracted plutonium remaining in French hands, the Soviet charges were hard to believe. Nevertheless, they prompted the United States, Britain, France and West Germany to take the matter up with Mr Vorster and his government.

The public debate was fuelled further last week when Louis de Guiringaud, France's foreign minister, announced in an interview that France also has new information indicating that South Africa was preparing to test a nuclear device. Dr Guiringaud warned that such a test would have "grave consequences", which would presumably include shelving the sale of the two French reactors and further political and economic reprisals.

Although few details of the basis for de Guiringaud's announcement have been revealed, it is widely believed that the concern was based on evidence of extensive construction work in the Kalahari Desert. The evidence was reportedly obtained by satellite and may have sparked the original Tass pronouncement.

South Africa's response to all of these charges was initially to protest that its nuclear activities are entirely peaceful, a protestation that left room for doubt since it

should be recalled that India's explosion of a nuclear device in 1974 was also labelled as peaceful. At his press conference on 22 August, however, President Carter announced that the United States has been given a more complete assurance that South Africa is not intending to develop nuclear weapons.

Specifically, Carter announced that "in response to our own direct inquiry and that of other nations, South Africa has informed us that they do not have and do not intend to develop nuclear explosive devices for any purpose, either peaceful or as a weapon—that the Kalahari test site, which has been in question, is not designed to test nuclear explosives, and that no nuclear explosive tests will be taken in South Africa, now or in the future". Carter added, however, that the United States would "continue to monitor the situation very closely".

Some comfort should be taken from the fact that as soon as allegations were first raised about South Africa's nuclear intentions, four major western powers moved swiftly to put South Africa on notice that a nuclear explosion would have grave political consequences, and that South Africa would not be allowed to hide behind the pretence that an explosion could be called peaceful. South Africa's unequivocal denial of plans to develop nuclear explosives is a welcome statement.

Nevertheless, until South Africa agrees to submit its nuclear facilities to international inspection, suspicions will linger. It should be noted, for example, that the country has large reserves of uranium and a pilot-scale enrichment plant, perhaps capable of enriching uranium to weapons grade. Experts in the United States have publicly stated that they believe South Africa has the technical ability to produce explosives, given sufficient concentration of funds and manpower on the effort. South Africa has now been put firmly on notice that any such attempt would lead to grave political consequences including possible abrogation of trade and economic agreements, but Carter also noted last week that the United States would "renew our efforts to encourage South Africa to place all their nuclear power production capabilities under international safeguards and inspections". That should be a major political priority. □

Technik: the relevance of a missing concept

M. Fores, of the UK Department of Industry, and I. Rey, previously of the Swedish Ministry of Industry, offer their personal suggestion that the absence of an English word to describe the 'useful arts' of manufacture leads to unnecessary confusion in talking about the world of manufacturing

WRITERS in the English language often use words in such a way that 'science' and 'technology' are considered as key features affecting manufacturing. Sometimes they are even wrapped together and simply known as 'science'. Hilary and Steven Rose, for instance, do this in their book *Science and Society*. Such a usage incorporates a number of errors concerning what was once known as the 'useful arts' of manufacture. To show this, we introduce the word *Technik* which is widely used and understood in Germany. It concerns the functioning of natural and man-made things, including the set of principles according to which artifacts work and the methods used in making them. Most continental European languages include such an idea.

One English language study which erred over the general influence of 'science' in the culture was Bronowski and Mazlish's *The Western Intellectual Tradition* (Penguin). They wrote:

What is needed is that in the general history books the development of science should take its place along with political and economic developments. The steam engine helped to shape the modern world at least as much as Napoleon and Adam Smith, but only rarely do historians admit the fact . . .

Few would dispute that the steam engine has had an important influence on the modern world; but it cannot sensibly be thought of as a 'product of science'.

In Germany, if there are two cultures or sub-areas of the general culture, they are not the science and humanities of the English split. They are *Wissenschaft*, concerned with all knowledge and all the subjects of the classical university, and *Technik*. Normally, however, there are three sub-cultures: *Kunst* 'art', the English 'fine arts' and performing arts; *Wissenschaft*; and *Technik*, widely accepted to be separate from both. *Wissenschaft* means the art of handling knowledge, akin to 'gamesmanship' and seamanship'.

The importance of *Technik* stems from the fact that it has been a prime concern of the species from earliest times. Some of its first products were stones sharpened to kill animals for food, or to ward off dangerous animals. Man became a tool-maker and tool-user through *Technik*.

The history of *Technik* is not, however, the conquest of nature. Problems of *Technik* are not seen in terms of nature but rather of a specific job to be done or an artifact to be made. And more complex contrivances strayed further from the natural state of their component materials. A stick, as a lever, was made of wood, directly from the natural world. But a potter's wheel could be made from various materials, probably in combination. And a governor to control the speed of a rotating machine is a contrivance to control a contrivance. Thus the exponent of *Technik* must be obsessed with the artificial, rather than the natural. If the operations of *Technik* shade into those of either of the other two cultural areas, they shade into *Kunst*, rather than *Wissenschaft*. Metal-working skills are an obvious example of craft skills that have been shared between making the products of fine art and useful artifacts.

The 'applied science' concept is a peg on which some have hung their views about manufacturing. Only in English, of the major European languages, can the concept exist, since only English has a meaning for the word science which only includes knowledge of natural pheno-

mena, or the process by which this knowledge is derived. By use of the 'applied science' phrase, the process of *Technik* is made to seem closer to the natural world than it is in practice.

The applied science concept is misleading for other reasons. It implies that a contrast can exist with pure science. Yet there can never be any body of pure science at all, if the criterion used is lack of utility, or lack of applicability of knowledge. All scientific knowledge is put out freely for verification of falsification—so all can be used freely and with no attribution. The most basic ideas of natural science, such as Newton's laws of motion, turn out to be widely applicable in practice. So the bizarre conclusion must be drawn that the purest of science is more widely applicable in practice than work which is done for particular uses, so-called applied work. Applied science as a sensible near-alternative to *Technik* fails because manufacturing cannot be well characterised as the application of scientific knowledge. Furthermore, studies have shown that the rate of technical progress in manufacturing cannot be equated with the rate of adoption of science.

Continental-style *technologie* invariably means what one would expect from its Greek roots (*techné* means art; *logos* means word or law), so it is not the same as English-language technology. British official statistics show that most technologists are engineers; they are not like other types of '-ologists', students of events. The use of the concept of *Technik* avoids this misunderstanding.

Another difference between perceptions of *Technik* and of technology comes with the idea of utility. Acts of *Technik* are accepted to be pointless if there is no demand, within the existing cost pattern, for the useful artifact being made. There are no imperatives of *Technik* along the lines of technological imperatives of the English-language, whereby critics complain that because something can be made it will be made.

Another feature is that *Technik* does not rule well in an aristocratic or a dictatorial situation, if only because it depends more on detail than on principle. The best conception for a contrivance, or the best design for it, is of no use if the detailed manufacture of component parts is not up to standard. The whole may not be greater than the sum of the parts; but if one of the parts is defective, the whole cannot function.

Continental *Technik* is accepted to be a separate cultural area with codes of behaviour, responses and a dignity of its own. The competent *Techniker* does not feel that he has constantly to look over his shoulder at science or the arts, as the exponent of technology often does. Science and arts do not care much for utility and they have a different grasp of the artificial. *Technik* remains a missing concept in English; its absence is the English speaker's loss. □



Potter's wheel: an example of Technik

The French 'nuclear war'

Les écologistes are gaining a voice in French politics. Alexandre Dorozynski outlines their growth

THE French government is currently faced with a political issue it would doubtless have preferred to keep out of the public forum: that of its "all out" nuclear power development programme and, more particularly, of the fast breeder Super-Phénix reactor at Malville, near Lyon. The issue has become political as the campaign preceding the nationwide legislative elections to be held in March next year has got underway. Polls indicate that the election may well give the Socialist-Communist coalition an edge in the National Assembly, and it now seems likely that the 'nuclear weapon' will be used in the forthcoming 'battle'.

Socialist leader François Mitterand has already suggested that a national referendum be held on the nuclear programme. And a loosely-knit group, termed somewhat vaguely and sometimes disparagingly as *les écologistes*, are beginning to represent a political power to be counted with. Under a programme termed *Écologie '78*, they are to be represented at the municipal elections next year. Seven ecological groups will put forward candidates.

Les écologistes first emerged as a political group in 1974 under the leadership of agronomist René Dumont, who received 1.34% of the votes cast during the presidential election that year. Since then, their numbers have increased rapidly: during the municipal elections earlier this year, they mustered an unexpected 10% of the votes. So far, they have refused to support any of the existing political parties, but if they decide to throw their weight one way or the other, they could well alter the balance between right and left.

But who are *les écologistes*? According to the Ministry of the Environment, created in 1971, there are nearly 15,000 ecological associations in France. They

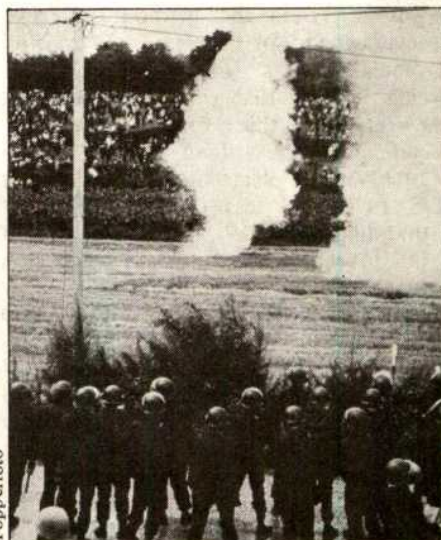
include such groups as the National Federation for the Protection of Nature, created in 1968, now with a membership of about 100,000; the Association for the Rights of Pedestrians (15,000); the French Association for the Protection of Water (about 1,000); and smaller, regional and special interest groups such as the Friends of the Trees of the Loire (500) and the Committee against Atomic Pollution of La Hague (150). Some of these associations overlap, and total membership is probably no more than two million. The first associations were started with specific, sometimes narrow goals, but interest gradually grew to include more general issues concerning modern life; consumerism, means of production, housing, and so on.

Nuclear power concern

One of the major issues now concerning *les écologistes* is the nuclear power programme and the construction of Super-Phénix, which was the bone of contention of some 50,000 marchers who converged on the farming region of Malville, 40 miles from Lyon and 30 miles from Geneva on Sunday 31 July. They clashed with a few thousand police and a paratrooper unit in what has been called the first battle of the French 'nuclear war'. The battle had its casualties—about 100 wounded and one dead. The dead man, Vital Michalon, was a quiet, young teacher of physics and chemistry (and a reserve officer) whose death has been variously attributed to heart failure, to the explosion of a concussion grenade used by police, or to that of an amateurish explosive device put together by the less peaceful *manifestants*.

The 'march on Malville' might have been dismissed as the kind of incident likely to occur during a hot summer (even though the summer was cool and the marchers were soaked by rain) but public polls, in which Frenchmen participate eagerly, indicate a mounting popular concern which the government cannot dismiss. In 1974, three-quarters of representative samples of Frenchmen were "very much in favour of or rather in favour of" the development of nuclear energy. At the beginning of this year, the percentage in favour had fallen to 50%, while that against had risen from 17% to 41%.

A poll carried out for the weekly news magazine *Le Nouvel Observateur* after the 'march on Malville' showed up the somewhat conflicting views of the French on the nuclear issue. Although nearly 70% considered an



Malville: the first battle

increase in energy consumption to be indispensable to the improvement of their way of life, nearly 50% would be "willing to change their consumption habits and way of life if this could stop the construction of nuclear power plants". A majority (56%) approved of Mitterand's suggestion for a referendum on nuclear power. Of those questioned who identified themselves with the Communist party, 76% approved of the proposed referendum, which Georges Marchais, the Communist leader, has since vigorously condemned.

It now seems impossible to keep the public debate from spreading, and politicians, right and left, may be called upon to take sides. The Ministry of the Environment is bound to go along with the government's pro-nuclear stand, especially as Michel d'Ornano (he is the one who lost the Paris mayoralty to Jacques Chirac), now Minister for Culture and the Environment, used to be, as Minister for Industry, one of the proponents of the "all nuclear" power plan. And the importance of the forthcoming debate has grown in the light of recent talk of a moratorium on the construction of new nuclear power plant in Germany.

So what do the ecologists really want? Few of them will deny that, in view of the poverty of French resources in oil and coal, and the built-in limitations to the development of hydro-electric power, some of France's energy should be nuclear. But most consider the intensive programme unreasonable, and the development of Super-Phénix risky. The government, on the other hand, views nuclear power as a means of stopping the outward flow of currency, and the fast breeder as a major trump to play in case of uranium scarcity in the future (and, also, as a potentially marketable technology).



Michel d'Ornano, environment minister

It is certain that without intensive development of nuclear power, the 'official forecast' of 370×10^9 kWh of electricity by 1985 will not be reached, by far. But is this a forecast or a policy statement? Less than half of 370×10^9 kWh of electricity was produced in 1975, and less than one-tenth of that figure in 1950. Ecologists do not fail to remark that Eurodif, Europe's first uranium enrichment plant, at Tricastin, is itself expected to consume some 30×10^9 kWh a year, which is almost as much as the total electrical energy output in 1950. And they also argue that a less spectacular increase in energy production may not mean, as hawks have hinted, a return to the Middle Ages.

Marching for other issues

But nuclear power is not the 'ecologists' only concern. On 15 August, protesters marched on the Larzac plateau, in the southern part of the Massif Central, against the Army's proposed take-over of more than 10,000 ha of land for training and testing. The expansion was planned in 1970 and would have been completed last year, were it not for the deter-

mined opposition of local farmers, who have succeeded in mustering considerable support. In six years of stubborn but largely peaceful opposition, the farmers have become well organised and have learned many tricks. For example, they published a study of cattle and sheep farming in conjunction with an effective public relations operation which included releasing a flock of sheep under the Eiffel tower in Paris; on another occasion they organised a symbolic harvest for the Third World which opened a debate on malnutrition.

The success of the Larzac farmers, who have joined the loose ranks of *les écologistes*, is measured by the fact that the Army has not been able to acquire half of the land it wanted, and that sheep are still squatting on some of the Army-owned land in virtue of ancient pasture rights. Conscious of public opinion, the Army has seldom resorted to expulsion by force.

Les écologistes have also contributed to the sinking of a proposal to construct a super-highway in Paris (the *Radiale Vercingétorix*, named after the famous Gaul warrior), to preserving some sites from urbanisation, to

promoting legislation against water pollution, to imposing controls on the asbestos industry, and to banning the 2-4-5T defoliant. But it should not be forgotten that they are a variegated group, which includes the scientifically inclined, philosophers, and dreamers who yearn for the unpolluted paths cherished by Jean Jacques Rousseau and probably gone forever.

Many of them, and the public, simply do not recognise the boundary between dream and reality because they are not well enough informed. Most, however, appear to have a genuine concern about the environment as a part of society that has changed irreversibly, and believe, as voiced by Giscard d'Estaing at a ministerial council last July, in "the importance of environmental policy as a factor of transformation of society".

As Frenchmen shift to high gear in their semi-permanent political campaigning, the hope is for a reasonable, informative debate. Perhaps then the traditionally central authority, and the inevitably peripheral, but increasingly concerned, citizen will find a common point. □

USA

New DNA draft

An important new factor has been injected into the long-tangled process of developing legislation to control recombinant DNA research in the United States. Colin Norman reports

WHEN members of Congress left Washington for their summer vacations a month ago, two separate versions of legislation to control recombinant DNA experiments were under consideration. In the Senate, a bill developed by Senator Edward Kennedy's Health Subcommittee was awaiting consideration by the full Senate, while in the House, a bill drafted by Representative Paul Roger's Health Subcommittee was pending before the House Commerce Committee. When Congress returns next week, however, a third version will have to be taken into serious consideration, and the already murky legislative outlook has become even more opaque. In fact, there is now a real possibility that Congress will not complete its work on the legislation this year.

The new version has been drafted by Senator Gaylord Nelson, a Democrat from Wisconsin, in response to concerns which a number of scientists have voiced over the Kennedy bill. Nelson

says he plans to offer his bill as an alternative to Kennedy's when the legislation is brought to a vote in the Senate, and the move will guarantee a full-scale debate on some of the key issues.

The Kennedy bill has aroused some concern because it would establish a Presidentially-appointed commission to draft new rules for recombinant DNA experiments, licence facilities and approved experiments, an arrangement which many scientists view as a recipe for bureaucratic delay. The bill would also establish hefty fines for those who violate the rules and it would allow state and local governments to write regulations which would be stricter than the federal controls. The House version would be less restrictive since it would leave most of the responsibility for implementing controls in the hands of local biohazards committees, and it would also place considerable restraint on the authority of local governments to set their own recombinant DNA regulations.

Until recently, the Kennedy bill was moving through the Senate without encountering much Senatorial interest or opposition, and it was scheduled for a vote in the full Senate shortly before the August recess. It was shunted aside by more pressing legislation, however, and it is still pending. The delay has

enabled a number of scientists to step up their lobbying, and their concerns have met with a sympathetic reception in Nelson's office. Nelson's proposed bill is very similar to the House version, though in some respects it is even less restrictive.

Though Nelson has not actively sought supporters for his bill, his office has been receiving a number of calls from other Senate offices expressing interest. In addition, other Senators, including Moynihan of New York, Eagleton of Missouri and Chafee of Rhode Island, have voiced concern about Kennedy's proposal to establish a new recombinant DNA commission. In short, Nelson's bill is likely to gather considerable support. Nevertheless, if it is put to a vote and it goes down to a heavy defeat, those who support the Kennedy bill would have their hand considerably strengthened when negotiations begin to iron out differences between the House and Senate bills.

Meanwhile, the Roger's bill is not likely to be approved by the Commerce Committee much before the end of September, and it may then be referred to the House Science and Technology Committee. That would delay passage by the House until mid-October at the earliest, which means that there would be little time to reach a compromise with the Senate version before Congress goes into recess before the end of October. □

IN BRIEF

EEC research

The European Commission has recently sent proposals for a new European science and technology policy to the Council of Ministers. In a communication called *The Common Policy in the Field of Science and Technology*, it suggests a new structure for cooperation and some areas where collaborative research should be increased.

Since 1973, energy has taken 64% of the Community R&D budget. The proposals say that this balance should be redressed and suggest that a new programme of forecasting and assessment in science and technology (FAST) be set up. FAST will be a collaborative effort between research groups in Europe and international bodies with an initial budget of 4.4 million ua (1ua=£0.66).

ICRF sues for libel

Lawyers for the UK Imperial Cancer Research Fund (ICRF) have recently issued writs for libel against Daily Mirror Newspapers Ltd. The legal action has arisen over an article published in the *Sunday Mirror* on 14 August, questioning whether some of the £23 million which the ICRF has invested could be put to better use.

The ICRF replied to the article in a statement issued on 15 August which explained that such a high level of investment was necessary to support long term research. The decision to sue for libel was taken after the *Sunday Mirror* refused to publish an apology.

Engineering inquiry

Following hard on the heels of the UK

Department of Education and Science's (DES) discussion paper *Industry, Education and Management is Education, Engineers and Manufacturing Industry*, a report prepared for the British Association for the Advancement of Science (BAAS) under the direction of Dr Joseph Pope, Vice Chancellor of Aston University. Like the DES publication, the BAAS report is the outcome of an investigation into ways of encouraging able professional engineers to enter British industry.

The report puts some of the blame for industry's ills on the low status of engineers, which discourages young people from entering the profession. It recommends that universities and polytechnics accept candidates without 'A' level physics. More women should also enter engineering.

THE principal mountains of California, the Sierra Nevada, run in a north-south chain. They are unbroken by highway crossings for 165 miles of their greatest height. Within this vast area, about 40 miles wide by trails, are two National Parks, Kings Canyon and Sequoia. North of Kings Canyon Park is the equally splendid Sierra National Forest. The flank of the Sierra lying east of the summit crest is outside the park boundaries, and within Inyo National Forest. Much of the terrain outside the parks has been designated as Wilderness Area, administered by the US Department of Agriculture. Happily, there is collaboration between the Departments of the Interior and Agriculture in administering this scenic treasure, which in recent summers has swarmed with knapsackers. In 1976, for example, it received 1.1 million visitor-days of use.

"The Earth was created by the assistance of the Sun, and should be left as it was". So, we are told, said Chief Joseph of the Nez Percé. Much of the Earth is no longer as it was, but Chief Joseph's concept is still the guiding principle for managing wilderness. Detailed instructions of the observation of this principle are given to human invaders at the entry points to the wilderness, which are monitored. Yet there are anomalies. One of them is the presence of saddle horses and pack mules, whose excretory reflexes seem to be stimulated while wading through streams. These animals, however, are allowed to mar the wilderness; they have a strong hold on western American traditions.

Another interesting problem is that pet dogs are permitted in wilderness

areas that lie outside the boundaries of national parks. I have just returned from the John Muir Wilderness Area and its visiting dogs. One of the most prominent breeds was the Labrador retriever, a dog that

Wilderness areas



THOMAS H. JUKES

enjoys jumping in a mountain lake to fetch a piece of wood. The name of the canny Scotsman, Muir, has been given to many other California landmarks, including schools, a hospital, a national monument and a famous trail. Perhaps Muir's wilderness area should be re-named for his dog, 'Stickeen'. An even better idea would be to exclude the dogs, so that native mammals would be less harassed.

A further anomaly: hunting, in season, is permitted in wilderness areas of national forests, but not in national parks. Sharpshooters lie in wait just outside the park boundaries

to pick off deer that heedlessly step outside the demilitarised zone. The official instructions issued to wilderness users remind them, almost plaintively, that target practice with firearms is illegal, but hunting is not.

Our camp was evidently in the territory of a marmot. These beasts usually greet intruding hikers with a shrill whistle, and then scurry into holes. This one walked into camp, apparently to be photographed, after which it fixed us with a withering stare, and refused illegal offerings of food. Another frequent visitor to camps is a raucous bird, Clark's nutcracker. Now I know that the regulations say that the entrails of fish should be carefully buried at least 100 feet from water, but Clark's nutcracker would much rather devour them on the spot, and I could not resist his supplication. Next morning, at daybreak, he awakened me in the whitebark pine just above my head with a loud scream to demand breakfast.

At timberline, about 11,000 feet, the flowers make a mockery of all amateur and professional gardeners. Last week, in Pioneer Basin, it was the time for blue, mauve and pink penstemons: small blue-and-white lupines, golden bush potentillas; rose epilobium, and both large and tiny yellow monkey flowers. Eastern brook trout (*Salvelinus fontinalis*) in the high lakes scorned, for the most part, the offerings of anglers. There were some splendid afternoon thunderstorms, the nights were clear and star-filled, and the mornings blazed with sunlight. In general, the visitors, with their small tents and bright clothing, were heedful of Chief Joseph.

correspondence

Sinking of 'Cavtat'

SIR,—With regard to the report by Alastair Hay (26 May, page 301), on the sinking of the Yugoslav ship 'Cavtat', off the coast of Otranto in southern Italy, I understand that there is no evidence that poisonous lead derivatives have leaked from the drums in which they are sealed.

The article, however, is decorated with the picture of an Italian fisherman holding fish, with the caption "Poisoned fish caught in the Gulf of Taranto". That picture and caption will certainly capture the attention of most readers of *Nature*, and it will mislead them into thinking that if they visit that area, they will be at high risk of being poisoned. Because this is clearly not the case, the above picture seems ungenerous toward the people of the area, who are heavily dependent on tourism and may suffer from the effect of an unfortunate picture and caption published in an authoritative journal like *Nature*.

FRANCESCO BRESCIANI
Department of Pathology,
University of Toronto, Canada

Effects of marine research

SIR,—Each time I re-read Desmond Scott's assessment (30 June, page 762) of the effects on marine research of the Third United Nations Conference on the Law of the Sea, I get more annoyed. It is not that his forecast seems improbable—coastal states are indeed likely to exert total control over all scientific research on their continental shelves and within 200 miles of their shores. But the account of how this situation arose and how it should be cured exhibits a curious bias.

Scott appears to attribute all political difficulties between developing and developed countries, especially as they pertain to ocean resources and jurisdiction, to the marine scientists. His view of them is surprisingly bitter and unfortunately misleading. I contend that the scientists had little to do with creating the problem and can do little to solve it. In the confrontation between the developed and developing countries, related as it is to the history of colonialism and its children, post- and neo-colonialism, and accompanied by the emergence of the 'new economic order', marine science has played a pretty trivial role.

The issue of control over marine

science was initially evoked for bargaining purposes, could well have been dropped once the question of jurisdiction over coastal resources was settled, and now refuses to go away, to the great eventual cost of all who use the ocean and its resources or are affected thereby. International interactions under the new law of the sea will be among governments, not individuals, and there are few governments whose actions are determined by scientists, especially when larger political forces are at play.

WARREN S. WOOSTER
Institute of Marine Studies,
University of Washington, USA

Harvesting whales

SIR,—In your 'in brief' columns (16 June, page 576) there is a confused and inaccurate account of some analyses I have done on the dynamics of harvested populations. Particularly misleading is a belief attributed to me that certain whale species are under the threat of extinction due to the dynamic instability of the harvesting strategy. I have never made any statement to this effect and do not believe it to be true. Indeed the idea is absurd.

JOHN BEDDINGTON
Department of Biology,
University of York, UK

Darwin and his flowers

SIR,—In view of your reviewer's acceptance of "the role of plants in Darwin's scientific life," (21 July, page 273) it may be of interest to mention that in a collection of unpublished letters from eminent botanists and gardeners, written to the late William Robinson, editor of *The Garden* (1871–1927), and preserved by a member of this Association related to Robinson, are some written by Charles Darwin from Downe, Kent in 1875.

On 4 January he wrote: "I am much obliged for your kind gift of six copies of *The Garden*, with a drawing of myself and a notice of my works. This notice is drawn up in the most generous spirit and is highly honourable to me. I thank the writer very sincerely."

In a further letter he wrote: "I may mention (though it is improbable) that you can aid me on one point. If you have some *Euryle ferox*, and if it produces more than one flower at a time, I wish you would cross some and fertilise some others with their

own pollen, in order to see, when their seeds are counted, whether the cross aids at all in increasing their fertility. The *Euryle* is dead at Kew where they would have made the trial on a large scale for me. Professor Carpavy (?) has advanced this plant as a case of self-fertilisation for many generations with unimpaired fertility."

He also asked for some experiments with two distinct plants of *Nymphaea* which were protected while expanded from insects. On 5 May he wrote: "I write . . . to thank you and to say that it will be superfluous to castrate the flowers which are crossed with a pollen from a distinct plant, grown under as different circumstances . . . as maybe. . . . The flowers fertilised with their actually own pollen should certainly be protected from insects. . . . I was glad to see you elected to the Linnean Society."

Charles Darwin's chief contribution seems to have been to make the world realise that plants have a history as well as a structure which had been the sole concern of most botanists.

ERIC HARDY
Merseyside Naturalists' Association,
Liverpool, UK

Grants for social science

SIR,—The article 'Research on research' (4 August, page 398) refers to the sum of £25,000 transferred to the Social Science Research Council (SSRC) from the Council of Scientific Policy (CSP) for science policy studies. You state that the research grant to the Science Policy Research Unit (SPRU) at the University of Sussex "means that other applications are denied". This is untrue, and is a misunderstanding of SSRC's procedures.

The grant made to SPRU is under the SSRC's research grant scheme, and the funds come from the general research grant allocation. The sum transferred to SSRC from CSP for science policy studies is at the disposal of the Research Initiatives Board (RIB) for research contracts. Should the RIB and Council decide to give greater priority to science policy studies it is up to them to take a further initiative in this area.

It is therefore untrue that the research grant to SPRU closes the door to other applicants.

DAVID WAINWRIGHT
Social Science Research Council,
London, UK

news and views

Nucleosome core structure

from Michael Spencer

THIS issue of *Nature* (page 29) contains the first results of an examination of crystals made from chromatin core particles. The analysis, by Finch *et al.* of the MRC's Cambridge laboratory, is an impressive result of applying all the resources of a multidisciplinary group to rather limited data, so as to extract from it the maximum amount of information.

The core particle is a repeating unit containing 140 base pairs of DNA and an octamer of basic proteins, and is a degradation product of the complete nucleosome which (in rat liver) contains 200 base pairs and an extra protein. Earlier workers on nucleosomes have used electron microscopy, X-ray and neutron diffraction, and biochemical techniques, to build up a tentative picture of a somewhat asymmetrical particle with a hole in the middle, the DNA being wrapped round the outside. A model which anticipated some of the features seen by Finch *et al.* has been described by Richards *et al.* (*Cell Biology, Int. Rep.* 1, 107; 1977). Finch *et al.* have now established a model of this type in a rigorous manner and filled in some new details, by developing a large scale preparation method for homogeneous core particles and obtaining single crystals.

Crystals were first obtained in 1975 by Bakayev *et al.* (*Nucleic Acids Res.* 2, 1401), but they could only record powder diffraction patterns because the crystals were too small. Even now the analysis is restricted to details larger than about 20 Å, either because of the disorder within the crystals or because there is a combination of small crystal size and large unit cell. It is enough, however, to define a probable path for the DNA and to suggest some features of the protein core. An interesting new technique, which will surely be applied

to other large structures in the future, has been the use of electron microscopy to deduce the phases of the X-ray reflections. This vital information, without which a structure cannot be solved, is often deducible only with the aid of isomorphous derivatives which in the present case were not available.

Finch *et al.* got round this difficulty by taking electron micrographs of small fragments of single crystals, and obtaining optical diffraction patterns from the micrographs. Some of these patterns could be identified with the three different X-ray patterns obtained with each of the unit cell faces at right angles to the beam (the 'principal projections'). Having done this, they calculated Fourier transforms from the density distributions in the micrographs and used these to deduce phases (or signs) for most of the X-ray amplitudes. The remaining signs, which were needed to extract the fine detail information present in the X-ray patterns, were deduced by trying different combinations of signs until the three independent particles in the unit cell looked as similar as possible in the calculated electron distribution.

The picture that emerges is of a roughly disk-shaped particle of diameter 110 Å and thickness 57 Å, divided into two symmetrically arranged halves along its short axis and somewhat wedged-shaped, like a half-open oyster. The authors have, with Cantabrigian erudition, coined the name 'platysome' to describe it. The DNA cannot be positively located, but the electron distribution suggests that it forms a superhelix of pitch 28 Å round the outside of the protein core. Now enzyme digestion studies by a number of workers (for example Simpson & Whitlock *Cell* 9, 347; 1976) show that nucleosome DNA chains are most sensitive to attack at intervals of 10 bases along their length. This is consistent with a DNA double helix of 10 base pairs per turn wrapped round a protein core. There are, furthermore,

tracts of relatively low sensitivity which suggest that the protein is in closer contact with DNA at two points in each turn of the superhelix. From this Finch *et al.* deduce that the DNA occupies $1\frac{1}{2}$ turns of superhelix per particle, and that the digestion pattern is consistent with the core possessing a dyad or axis of symmetry.

One further feature of the model is of interest. It has been known for some time that when closed circular DNA interacts with histones to form synthetic nucleosomes, the DNA appears to twist up to form $1\frac{1}{2}$ turns of left-handed superhelix per nucleosome (for example Germond *et al. Proc. natn. Acad. Sci. U.S.A.* 72, 1843; 1975). Since a single turn of superhelix would be too large to account for the known size of a nucleosome, various ingenious schemes have been postulated involving folds or kinks in the DNA to make it more compact. Now, however, Finch *et al.* believe that the DNA is not kinked, but that the Watson-Crick helix winds up slightly on interaction with the histones. (It is not suggested, as eager iconoclasts might conclude from the paper's abstract, that there is any change in the sense of the helix). If the histones were removed the DNA would then unwind from 10 to between 10.4 and 10.7 base pairs per turn. The fact that purified DNA fibres show exactly 10 residues per turn in the B form could arise from a distortion due to crystal packing, and Levitt (one of the paper's authors) has done energy calculations that support this view.

What next? Clearly, there is hope that better crystals will give data at higher resolution. The best crystals obtained so far have, paradoxically, come from particles in which there was considerable protein degradation. This is clearly unsatisfactory, but the authors give reasons for believing that the gross structure is similar to that of intact particles. It does not follow that intact nucleosomes do not have identical structures; little is known about why

homogeneous preparations can still refuse to crystallise, as many workers on tRNA know to their cost. In any case the present work is a promising

start, and we can look forward to following a period of intensified competition between the interested laboratories. □

Can surfaces of metals spontaneously distort?

from John Pendry

THE simplest model of atomic arrangements at clean surfaces assumes that the surface is created by removing all atoms on the vacuum side of the surface plane whilst freezing the positions of those on the crystal side. Such a model belies the complexity of forces at surfaces: structure is determined by the electronic energy levels which are known to be severely distorted by the presence of a surface, and the wonder is that so simple a model should ever work rather than that there are exceptions to it!

Deviations from the model are particularly easy to detect when they involve structure of new symmetries, that is, puckering of the surface. In that instance the diffraction pattern obtained using a beam of low energy electrons changes and acquires extra

details clearly visible on the fluorescent screen used to detect the electrons. It was in this way that two groups (Felter, Barker and Estrup at Brown University (*Phys. Rev. Lett.* **38**, 1138; 1977) Debe and King at Liverpool University, (*J. Phys. C* **10**; 1977)) working independently, discovered a structural rearrangement on the (001) surface of tungsten. A similar rearrangement on the (001) surface of molybdenum was also observed by the Brown group.

The rearrangement occurred below 300 K and the transition appeared to be perfectly reversible. In a subsequent preprint Debe and King report a determination of the space group of the new structure, and are currently analysing intensities in their diffraction pattern in order to find the detailed atomic configuration.

Although observation of a structural rearrangement is relatively simple it is no trivial matter to be sure that it was not induced by impurities. In fact hydrogen is known to induce a similar structure on a tungsten (001) surface. The present structure was probably originally observed by Yonehara and Schmidt (*Surface Sci.* **25**, 238; 1971) but the question of hydrogen contamination could not be resolved at that time. The present work establishes conclusively that the structure is a clean surface effect. The Brown group also demonstrate that it is not due to a phase transition in the bulk of the material; chromium is known to have such a transition, but molybdenum and tungsten were both shown to be free of bulk transitions by X-ray analysis which is sensitive only to bulk effects.

It has been clearly shown that the distortion is due to an intrinsic property of the clean tungsten surface but it has yet to be established which particular property is responsible.

The stable structure of a system is always the one with lowest free energy (E) so that if we plot E as a function of, say, the amplitude (d) of a periodic displacement of the surface atoms it will appear as shown in Fig. 1. Two cases are shown. In Fig. 1a the free energy has two minima which vary as functions of temperature. At the transition temperature the second minimum overtakes the first and the stable structure jumps discontinuously to one with a finite-amplitude periodic displacement of the surface atoms. For the surface to achieve the newly stable configuration many surface atoms must jump across the potential barrier between the metastable and stable structures. It is a characteristic of such transitions that they are 'sluggish' tending to show supercooling and superheating effects.

No such hysteresis was observed in this experiment and it is more likely that the transition was of the so-called 'soft mode' type shown in Fig. 1b. The restoring force for the displacement in this type of transition goes continuously to zero as the temperature is lowered to the transition point. Hence there is a precursor to the transition in the form of a vibrational mode whose frequency goes to zero, that is to say goes 'soft', as the temperature is lowered. Below the transition point the amplitude of the periodic displacement becomes finite, increasing with decreasing temperature. The period of the distortion need not be commensurate with the original lattice spacing but in the instance of the tungsten (001) sur-

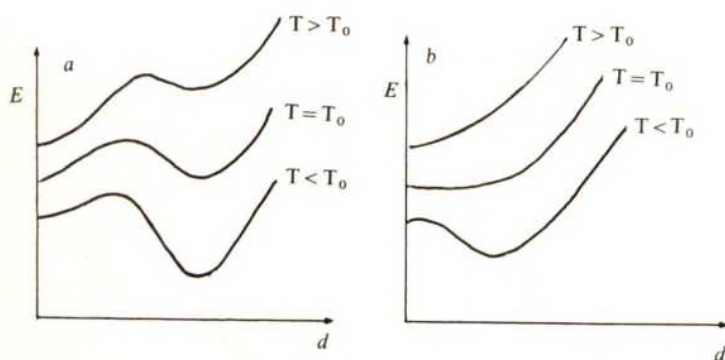


Fig. 1 Free energy plotted as a function of d the amplitude of a periodic displacement of surface atoms; a, for the case of two minima; b, for the case when a soft mode appears.

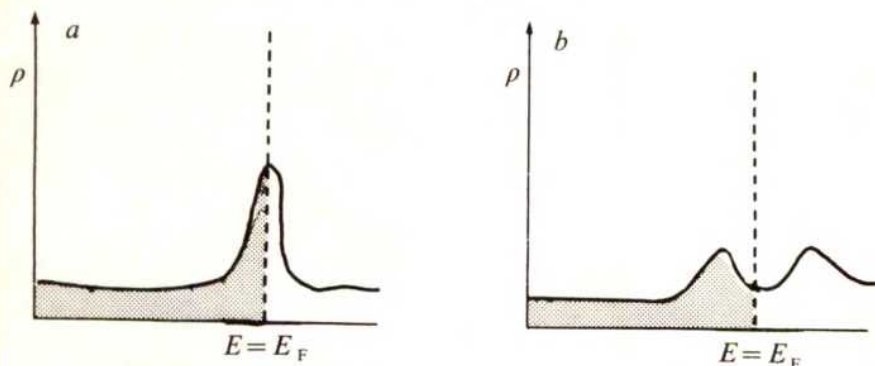


Fig. 2 a, Schematic density of electronic states with a large peak near the Fermi energy which favours a transition that splits the peak as shown in (b), thus lowering the average energy of electrons.

John Pendry is Head of the Theoretical Physics Group at the Daresbury Laboratory.

face it appears to be so, the new periodicity being double the old one.

A common mechanism for producing soft modes is the occurrence of a high density of electronic states near the Fermi level, giving rise to a so-called Peierls instability. The argument is that distortions of lower symmetry split the electronic levels, driving some above and some below the Fermi level. Figure 2 shows the general idea. If the density of states at the Fermi level is high enough the lowering of energy in the occupied states will cancel other positive contributions and make the distortion energetically favourable.

It seems likely that a theory of the clean surface reconstruction will also shed light on chemisorption of foreign atoms. The Brown group observed that adsorption of hydrogen produces diffraction patterns that though different from the reconstructed clean surface pattern, have certain features in common with it. They speculate that hydrogen induces the surface to reconstruct even when the temperature is above the clean surface transition point. If this is correct it is to be expected that the soft mode responsible for the reconstruction will affect the energies of chemisorption.

These experiments challenge our understanding of surface electronic structure and its role in creating soft modes. Densities of states at surfaces are not easy to determine with the precision demanded here, but on the other hand as much experimental information is available about the electronic structure of tungsten as almost any other material and some interesting developments can be expected. □

Disulphide bonds and protein folding

from M. J. Geisow

THE study of the mechanisms by which proteins fold seeks to unravel the stereochemical determinants of tertiary structure, with the commendable long-term goal of predicting three-dimensional structure from amino acid sequence. Advances in the 1970s, both in formulations of concepts and in theoretical and experimental treatments of protein folding have been comparable with the rapid development of protein crystallography in the 1960s. By analogy, the next decade should see the first predicted structures, a preview of which might be the successful approximation of the pancreatic tryp-

M. J. Geisow is a member of the scientific staff at the National Institute for Medical Research, London.

Globin hnRNA = pre-mRNA

from Bob Williamson

DEFINITIVE work proving the existence of a high molecular weight precursor of mRNA has at last appeared, confirming the suspicions of a decade. Readers of these columns will know that for years the argument has proceeded between those who thought each high molecular weight RNA molecule an aggregate, and those who thought each cleavage due to inadequate purification techniques and omnipresent and omnipotent ribonucleases. Recently several groups, and in particular P. Curtis and C. Weissmann in Zurich, have shown a 15S precursor double the size of globin mRNA in induced mouse Friend erythroleukaemia cells, but this does not satisfy the true exponent of precursors. We need hnRNA molecules 10 times larger than the final product, for a given mRNA, to fit our favoured theory.

Bastos and Aviv have now provided the data (*Cell* 11, 641; 1977). They have looked at nuclear RNA from both mouse Friend cells and mouse spleen, and have found a nuclear RNA containing globin sequences at least 27S in size (approximately 5,000 nucleotides long), or seven times the mRNA length. Aviv's group identified this RNA species using a column of globin complementary DNA (cDNA) bound to cellulose. Since the cDNA was present in large excess the kinetics of hybridisation were favourable even for the small amounts of globin-specific hnRNA present.

After a 5 min pulse of labelled uridine, over two-thirds of the counts are in an RNA molecule sedimenting at 27S. It is processed with a half life of 5 min, first to an RNA of size 15S, previously shown by others, and then to the final mRNA. (There may be a small pool of mRNA in the nucleus,

which takes time to equilibrate to the cytoplasm; there is room in Aviv's data for yet another control step at this point). The scheme seems very similar for Friend erythroleukaemia cells and normal spleen, which is gratifying, since there is always residual concern when a generalisation is based on as atypical a model as a cell in continuous culture transformed by an RNA tumour virus and induced into erythropoiesis by an organic chemical acting on membranes.

The other interesting result presented is that the large 27S precursor has no poly(A) tail; this only appears after cleavage to the 15S molecule. Therefore poly(A) addition is not only a post-transcriptional event; it also occurs as part of intermediate processing. It should be possible shortly to determine whether the poly(A) is added internally and what is the position of the mRNA sequence in the 27S precursor.

Another group has also studied mouse spleen cells in culture using DNA bound to cellulose, and using very rigorous denaturation conditions with treatment by formaldehyde, which breaks secondary structure irreversibly (Kwan, Wood & Lingrel *Proc. natn. Acad. Sci. U.S.A.* 74, 178; 1977). In this case the RNA, pulse labelled for 10 min and prepared by several different techniques, was isolated and run on gels, and showed the 15S precursor peak but no 27S RNA species. The reason why some groups fail to find this larger species while others do is unclear, but may be related to the very rapid processing of nuclear RNA, even while it is synthesised, in some culture conditions.

Bob Williamson is Professor in the Department of Biochemistry at St Mary's Hospital Medical School, London.

sin inhibitor structure by Levitt and Warshel (*Nature* 253, 594; 1975).

Many reports of the change of protein structure consequent upon denaturation and refolding have been based on kinetic measurements using optical, chiroptical or magnetic resonance properties of the amino acids to monitor the microenvironment. Typically, these experiments indicate biphasic or multiphasic pathways, which point to the existence of significant intermediate states. Thermodynamic observations are often more simply explained by an equilibrium of native and unfolded forms, suggesting

cooperativity in protein folding. Such a hypothesis is almost certainly an oversimplification for whole proteins, but might well describe the formation of component elements of secondary structure such as β -pleated sheet. The pattern of formation of hydrogen bonds in this structure would seem to provide an excellent physical model for a highly cooperative folding process.

The classical method of studying mechanism in chemistry is to trap intermediate states and infer the pathway from the structures of these intermediates. In disulphide proteins nature has provided just such potential inter-

mediates, since the order of formation of disulphide bridges must reflect the folding pathway. 'Freezing' the reaction by acid or alkylating reagents then provides covalent intermediates for study at leisure.

A prerequisite for the extrapolation of results from the particular disulphide proteins studied to general notions of folding, must be the demonstration that disulphide formation does not provide of itself the dominant driving force for protein folding. The consensus is that the peptide chain promotes rapid and specific disulphide formation and not *vice versa*. For example, in denaturants such as guanidinium chloride, correct disulphide bridging does not occur, even in the presence of thiols to allow disulphide exchange to correct 'wrong' bridges.

Having said this, it is also certain that in the absence of denaturants, the initial disulphide bridges formed are not necessarily native. Workers usually refold proteins in the presence of redox systems such as dissolved O_2 , oxidised and reduced glutathione or even disulphide exchange proteins (reshuffleases) to prevent the accumulation of non-native forms. In all of these oxidation methods it is clear that thiol-disulphide exchange is the principal process which leads ultimately to the native forms. Since a third factor (an oxidised thiol) must participate in the formation of each disulphide bond a question of accessibility arises, particularly for disulphide bridges which are deeply buried in the final structure. The degree to which the oxidative mechanisms influence the folding pathways is not known, but many workers have nevertheless found it relevant to ask what light disulphide bridges can shed upon the folding process.

The questions then are: which are the initial disulphides, what regions of tertiary structure do they connect and are there early native disulphides or do they consist of predominantly non-native forms? The principal answers to these questions have been sought in studies of ribonuclease A (RNase A), bovine pancreatic trypsin inhibitor (BPTI) and lysozyme. These are arguably an unrepresentative group because they are all very small and stable proteins, but they are sufficiently well characterised for precise study.

Two general mechanisms have emerged. The first is a random search mechanism in which a rapid phase occurs where there is formation of many randomly-paired disulphides and mixed disulphides, followed by a reshuffle to the native form. This folding pattern was deduced for RNase A (Hantgan *et al.* *Biochemistry* **13**, 3421; 1974).

The second is a limited search mech-

anism where there is rapid formation of a few specific disulphides, followed by slower reoxidation and exchange of the remainder to produce the native structure. Anderson and Wetlaufer (*J. biol. Chem.* **251**, 3147; 1976) found that the first two native disulphides of reduced lysozyme formed eight times more rapidly than the second two, implying an ordered progression to the final structure. Creighton (*J. molec. Biol.* **95**, 167; 1975) observed that the refolding of BPTI could only take place through the formation of two of the four major disulphide intermediates present initially, suggesting an obligatory folding pathway.

The latter mechanism is certainly more consistent with the idea of nucleation regions which, formed early during folding, predispose to particular disulphide bonds. Creighton notes that the predominant cysteine residues involved in early disulphides in BPTI are at the centre of an α -helix and an antiparallel β -pleated sheet respectively; secondary structure is the favoured site of nucleation in folding proteins. Even if each of these mechanisms is correct for the respective protein, the experiments rule out the 'all or none' or co-operative picture of protein folding, since disulphide formation is far more rapid than the return of biological activity.

More recently an ingenious thermodynamic approach has overcome the technical problems of 'trapping' the rapidly reoxidising disulphide bridges. Acharya and Taniuchi (*Proc. natn. Acad. Sci. U.S.A.* **74**, 2362; 1977) have allowed randomly alkylated lysozyme containing one blocked cysteine residue per mol of protein to refold over an extended period. Enzymically active material isolated after refolding contained eight isomers representing each of the four possible ways of forming three native and one open disulphide bridges. There were similar amounts of these four forms. Such an observation clearly rules out an obligatory pathway for the formation of correct disulphide bonds, since the folding routes must have been independent of each disulphide in turn, although ultimately producing near-native molecules. Acharya's and Taniuchi's result does not fix the sequence of events leading to the correct structure and therefore cannot rigorously distinguish between a random or nucleation folding mechanism. However, the inability of a particular disulphide to form did not appear to influence the final fold. This lends some support to the notion that the early folding events reflect what the peptide chain is trying to do, rather than simply the need of the cysteines for oxidation. The truth, of course, is probably a bit of both.

The recent topological analysis of protein structure by Levitt and Chothia (*Nature* **261**, 552; 1976) points to the presence of a limited set of folding units in globular proteins, composed of adjacent elements of α -helix and β -structure. The elements of secondary structure concerned are also close in linear sequence, suggesting that they could quickly form appropriate interactions to produce units of tertiary structure. Such folding units apparently occur with a statistically very significant frequency in the 31 proteins examined. The authors argue that the folding of proteins is kinetically determined by the high probability of formation of these folding units, which subsequently interact with each other to produce the native structures. This attractive hypothesis might be testable in disulphide proteins. The identification of cysteine residues which would be brought into proximity within a Levitt-Chothia folding unit should coincide with the experimentally observed early disulphides, even though these might not be the final native ones. □

Weather on Neptune

from Garry E. Hunt

STUDIES of the weather systems of planetary atmospheres provide a greater insight into the meteorological processes involved in a wider range of conditions than can be achieved through investigating the Earth alone. In addition to the direct spacecraft observations, Earth-based measurements of the changing cloud patterns of Venus, Mars, Jupiter and Saturn have provided a wealth of data on their atmospheric phenomena. The same approach cannot be applied to atmospheric studies of Uranus and Neptune since these planets are too distant for direct telescope observations of any cloud systems to be possible. In these conditions it is necessary to adopt a subjective approach such as monitoring the planetary brightness spectrum or changes in the absorption spectra formed in the cloudy planetary atmosphere. Certainly patrols of the hydrogen features in the Jovian atmosphere have been shown by Hunt and Bergstralh (*Icarus* **30**, 511; 1977) to be good identifiers of planetary motions and variabilities in the cloud layers. A patrol of the Neptune reflectance by Joyce *et al.* (*Astrophys. J.* **214**, 657; 1977) indicates a substantial but unexpected increase in the planet's reflectance between 1 and 4 μ m, between April 1975 and March

Garry Hunt is in the Department of Physics at University College, London.

1976, with an increase of a factor of 4 at $3.5\ \mu\text{m}$ (L band). Pilcher (*Astrophys. J.* **214**, 663; 1977) suggests that these measurements are the first direct evidence of weather on Neptune caused by rapid variations in the cloud structure. Paradoxically, the simultaneous observations of the nearer planet Uranus show no variation during this observational period. Could Neptune have weather and Uranus not?

The radiative time constant is the time taken for a mass of air to warm up or cool by radiating in the infrared portion of the spectrum. Stone (*Space Sci. Rev.* **14**, 444; 1973) suggests that for Uranus this parameter is about 600 Earth years which is long compared with both its orbital and rotational periods of 84 yr and 22 ± 2 h. Similarly for Neptune the relaxation time is about 2,200 yr which is again enormously long compared with the orbital periods of 164 yr and 21 ± 2 h respectively. Consequently, this would suggest minimal diurnal and seasonal changes in the atmospheric structure of the visible cloud layers. Furthermore, we would expect the planets to behave in a similar manner, which, at first sight, is not borne out by the measurements of Joyce *et al.* (*op cit.*). Is it possible for Neptune to possess a more active weather system or more variable cloud layers?

The increase in the Neptune reflectance observed by Joyce *et al.* must be due to a corresponding increase in the planetary albedo. Correspondingly, it would also require a reduction in the atmospheric absorption, which is substantiated by the smaller amounts of methane and hydrogen they derive, compared with the previous studies by Owen (*Icarus* **6**, 108; 1967) and Encenaz *et al.* (in *Exploration of the Planetary System* ed. Woszczyk, A. & Iwaniszewska, C., Reidel, 1974) respectively. Does this imply a variable cloud structure affecting the level where the absorption lines are formed?

There seems little doubt that the Neptune atmosphere (and Uranus too) will possess layers of cloud. Prinn and Lewis (*Astrophys. J.* **179**, 333; 1973) predict a layer of methane haze in the upper troposphere of both these planetary atmospheres, which may be more substantial in thickness in the colder Neptune environment. At the lower levels, layers of NH_3 , NH_4SH and aqueous NH_3 could possibly form but they are unlikely to affect the spectroscopic or photometric observations. What could cause the methane cloud opacity to vary, since this is the most likely candidate to explain the paradoxical observations of Joyce *et al.*?

Morrison and Cruikshank (*Astrophys. J.* **179**, 329; 1973) found that at $20\ \mu\text{m}$ Neptune has a higher brightness

temperature ($\sim 58\ \text{K}$) than Uranus ($\sim 53\ \text{K}$). Does this mean that Neptune (and not Uranus) may have an internal heat source? It would then be possible for this energy source to produce convective activity in the Neptune atmosphere which would be important in the planet's meteorology. The solar energy at the distance of Neptune is extremely weak, and would therefore have a limited effect in suppressing the atmospheric convection, as is apparently achieved in the region of Jupiter bounded by $+45^\circ$ latitude (Ingersoll *Space Sci. Rev.* **18**, 603; 1976; Hunt *Adv. Physics* **25**, 455; 1976). It would then be possible for this redistribution of atmospheric heating to modify the upper troposphere thereby affecting the condensation processes. The resulting variability in the cloud structure would then create the changes in reflection and absorption properties observed. However, these measurements are for the whole planetary disk, so that it is possible that global scale features are being observed. Perhaps this then suggests that Neptune may not possess belts and zones which are so familiar on Jupiter and Saturn.

But why should Uranus not show similar variations during this time period? The $20\ \mu\text{m}$ observations of Morrison and Cruikshank (*op cit.*) could be interpreted as suggesting that Uranus may not have an internal heat source. This would then imply less convective activity in the Uranus atmosphere, so that perhaps one would not expect to observe variability in the cloud layers. But perhaps Uranus does have an internal heat source. Then it is quite likely for the atmosphere to exhibit some variability, but the time scale may be affected by peculiar inclination of the rotational axis. The level of activity may depend upon the portion of the disk that is observed, so that it is essential for a patrol of the Uranus atmospheric features to be carried out during the next few years.

The brightness of the outer planets, Uranus and Neptune have been patrolled by Lockwood (*Science* **190**, 560; 1975) to study possible variations in the solar constant, which would obviously affect every planetary atmosphere. If these planets, Uranus and Neptune, possess variable cloud layers, which could modify the planetary brightness, then it may be necessary to reconsider this type of study. However, there is little doubt that future studies of the meteorology of Neptune provide a further environment to study atmospheric processes very different from those of the Earth's atmosphere, so that there is a great deal to be done with our telescopes and computers before the planet is visited by Voyager in 1989. □

Structural gene for myosin

from Carol K. Klukas

In a recent issue of the *Journal of Molecular Biology* (**114**, 133; 1977) A. R. MacLeod, R. H. Waterston, R. M. Fishpool and S. Brenner report their identification of a structural gene for a myosin heavy chain in *Caenorhabditis elegans*, a small nematode worm. Their report represents another step in the long term project being undertaken in Sydney Brenner's laboratory to dissect the genetic specification of this creature, a representative and yet manageable eukaryotic organism.

The basic approach is that used to dissect the much simpler genetics of bacteria. In bacteria genetic analysis of mutants provides an outline of the genetic organisation: how many genes are involved in any particular process and how they are arranged on the chromosome for example. Biochemical measurements (such as assays for activity of specific enzymes in various mutants) then can provide information on precisely how the genes work together to specify a particular biosynthetic process within the bacterial cell.

But the problem of deciphering and dissecting the genetic system which dictates the complexities of a multicellular organism, in which different cells each with their particular biochemical processes, must be positioned in a correct spatial relationship and must function in a correct relationship temporally, seem daunting. However, the 1-mm long *C. elegans*, which consists of a total of only some 600 cells and has a life cycle of only $3\frac{1}{2}$ days is an organism uniquely suited to such an attempt.

The DNA content of this worm's genome is only 20 times that of *Escherichia coli*, and of this DNA only 6.7×10^7 base pairs code for unique sequences, enough to code for about 6.7×10^4 average-sized genes. This number is large enough, but is considerably more manageable than that of most other eukaryotes. Furthermore, because *C. elegans* is hermaphrodite, recessive mutants are easy to isolate and since the defect is reliably transmitted to the offspring a ready supply of recessive mutants for study is assured. Although *C. elegans* does not have many external features in which abnormalities can be detected, useful behavioural mutants can be quite easily detected visually whose

Carol Klukas is in the Department of Biophysics at King's College, London.

abnormalities often directly indicate mutations in the nervous system or musculature.

With these advantages in mind, Brenner's group has set about the slow task of dissecting the genetic specification of *C. elegans*, especially that of the nervous system. The work involves the characterisation of mutants such as E675 which maps in the *unc-54* gene and was used in the recent *J. molec. Biol.* paper to identify the *unc-54* gene as the structural gene for a myosin heavy chain present in a major fraction of the total nematode myosin. All worms with mutations in the *unc-54* gene, including deletion mutants, have disorganised muscles due to a drastic decrease in the number of thick filaments in the body wall musculature, but these mutants survive because the pharyngeal muscle continues to function normally. In E675 the myosin has a molecular weight of 2×10^5 rather than the normal 2.1×10^5 component. By means of cyanilation and cyanogen bromide cleavage the authors show that the mutant heavy chain is an altered form of the wild-type myosin

and thus conclude that *unc-54* is the structural gene for this heavy chain.

There are always some thick filaments visible in the body wall cells of *unc-54* mutants, even in the deletion mutants. This indicates the presence of multiple myosins in *C. elegans* as has been noted in higher organisms. In normal worms do other myosins associate with *unc-54* heavy chain to form hybrid myosin molecules or do thick filaments containing mixed myosin populations occur? The suggestion from observation of mutants is that mutant heavy chains can interfere with the production of normal myosin in some way and it will be part of the *C. elegans* puzzle to work out this interplay between myosins and the genes which direct their synthesis.

The whole puzzle is very complex indeed, but each piece which is fitted in by Sydney Brenner's group brings closer a complete picture of just how the information specifying a higher organism is stored in its genes and unravelled in the correct sequence to dictate the development of an organism such as *C. elegans*. □

plate carrying the South Asian sub-continent moved northward and collided with the front edge of the Eurasian plate. It obliquely compressed and in stages ploughed into the ancient Asiatic continent, and in fact is still moving at a velocity of $\sim 0.5 \text{ cm yr}^{-1}$. Palaeomagnetic measurements suggest that the suture of the two plates is located roughly along the Yalu-Tsauo valley.

Gravity and earthquake data suggest that the thickness of the Earth's crust at Mt Jolmolungma (Mt Everest) is $\sim 48 \text{ km}$, much less than that in the vicinity of Lhasa which is $\sim 70 \text{ km}$. Geophysicists found, on the other hand, intense hydrothermal activity along a belt south of the Gangdise Range and north of the Himalayas. The initial interpretation is that this widespread geothermicity is the result of lava activities.

Data were collected along built and planned highway routes, on the occurrence and development of mud-and-stone flows. Glaciation was studied over large areas. Chinese glaciologists are now working out laws of motion pertaining to a rare oceanic (warm) type of glacier, the distinguishing features between this type and the continental (cold) type, and the changing distributions of both on the Plateau. Wave-like glacial movements were also observed for the first time. After long years of slow movement, some glaciers show a sudden burst of speed in their advance. The momentum change is frequently so large that the middle moraines on the surface of the ice are twisted and the ice surface shows cracks like a spider's web.

It is reported that the large quantity of good data are subjecting to severe tests many views currently adopted in the West. For instance, one hypothesis maintains that the Plateau was totally glaciated during the Quaternary Period and that few plants and animals escaped extinction to survive today. The survey, however, indicates that the Tibetan flora and fauna consists of many species, some of which are left over from the Tertiary Period. Tibet has more than 4,000 species of higher plant. Geological data show that during the glacial period in the Quaternary, the glaciers were indeed much larger than at present. At that time the Himalayas could not yet completely block winds coming from the Indian Ocean, and precipitation in most parts of Tibet was greater. However, data indicate that the Tibet Plateau has never been completely covered by glaciers. Another common belief in the West is that northern Tibet is in a drought belt. The survey establishes that the vegetative cover there is basically the grassland type

Surveying the roof of the world

from T. B. Tang

MANY large-scale geographical and scientific surveys have been conducted in China since 1949, particularly in the past five years. In 1973–75, for example, the State Department of Agriculture and Forestry undertook a land resources survey of the Heilungkiang (Amur) region, in which scientists cooperated with the ordinary citizen. The Department of Geology carried out in 1974–77 an airborne geophysical survey of the southern Yellow Sea, East China Sea, and South China Sea. The largest and most comprehensive survey to date must be, however, the four-year expedition to the Chinghai-Tibet Plateau, organised by the Academia Sinica and started in 1973. The Plateau, sometimes referred to as the 'third Polar Region' or the 'roof of the world', is of immense scientific and other interest. In 1951 and 1960, local and specialised surveys were carried out in that vast area, and their results were used in the planning of the present expedition. More than 400 scientists, from 45 institutes and specialising in some 50 disciplines, took part at one time or another. The field work now completed, the programme is entering the stage of laboratory analysis, data processing, synthesis of

results, and theoretical interpretation. In June the Academia Sinica arranged a conference at which selected fossils, photographs and films were shown, and papers describing preliminary results were discussed (*Hsinhua News Agency* and *Ta-kung-pao*).

It has long been known that the Plateau used to be under sea-level, but little was ascertained as to exactly when and how the uplift occurred. That during the early and middle Jurassic period Tibet was a shallow sea or depression was confirmed by the present survey from palaeontological evidence. More than 6,000 fossils have been collected, dating from the Proterozoic and Cambrian periods onwards, and including those of a Hipparion (exhibited at the conference) and a sauropod dinosaur. It is the first time that dinosaur fossils have been found at places now at such a high altitude. (A preliminary regional stratigraphical map has been published.)

In the Pliocene Tibet became low-lying dry land with subtropical conditions. Then, in the ensuing period of several million years, a tremendous uplift took place. The geophysical model most discussed at the conference proposes that the upheaval was generated mainly by the spreading of the Indian Ocean floor, so that the

which is, or can be made to be, good grazing.

Water resources have also been investigated. The survey determines that Tibet has 1,000 lakes, about half of the total in China. Most are tectonic lakes, the rest mainly formed by glaciation. They contain abundant deposits of salt, soda, sodium sulphate, and borax. The lakes have significant effects on the local climates and the areas around them possess great potential for agriculture and animal husbandry. The fish in them are chiefly Schizothoracinae of the Cyprinoid family and barry loaches of the Cobitidae. They are different from those on the plains, preserving many of their primitive features. It is thought that they have evolved from those existing in the late Tertiary Period, and have undergone some adaptive changes during the uplift of the Plateau.

The Chinese collected over 50,000 specimens of higher plants, and over 70,000 of insects, birds, animals, fish and other aquatic life. Preliminary identification work has revealed more than 100 new species of plants, some presumably having developed from known species (during the upheaval)

and some being entirely unique to the Plateau. Identification of the detailed natural-geographic zones is now at an advanced stage. Incidentally, as a result partly of the survey but mainly of research started in 1971 by the Chinghai Institute of Biology, a Tibetan medical pharmacopeia was published in July. It is in three volumes, the first two dealing with herbal drugs and the third with animal products having therapeutic applications. 455 drugs are listed, an outstanding example among which is saussura, a herb with a white pappus, of great medicinal value, but scarce in other parts of China.

Besides providing a wealth of unique scientific data, the survey has located many important mineral, water, geothermal energy, and other resources, and showed that the Plateau possesses agricultural and cattle-raising potential. No doubt the development of Tibet will continue at an ever increasing pace. An oil pipeline is already in existence from Golmo in Chinghai to Lhasa, and work has started on a 2,100-km railway line to link up with the national network.

pointed out by R. C. Staples (Boyce Thompson Institute) in his introductory talk, this approach has led to the poorest farmers often benefiting least from the 'Green Revolution'. Following up this point, F. N. Ponnamperna (IRRI, Philippines) emphasised the tremendous potential for increasing food production without requiring large increases in inputs which is offered by the breeding of new varieties tolerant of stress. He gave examples of the recent advances achieved by the International Rice Research Institute in breeding lines tolerant of soil conditions such as salinity, alkalinity and zinc deficiency.

The conference was significant in that it was probably the first major attempt to review progress in the application of fundamental plant physiological research to the breeding of crop varieties adapted to a range of stressful environments. Although a wide range of stresses including disease, aluminium toxicity and air pollution was covered in the meeting, the majority of papers were concerned with temperature or drought.

Among several interesting contributions was one by M. J. Jaffe (Ohio University, Athens, Ohio) on a commonly neglected stress. He reported a series of studies on the effect of mechanical stimulation, such as might be experienced by plants brushing against one another in the wind, on plant growth and development. Even slight rubbing of expanding internodes was shown to reduce their extension dramatically. G. F. Somers (University of Delaware, Newark) described some speculative work where irrigation with sea water is being used in a programme designed to select new salt-tolerant crops from among wild species native to salt marshes. If successful, this approach alone could greatly increase the world's arable area, since there are large areas with saline soils or where saline irrigation water only is available.

The general problems of improving crop tolerance of stress were illustrated by the contributions in the field of drought resistance. These ranged from reports of fundamental studies at the molecular level to reports by plant breeders actually concerned with breeding new varieties. The present state of the art in breeding for drought resistance in wheat was outlined by F. T. Townley-Smith and E. A. Hurd (Agriculture Canada, Saskatchewan), who described the techniques adopted in their own programme. Although they make some selections on the basis of 'physiological characters' such as the rate of wilting of cut leaves or the length of roots in seedlings, their programme relies heavily on yield measurement as the main selection criterion. Physiologically based screen-

Stress physiology of crop plants

from Hamlyn G. Jones

An international conference on Stress Physiology of Crop Plants sponsored by the Boyce Thompson Institute for Plant Research and the Rockefeller Foundation was held at the Boyce Thompson Institute, Yonkers, New York, on 28-30 June, 1977. The conference was organised by H. Mussell and R. C. Staples.

It is the tropical countries which have the greatest potential for providing the extra food which will be necessary to feed the growing world population, since they have extensive areas of potentially cultivable land where crops either are not grown at all or only yield poorly because of stresses such as drought, non-ideal temperatures or adverse soil conditions. This was the message in the first paper of the conference, given by M. N. Christiansen (USDA, Beltsville).

In the past, much of the effort towards improving crop yields in these situations has involved the breeding of high yielding varieties suitable only for near optimal conditions, with the consequent need to ameliorate the stresses by installation of expensive irrigation systems or by using high inputs of fertiliser or pesticide. As was

Hamlyn G. Jones is a Lecturer in Botany at the University of Glasgow.



A hundred years ago

With reference to Galileo's claim to be the inventor of the telescope, M. Wolf quotes (*Annalen der Physik und Chemie*) from a manuscript of Scheiner (1616) in a library in Zurich, a curious passage, of which the following is part: "It must be allowed first, considering what the telescope does, that Baptista Porta has better right to be thought the inventor, because he describes, after his own way, in obscure words and puzzling expressions, an instrument like the telescope. But secondly, if we speak of the telescope, as it is now used after general perfection, we must say that neither Porta nor Galileo is the first discoverer of it, but the telescope in this sense was discovered in Germany, among the Belgians, and that accidentally by one Krämer, who sold spectacles, and either for amusement, or experimentation, combined concave and convex glasses, so that with both glasses he could see a quite small and distant object large and near; at which success being rejoiced, he united several similar pairs of glasses in a tube, and offered the combination at a high price to wealthy people. Thereafter they (the telescopes) became gradually more common among the people, and spread to other countries. In this way two of them were brought for the first time by a Belgian merchant to Italy.

from *Nature* 16, 30 August, 390; 1877.

ing is not emphasised in their programme, partly because the tests tend to be too complex to use on large numbers of lines and partly because most such tests tend to be poorly related to field performance. This paper highlighted the somewhat depressing fact that, to date, the physiological approach has not made much contribution to the development of new stress-tolerant varieties. In spite of this there was general agreement that really large and rapid improvements in stress tolerance will only come about by cooperation between breeders and physiologists.

- Part of the difficulty is that in the past many laboratory physiologists have been reluctant to face the complexities of the field situation, which is, after all, where their ideas must ultimately be tested. An encouraging feature of the conference was the surprisingly large number of contributions from workers who have been facing this challenge and have begun to study stress physiology in the field and to apply this work to plant breeding. N. C. Turner (CSIRO, Canberra), for instance, reviewed recent work on the mechanisms whereby plants adapt to drought stress in the field. The important message was that the responses observed in the field were frequently quite different from those found in controlled environments so that much laboratory work may be misleading.

It was apparent from the various papers and discussions during the meeting that we are further advanced in our understanding of and ability to breed for tolerance of some stresses such as cold, specific ion toxicities or more particularly disease, than we are for drought. This may be largely a function of the complexity and variability of droughts and the range of possible tolerance mechanisms. Surprisingly, perhaps, those such as A. Blum (Volcani Institute, Bet Dagan, Israel) and C. Y. Sullivan (USDA, Lincoln, Nebraska) who work in the most arid regions, tended to be the most optimistic and appeared to have made most progress in developing physiologically-based screening tests for drought resistance. Sullivan, for instance, reported good correlations between field performance of sorghum and several physiological tests including the ability of disks of leaf tissue to withstand osmotic stress or short periods at high temperatures.

The basic optimism and philosophy both of the conference organisers and of the vast majority of participants is probably best summed up by this extract from the 1976 annual report of the Boyce Thompson Institute: 'We believe that there is no more important task for research to undertake than

providing the technology to feed (and clothe) the continually expanding world population, . . . and that understanding plants and pests at the basic physiological and biochemical level is essential for the future breakthroughs necessary. . . .'

Earthquake prediction

from R. P. Adams

Discussions on earthquake prediction remain one of the drawcards at seismological meetings. It was appropriate that on 16 August a full-day symposium was devoted to this topic at the General Assembly of the International Association of Seismology and Physics of the Earth's Interior, being held at Durham.

INTERNATIONAL interest was evidenced by the fact that authors from seven different countries contributed the fifteen formal papers. It was fitting that three papers were given by scientists from the People's Republic of China, as this was their first chance to appear at meetings sponsored by the International Union of Geodesy and Geophysics since China's admission to that body 10 days earlier.

In coming from the only country yet to have successfully predicted major earthquakes, the Chinese have a clear lead in this field. Their first paper, presented by Chang Yu-ming on behalf of a group of 10 authors, discussed in detail the general seismotectonics of China, while two later papers dealt with detailed descriptions of precursory phenomena associated with known earthquakes. Tung Sung-sheng spoke of variations in seismic velocity in Yunnan Province that enabled a general warning to be issued a year before two earthquakes of magnitude 6.7 and 6.4. Chen Yun-tai reported observed changes in gravity measurements of up to 350 μ gal before and after the successfully predicted Haicheng earthquake of 1975, and smaller gravimetric changes associated with the catastrophic Tangshan earthquake of 1976, that was not predicted. It was suggested that these surprisingly large differences could have arisen from the movement of ground water. In the general discussion it emerged that although the immediate warnings before earthquakes were usually dependent on the occurrence of foreshocks, there has been at least one

case in Yunnan Province where this was given by other methods, such as variations in measurements of radon concentration in ground water, and resistivity.

Little was said about short-term precursors, but a new concept appears to be emerging for identifying 'precursory swarms' as a long-term indicator. This idea was most fully developed by F. F. Evison (Wellington) who elaborated an idea first published in *Nature* in April 1977. Evison's hypothesis is that a swarm of activity near the area of a forthcoming earthquake is followed by a 'precursory gap' which lasts until the occurrence of the 'major event'. As well as the position of the impending earthquake, its time of occurrence and magnitude are also indicated by the pattern of seismicity. It is still difficult to identify and interpret such swarms, and different criteria may be needed in different areas.

V. I. Keilis-Borok (Moscow) also spoke about the recognition of seismicity patterns before impending earthquakes. He postulated criteria for determining periods of 'activation' and 'quietness', rather analogous to Evison's concept, and also referred to precursory earthquake swarms. In reporting a detailed study of seismicity in the Aleutian Arc, Engdall and Kisslinger (Boulder, Colorado) also commented that they had observed similar patterns, and precursory variations in seismicity were observed by Talwani (South Carolina) in small earthquakes associated with a reservoir.

Other papers covered as diverse topics as the tilting of the Hawaiian Islands in relation to large circum-Pacific earthquakes, (Berg and Sutton, Honolulu), the prediction of intermediate-depth Romanian earthquakes by assigning time-series to historical events (Purcaru, Frankfurt), and the theory of deciding what constitutes an 'anomalous' reading (Jackson and Ergas, Los Angeles). It was also pleasing to note speakers presenting what seem to be negative results. Susan Raikes (Pasadena) reported a very thorough study of P-wave residuals in Southern California, revealing no anomalies. Dolbilkina and others reported that Soviet workers also often failed to detect precursory velocity anomalies, but a small change was found by a refraction survey near a large earthquake in Kamchatka.

Earthquake prediction still awaits a major breakthrough. It now seems however, that the best prospects remain the study of seismicity patterns, with the new concept of precursory swarms for the establishing of long-term predictions, and a fuller understanding of methods used by the Chinese for the issuing of final and short-term predictions. □

Robin Adams is Superintendent of the New Zealand Seismological Observatory.

review article

Decision making in animals

D. J. McFarland*

Animals must make decisions about when to feed, when to court, when to sleep, and so on, in such a way as to maximise as far as possible their chances of survival and reproductive success. It is possible to formulate in mathematical terms the optimal strategy for an animal to pursue. The theoretical optimum behaviour can be compared with the actual behaviour of the animal, and perhaps shed some light on the evolution of behaviour.

At any particular time there are many activities in which an animal could engage: feeding, courtship, sleep, and so on. It is usually impossible for an animal to carry out such different activities simultaneously, because the required movements are mutually incompatible. There must be some process or mechanism that determines which activity is to have priority at any particular time, and such a process is conveniently termed a decision process.

The decisions made by animals may be decisions on whether to continue with current activity, or to change to some other form of behaviour. They may also be decisions between alternatives that present themselves anew. In each case, the animal has to assess a wide variety of factors. Consider, for example, the situation of a herring gull (*Larus argentatus*) incubating its eggs. Normally it will sit tight until relieved by its mate. Both parents incubate the eggs, and a nest left unattended is soon subject to predation by neighbours. It is important, therefore, that the sitting bird should not leave the nest before being relieved by its partner. This rarely happens under normal circumstances, unless the bird is flushed from the nest by a predator, such as a fox or a human. Studies of herring gulls, at the South Walney Nature Reserve in Cumbria, have shown that the non-sitting member of a pair is often foraging for food, or resting on the beach. It normally returns to the territory within a few hours of leaving. Sometimes, however, the return may be delayed as a result of some mishap, injury, or capture by a scientist. The sitting bird, with increasing hunger, should eventually decide to desert the nest, because herring gulls breed in many successive seasons and it may not be in the genetic interests of the individual to endanger its life for a single clutch of eggs. The timing of such decisions is a question that has interested students of animal behaviour in recent years.

The basic dilemma for the incubating gulls lies between the possibility that its partner may return at any time, and the increasing necessity to obtain food. The situation is complicated by the fact that the gull must assess the returns on time invested in foraging, which vary considerably with the weather, the state of the tide, and the time of day. Moreover, individual gulls tend to specialise in particular types of foraging, so that the sitting gull should take account of its own foraging skills in assessing the likely returns from time spent foraging in different places.

Two questions posed by this example are of particular interest to ethologists: (1) how can we account for the decision processes involved? (2) how good are animals at making decisions of this complexity? The basic approaches to these questions stem from

decision theory, on the one hand, and the theory of natural selection, on the other. In recent years there has been some convergence between these two lines of thinking.

Decision theory

The study of decision making provides a meeting point for many disciplines: statistics, economics, philosophy, psychology and control engineering. In classical decision theory there are two important classes of variable. One, the subject's evaluation of the relative attractiveness of alternative choices, is called the utility. The other, called the (subjective) probability, concerns the subject's evaluation of the consequences of making each choice. Thus a man may wish to make a decision between going to a restaurant or to a cinema, in terms of the utility of each. He may also take account of the probability of the restaurant being expensive, or the film being poor.

Reports of attempts to investigate these elements of decision making make up a voluminous literature, but only recently has this work become directly relevant to animal behaviour.

The empirical approach to the relationship between decision theory and animal behaviour was initiated by Logan¹, who studied rats living in a box in which they had to work for any water they received by pressing a bar. The animals had to work on some specifiable terms for the water rewards. The terms were manipulated by varying the force required to depress the bar, the number of times the bar had to be pressed to obtain a reward, and the size of the reward. Logan found that the rats were sensitive to the details of the terms, in that daily water intake was reduced when any of the three aspects of the terms was increased. In later experiments, Logan² gave rats a choice between a large food reward following a small delay, and a small reward following a large delay. He was able to establish equivalent combinations that were described by 'indifference' functions, and by equations describing the relative incentive value of different amounts and delays of reward. He also gave rats a choice between constant food reward and a varied amount, or varied delay, of reward; thus introducing the concept of 'subjective probability' for the rat. In general, Logan's results were similar to those that would be expected by classical decision theory. The evidence subsequently obtained by psychologists^{3,4} and ethologists⁵, although often confounded by measurement problems⁶, tend to support the view that animals are capable of consistently evaluating alternatives and of maximising their combined value. Consideration from first principles⁷ gives rise to similar conclusions, but draws attention to some formidable problems. A simple everyday example may serve to illustrate these.

There is widespread agreement that an understanding of decision making can be gained from (1) the formulation of a

*Department of Zoology, University of Oxford, South Parks Road, Oxford, UK.

maximising principle, (2) the recognition that there will inevitably be some trade-off among various aspects of the problem, which will necessitate (3) the formulation of an optimality criterion. As an example, let us consider a university committee set up to review applications for a lectureship. Their maximising principle is to choose the best candidate, but the requirements of the post are such that there will inevitably be a trade-off between the teaching experience T and the research ability R of any given applicant. Suppose that the committee arrives at an agreed short list of two applicants A and B, and that they are able to score these for teaching and research, so that for applicant A, $T = 9$ and $R = 1$; while for applicant B, $T = 2$ and $R = 7$. Any assessment of an applicant's overall strength of candidature must rest on the optimality criterion used to evaluate teaching experience in relation to research ability. For example, if the criterion implies that strength of candidature $C = T + R$, then applicant A would score $C = 9 + 1 = 10$, while for applicant B, $C = 2 + 7 = 9$. On the other hand, if we let $C = T \times R$, then A scores $C = 9 \times 1 = 9$, while B scores $C = 2 \times 7 = 14$. Clearly, by altering the optimality criterion, without changing the scores, it is possible to come to a different conclusion as to which is the best applicant.

We now have to ask what factors influence the optimality criteria. In the university context, factors such as government attitude, financial pressures, and so forth, are likely to determine policy concerning the balance between teaching and research. As far as the selection committee is concerned these are ecological factors. An overriding influence will be the

climate of ideas concerning the maximising principle. While in some universities the best candidate is seen in academic terms, in others it may seem to appoint the youngest candidate, the most politically conformist, and so on.

Translation of this example into terminology relevant to animal behaviour is not difficult at a superficial level. For example, let us suppose that there are two main factors contributing to feeding tendency: degree of hunger, in terms of physiological requirement for food, and strength of food cues, in terms of the animal's estimate of the availability of food. There will be an optimality criterion combining these into an overall feeding tendency, similar to that combining teaching and research abilities into an overall strength of candidature. Any tendency to feed has to be weighed against tendencies for other types of behaviour, because it is important that the criteria determining the feeding tendency be related to the animal's needs as a whole. We should therefore expect the decision criteria for feeding to be shaped by natural selection in accordance with the animal's ecological circumstances. For example, where food availability is erratic, more weight should be given to cue strength, while the weight attached to hunger should be related to the animal's physiological tolerance. We now come up against a problem that has dogged animal psychology for many years. While there has been a great deal of research on specific 'drives', such as hunger, aggression, and sex, there has been relatively little work on the interactions between such systems. Yet it is obvious that an animal's various needs have to be delicately counterbalanced, and that there is a problem for nature in weighing one against the other. We may call this the trade-off problem.

In considering what an animal ought to do in a given situation, we have to remember that the various possible activities have different consequences, and most have both costs and benefits associated with them. Let us take, as an example, the courtship behaviour of the smooth newt (*Triturus vulgaris*). This has been the subject of intensive study by members of the Oxford Animal Behaviour Research Group, supported by the Science Research Council, and aided by various university colleagues.

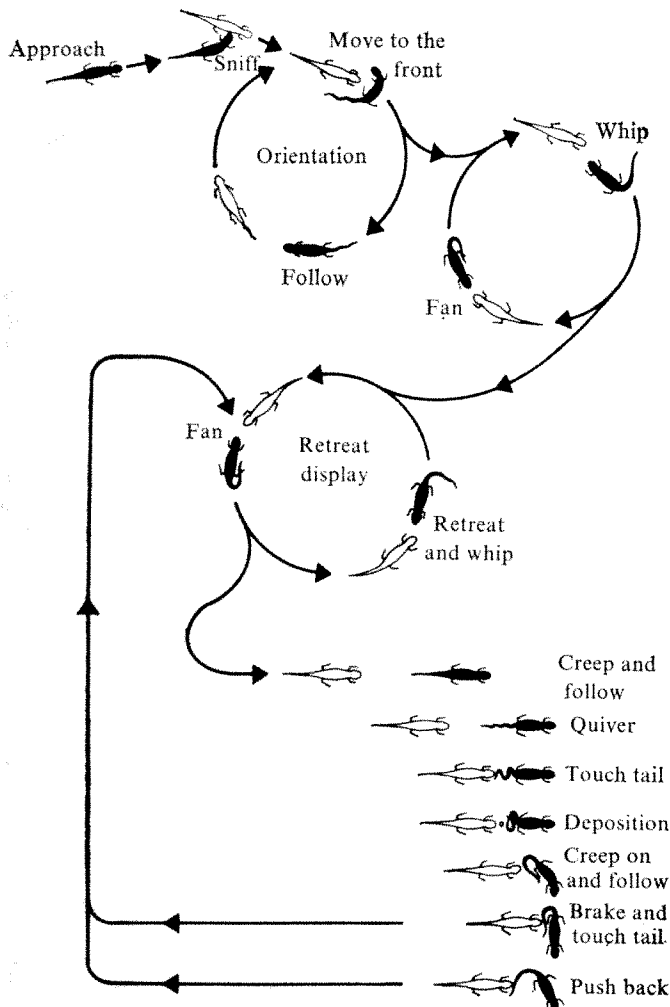
The courtship takes place on the bottom of a pond and is frequently interrupted by ascent to the surface of the water to breathe air. Observations show that the male newt breathes less often during courtship than he does normally. After the male has been to the surface of the pond to breathe he may be unable to find the female again when he returns to the bottom. Clearly, there is a cost associated with breathing during courtship, and it is not surprising that the males make breathing trips infrequently. However, although newts that are prevented from coming to the surface can probably remain alive by means of the oxygen dissolved in the water, they would be forced to become quiescent, so that courtship would no longer be possible. Thus there is also a cost associated with postponing breathing. The courtship of the male (Fig. 1) is designed to induce the female to pick up the spermatophore, which he deposits on the substratum. The female can only do this successfully if she follows the male closely. For this reason the courtship should not proceed too quickly, and the most successful courtships tend to be prolonged affairs. Thus, although the breathing problem can be alleviated by speeding up the courtship, this may make successful fertilisation less likely.

Mathematical formulation

Having touched on some of the problems involved in decision making in animals, we can now tackle the question of how we should aim to account for such decision processes.

It is difficult to foresee the day when the physiological analysis of behavioural control mechanisms will have advanced far enough to make much contribution to the explanation of decision making. Even if one knew all about the physiological mechanisms underlying breathing and courtship in newts, one would still not be able to predict when a male would decide to breathe rather than court a female. The decision will depend on

Fig. 1 A summary of the courtship behaviour of the smooth newt *Triturus vulgaris*. The black newt is the male. (From Halliday¹⁹.)



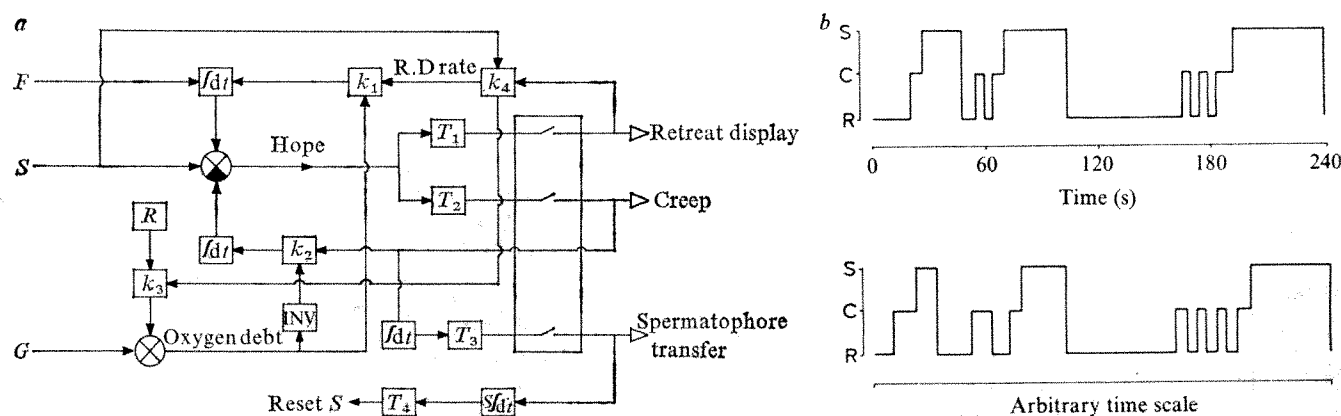


Fig. 2 a, A control model for the courtship of the male smooth newt. The following conventions have been observed: S, initial amount of sperm; F, initial sexual state of the female; G, initial value of oxygen debt; R, function representing the increase in oxygen debt with time; T_1 - T_4 , thresholds; k_1 - k_4 , modifiable parameters (modified from Houston *et al.*¹⁵); b, comparison of mean durations of various courtship activities of 22 newts (above) with that of the model (a). R, Retreat display; C, creep; S, spermatophore transfer phase (From Houston *et al.*¹⁵).

how the animal balances the two tendencies against one another. We would expect the balance to be influenced by both proximal circumstances and ultimate ecological considerations. The difficulty is that physiological knowledge is essentially piecemeal, whereas the decision problem is holistic.

The traditional behavioural analysis of preference and choice is equally inadequate in accounting for decision making as a function of time. The main reasons for this are that such theory takes no account of the ecological relevance of the behaviour, and is often attempting to account for processes which are in principle unobservable from the viewpoint of the psychological experimenter⁸.

Clearly, as has long been recognised by economists, some kind of abstract mathematical formulation is required to handle situations of such complexity. The difficulty lies in relating mathematical formulae to physiological and behavioural reality. In recent years there has been considerable progress in this area, by physiologists and behavioural scientists, who have embraced various types of systems analysis. The approach adopted by our research group is to represent the motivational state of the animal by the vector x , envisaged as a point in a multidimensional space. The state is influenced both by events internal to the animal, and by the external stimuli perceived by the animal. The changes in motivation induced as a consequence of behaviour trace a trajectory in the motivational space⁷.

It is traditional to view the motivational state of an animal as driving the behaviour, or as the input to a control system of which behaviour is the output⁹. However, an alternative approach is to regard the behaviour as the input which controls the state of the system by virtue of its consequences. In other words, the animal is seen as controlling its own internal state through the medium of behaviour, in accordance with built-in optimal design principles. Optimal performance is attained by reference to an optimality criterion that is embodied in an objective function, which is a function of the state x and of the behaviour u , where u is a vector representing the current behaviour in a behaviour-repertoire space. The objective function incorporates the optimality criterion represented as a set of trade-off relationships between the state and behaviour of the animal and their associated costs and benefits. In other words it specifies the (supposed) net cost of being in a particular state, and net benefit of doing a particular behaviour pattern under a given set of circumstances. The objective function is envisaged as a property of the individual animal, presumably genetically determined, and possibly modifiable by learning. Thus objective functions are expected to differ from one individual to another, although they may be similar in related individuals.

We can imagine the animal's brain as controlling the state of its body. Since every behaviour u results in a change in bodily

state x , the situation is akin to that found in man-made process-control problems, where the state of the plant x is influenced by extrinsic factors, and by the controller u , which is designed to achieve some optimal condition of the plant. For this reason the equations, which relate the ever-changing consequences of behaviour to the resulting changes in x , are called the plant equations.

The optimal control problem is to deploy the behaviour u in such a way that a particular state function, generally called a Hamiltonian H , is maximised at every point along the resulting trajectory. In other words, the animal is designed to deploy its behavioural options in a manner which continuously maximises the Hamiltonian

$$H = \sum_{i=1}^n \frac{\delta K}{\delta x_i} x_i - K = \text{plant equations—objective function}$$

The argument underlying this formulation is derived from decision theory, in which, as outlined above, the essential ingredients are the assumption of a maximising principle, and the formulation of an hypothesis relating to the objective function. This hypothesis can be tested by direct observation of the animal. As an example, let us return to the courtship of the male smooth newt (*Triturus vulgaris*). On the basis of a number of elegant experiments by Halliday¹⁰⁻¹², a deterministic control model was formulated by Houston *et al.*¹³, which accurately simulates the timing of the courtship sequence (Fig. 2). This computer model provides the information required to derive the Hamiltonian equations, which will predict the behaviour on the basis of its associated costs and benefits. The next task is to formulate the plant equations and find an objective function which satisfies the Hamiltonian formulation. On the assumption that the courtship of the male newt is designed to maximise the probability of successful fertilisation of the female, Dempster¹⁴ proposed the formulation illustrated in Scheme 1.

A prime feature of the courtship is that it takes place under water, and this means that the oxygen supply of the male is a most important factor. Indeed, the causal model (Fig. 2) shows that oxygen debt is the variable primarily responsible for modulating the temporal organisation of the courtship sequence, and this has been confirmed empirically¹¹. The importance of oxygen supply is reflected in the plant equation (Fig. 3) which specifies only one state variable: oxygen debt x . Because the male can replenish his oxygen supply only by terminating the

Scheme 1 Simplified version of Dempster's¹⁶ formulation of the temporal organisation of the courtship of the male smooth newt, in terms of optimality theory.

Objective: Maximise likelihood of successful fertilisation

$$\int_0^{\infty} \{ \exp[-\Phi S(t)t] S(t)u_1(t)x(t) + S(t)u_3(t)x(t) \} dt$$

Plant equation:

$$\dot{x}(t) = -x(t)^{-\rho}u_2(t) + \theta$$

Work during courtship:

$$\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \leq 1$$

Energy expended during courtship:

$$\int_0^{\infty} \left[\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \right] dt \leq E$$

Hamiltonian:

$$H = e^{-\Phi S(t)t} S(t)u_1(t)x(t) + S(t)u_3(t)x(t) - F(t) \left[-x(t)^{-\rho}u_2(t) + \theta \right]$$

(Costate) Hamiltonian equation:

$$\dot{F}(t) = \frac{\partial H}{\partial x} = e^{-\Phi S(t)t} S(t)u_1(t) - \rho \frac{F(t)}{x(t)^{1+\rho}} u_2(t) + S(t)u_3(t)$$

states: S = spermatophore count (0 - 3)
 x = oxygen 'debt'

controls: u_1 = rate of display behaviour (maximum rate δ)
 u_2 = rate of creep behaviour (maximum rate γ)
 u_3 = rate of spermatophore transfer (maximum rate τ)

parameters: Φ = basic discount rate of display behaviour
 ρ = control parameter for rate of decrease of x through skin respiration during creep behaviour
 θ = rate of increase of oxygen debt with time (during display and transfer behaviour)
 E = maximal energy expended in courtship
 F = costate variable = marginal value of likelihood of success per unit of x

courtship and visiting the surface of the pond to breath, there is effectively a fixed energy budget during courtship. Given that the courtship activities of the male have a "typical intensity"¹⁵, the fixed energy budget implies that there is a limited period of time available for effective courtship. During this time the male has to display to the female sufficiently to induce her to follow him closely during the spermatophore phase (Fig. 1), when it is essential that the female should correctly pick up the spermatophore, which is deposited by the male on the substratum. It is likely that coyness on the part of the females is a means of testing the suitability of the male as a genetic partner.

Early in the courtship there is a high probability that the female will depart from the scene to breathe, to feed, or to seek another male. As the male continues to display this probability decreases, and we can suppose that during the time available, R , this probability is given by $\phi e^{-\phi t}$. On this basis we can express the expected probability of fertilisation, given that the female is still involved in the courtship

$$\epsilon \int_0^T u_1(t) + e^{\Phi S(t)t} u_3(t) S(t)x(t) dt$$

This formulation implies that the importance of spermatophore transfer increases exponentially as the courtship proceeds.

The objective function (see box), therefore, takes account of the probability of the female departing, as well as the male's sperm supply S and oxygen debt x . The Hamiltonian, based upon this objective function and upon the plant equation specified below, gives rise to a Hamiltonian equation with a term for each of the three types of behaviour u_1 , u_2 , and u_3 . The optimal behaviour sequence is arrived at by the newt choosing to do that behaviour which has the larger term in the Hamiltonian equation. In doing this the newt is in effect choosing that activity which confers the greatest benefit in terms of maximising the probability of successful fertilisation.

This formulation has proved to be highly successful in predicting the timing of the courtship in a variety of conditions. It also makes some specific predictions about the physiology of the male newt. For example, during the vigorous display behaviour, the male's oxygen supply diminishes, but during creep behaviour the model requires that the oxygen supply increase to some extent. In fact, the creep behaviour is much less energetic than display, and it is entirely possible that oxygen supply can increase by means of skin respiration. This somewhat startling prediction implies that the male deploys the creep behaviour as a means of prolonging the courtship. Moreover, some such idea is necessary to explain the fact, illustrated in Fig. 2, that the male oscillates between creep and display more frequently in the later stages of the courtship session, when his oxygen supply is presumably lower. Of course the prediction that oxygen supply actually increases during creep behaviour should be put to the experimental test. It is reinforced, however, by the observation that this particular species of newt not only has a more prolonged courtship than related species, but also has a larger crest¹⁶, which probably enhances skin respiration.

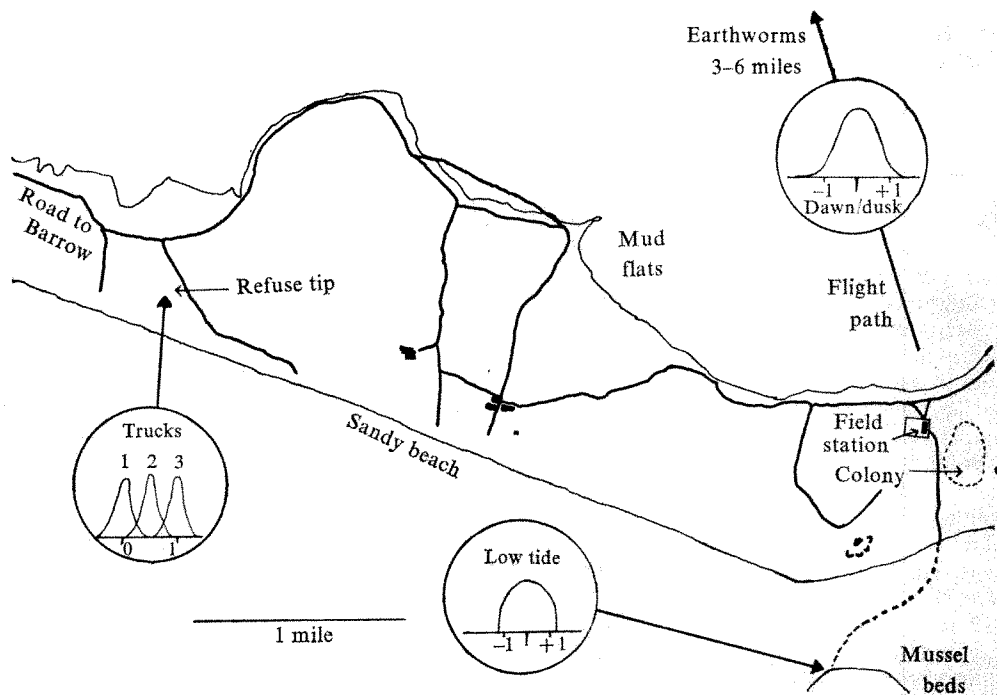
The reader may wonder why all this mathematical sophistication should be expended upon the courtship of newts. The answer is that so far newt courtship provides the most thoroughly worked test case of this general approach to animal decision making. This approach has widespread implications for theoretical biology, because it offers some insight into inverse optimality problems. Normal optimality problems pose the question of the best route to a goal, in particular circumstances. The inverse problem, which has been largely neglected by mathematicians, but is important for biologists, is that of what is being optimised by a particular structure, or a particular observed behaviour sequence. The general philosophy here is the following: if we know what a mechanism or system is designed to do, it will be much easier to find out how it works. In animal decision making it is essential to know that the decisions are about, before attempting to account for the mechanisms involved.

Natural selection

As biologists, we can expect natural selection to shape the decision-making processes of animals in such a way that the resultant behaviour sequences are optimally adapted to the current environmental circumstances. In other words, we can regard natural selection as a designing agent capable of producing the optimal design for any given set of conditions. Such considerations of optimal design apply just as much to the temporal organisation of behaviour as they do to the anatomical properties of animals. However, we cannot expect an individual animal always to behave optimally in its natural environment. Genetic variation between individuals, competition between individuals, the patchy nature of the environment, and changes in the nature of the environment, all combine to make it very unlikely that an individual animal can ever be perfectly adapted to its niche.

We can imagine, however, an 'ideal' animal that is perfectly adapted to its natural environment. The objective function of such an animal will be identical to a cost function, which is characteristic of the environment, in the sense that it reflects the selective pressures that moulded the objective function during the course of evolution. The cost function, therefore, specifies

Fig. 3 Map of part of Walney Island showing the positions of the most important feeding places in relation to the colony. Circles enclose graphs showing rough estimates of food availability.



the instantaneous level of risk incurred by (and reproductive benefit available to) an animal in a particular internal state, engaged in a particular activity in a particular environment. Such an ideal animal, perfectly adapted to its environment, will deploy its behavioural options so as to maximise the Hamiltonian, and thereby achieve that behaviour sequence which maximises its inclusive fitness in the prevailing circumstances.

In practice, we can distinguish two procedures by which optimal behaviour sequences can be investigated. In the first, a maximisation principle is assumed, and a particular form of the objective function postulated. This hypothesis is then tested in laboratory experiments. Ability to reproduce the behaviour of the animal is then taken as evidence in favour of the postulated objective function. This is the strategy used in the analysis of newt courtship outlined above.

In the second approach, an attempt is made to measure the components of the cost function empirically, by means of observations and experiments made in the field. The aim in this type of study is to determine whether animals in a particular natural environment are deploying their behavioural options in the optimal manner. The Hamiltonian formulation can then be used to generate behaviour sequences, or strategies, for a given environment, provided that sufficient is known about the cost function characteristic of the environment, and the plant equations characteristic of the animal.

As an example, let us consider the herring gull incubating its eggs, mentioned at the beginning of this article. This situation is being studied in detail by members of the Oxford Animal Behaviour Research Group, notably R. McCleery and R. Sibly, supported by the Natural Environment Research Council. The animals are studied intensively during the 4-week incubation period, particular attention being given to three main problems.

First, the factors influencing the internal state, of which the most important is hunger, and those affecting the animal's estimate of the environmental situation, of which the weather and food supply are of prime importance, are incorporated in the plant equations for the incubating bird. As part of the assessment of the animal's internal state, the whole nest including the sitting bird is weighed continuously by means of an electrically monitored balance buried in the ground. The measurements obtained in this way are capable of providing information about the loss of weight during fasting, and the gain of weight after foraging, with an accuracy of ± 20 g.

The gulls have a variety of possible feeding sites, at various distances from the nest, as indicated in Fig. 3. The availability of food fluctuates considerably in any one location, so that the bird has to choose among a continuously varying pattern of possibilities (Fig. 4)¹⁸. The availability of food as a function of time is computed from the tide cycle, the work routine of the men at the nearby refuse tip, the behaviour of earthworms in response to the weather, and so on. The extent to which the

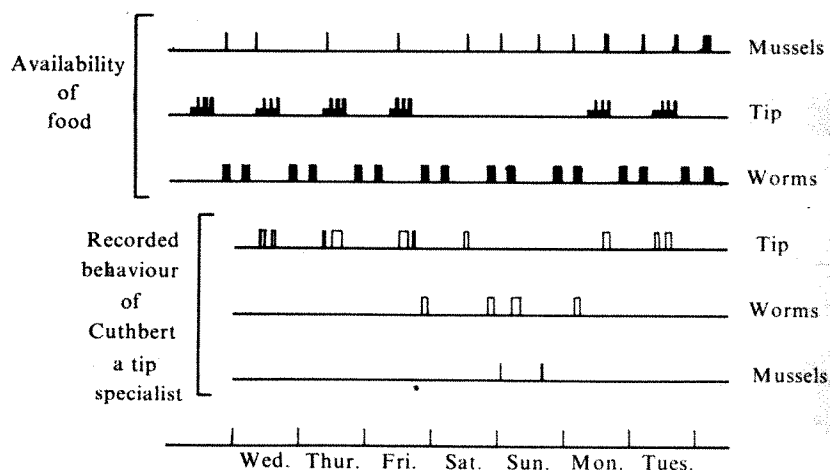


Fig. 4 Availability of food (above) during one week in the 1976 season, and (below) the foraging behaviour of one individual gull, record by radiotelemetry.

birds can exploit these opportunities depends on the willingness of the partner to take over the incubation duties, the probability of human interference near the feeding sites, and the vicissitudes of the weather. These variables are all monitored, either by direct observation, or by means of remote automatic monitoring devices. Weather variables, such as light intensity, wind speed, and so on, have a considerable influence on the foraging efficiency of the gulls, and must be an important factor in their foraging strategy. The returns that a gull can be expected to gain per unit time spent foraging are also estimated; by analysing samples of the food source, and checking this against regurgitations around the nest. All these factors taken together, when suitably calibrated and integrated with each other, can be used to obtain a good indication of the state of an individual bird in a given set of circumstances, and of the changes in state that are consequent on its behaviour.

• Second, the behaviour of the individual birds on the territory can be monitored by direct observation from hides. Each bird is fitted with a radio transmitter so that it can be followed in a Land Rover when it leaves the territory, usually to forage for food. The birds are conspicuously marked so that they can be recognised amongst the thousands of others. Typically, a bird will fly from the territory directly to its chosen feeding ground. It may spend one or two hours feeding, often in competition with other gulls. It will then often bathe and drink in a fresh water puddle, before taking a 'rest' on a quiet part of the beach, or in a field. Such rest periods are generally communal affairs, although interactions between individuals are few. A considerable amount of time is spent preening and the question of whether gulls sleep on such occasions is currently being investigated. After a few hours away from the territory, the gull invariably returns home and takes over the task of incubation, relieving its partner.

The third problem is the cost function, which is a function of both the internal state of the animal, and of its behaviour. Obviously, for an incubating bird there is a risk attached to being in a particular state of hunger. It is not that the animal is likely to die while sitting on the nest, but rather that it may not find food very easily when it leaves the nest. If the animal had a reliable supply of food it could afford to risk a higher level of hunger than if its foraging fortunes were uncertain. In our study we attempt to measure such risks experimentally by removing one of an incubating pair of gulls, and studying the condition and behaviour of the remaining bird.

Similarly, when a bird leaves the nest as a result of disturbance or when pressed by hunger, there is a chance that its eggs will be eaten by a neighbour. The risk of egg predation can be measured by removing both of the nest owners and measuring the survival time of the eggs. Every aspect of the behaviour also has risks and benefits attached to it. Thus a bird foraging on the refuse tip may be poisoned, a gull on the shore line may get oil on its feathers. There is also the energetic cost of each activity to be taken into account. By systematically evaluating the cost and benefits involved in the behavioural routines of both members of mated pairs of gulls we aim to arrive at a mathematical formulation of the cost function.

In real life, an animal has to decide whether to sleep, feed, groom, and so on, at any particular time. In addition to its motivational state and the strengths of its various behavioural tendencies, the decisions reached will be heavily influenced by the decision criteria embodied in the objective function. If I believe that animal behaviour is subject to natural selection, I must conclude that the objective function is shaped by natural selection, because the decision criteria strongly influence the order in which animals go about their daily tasks.

The animal can be seen as carrying out a cost-benefit analysis in making its decisions. If the animal is not closely adapted to its environment, it may not make the analysis correctly; that is its objective function may not conform to the cost function characteristic of the current environment. Nevertheless, the animal is still an optimising agent with respect to its own objective function.

I do not intend to suggest that the individual animal necessarily carries out the cost-benefit analysis in a cognitive fashion, although this may occur to some extent in intelligent animals. The form and pattern of the behaviour is the outcome of a design operation which aims at the optimal compromise between the competing selective pressures characteristic of the environment. Obviously in a non-stationary environment, there will be an advantage in organising the decision-making processes in a flexible manner. Thus we can expect the laws governing learning to be designed in such a way that they tend to shape the objective function of the individual, and make it more like the cost function of the current environment. To study such phenomena we have to know something about the make-up of the individual animal, and something about its ability to adapt to the environment. This is where the herring gull study is so instructive. There is no question that individuals differ greatly. Some specialise in foraging for mussels, others for crabs, using quite different techniques. Some specialise in stealing eggs and chicks from neighbours, and others have apprenticed themselves at the local corporation refuse tip. Indeed, an important aspect of the optimal decision-making approach, outlined in this article, is that attention is concentrated on the behaviour of individuals, in contrast to the more traditional approaches to the study of animal behaviour, which rely on statistical inferences based upon the study of large numbers of animals. This attention to detail is made possible by the fact that optimality theory provides a way in which precise mathematical functions can be used within the normal scientific framework of hypothesis, prediction, and test. Hypothesised functions, tailor-made for the individual animal, can be used in optimality computations to predict sequences of behaviour that should occur in observed circumstances and conditions, and these predictions can be compared with the behaviour observed in the field or laboratory.

What then is likely to be achieved by the application of optimality theory to animal decision making, or by empirical studies designed to test predictions of optimality theory? First, the development of the theory itself has proved to be extremely instructive in forging links between ecology and behaviour¹⁸, between behaviour and economics¹⁹, and between environmental physiology and behaviour⁶. As I have mentioned elsewhere²⁰, we are entering a new phase of interpretation of animal behaviour, in which the complexity of animals is taken as a challenge, rather than as an excuse for mysticism or for sceptical over-simplification.

Second, we cannot expect to understand decision-making processes without also appreciating the selective pressures that shaped them. In many areas of biology it is helpful to understand the function of the material being studied, but in decision making it is essential, because decisions inevitably reflect the trade-off and compromise among the design features of the animal. Optimality theory provides a self-checking procedure, which is a considerable improvement on the traditional open-ended approach to the survival value of behaviour⁹.

Third, empirical studies ought to be part of any mathematical approach to biology, where there is a strong temptation towards arm-chair theorising. These studies should tell us, not merely whether the theory is right or wrong, since it is bound to be wrong in some respects, but also something about the mental competence of the animal themselves. Already we have seen that herring gulls adapt individually to their complex, ever shifting, circumstances. When we know more about how to account for the apparent decisions we observe and how good the animals are at matching their decisions to the real world, we may be in a position to investigate the mechanisms used by animals in adapting their time budgets to changing circumstances.

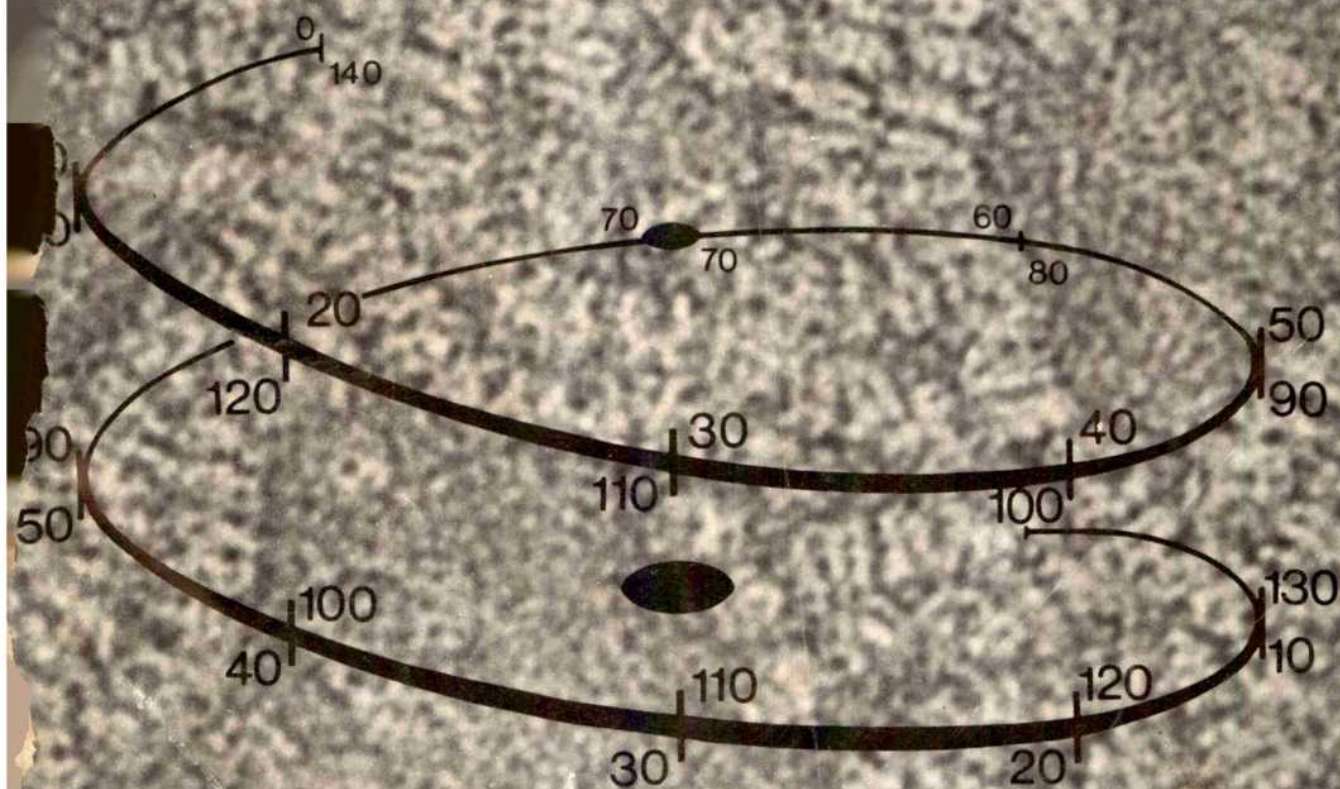
¹ Logan, F. A. *Nebraska Symposium on Motivation* 12, 99-129 (ed. Levine, D. (Nebraska University Press, Nebraska, 1964).

² Logan, F. A. *J. comp. physiol. Psychol.* 59, 1-12 & 246-251 (1965).

³ Lea, S. E. G. *J. exp. anal. Behav.* 25, 43-54 (1976).

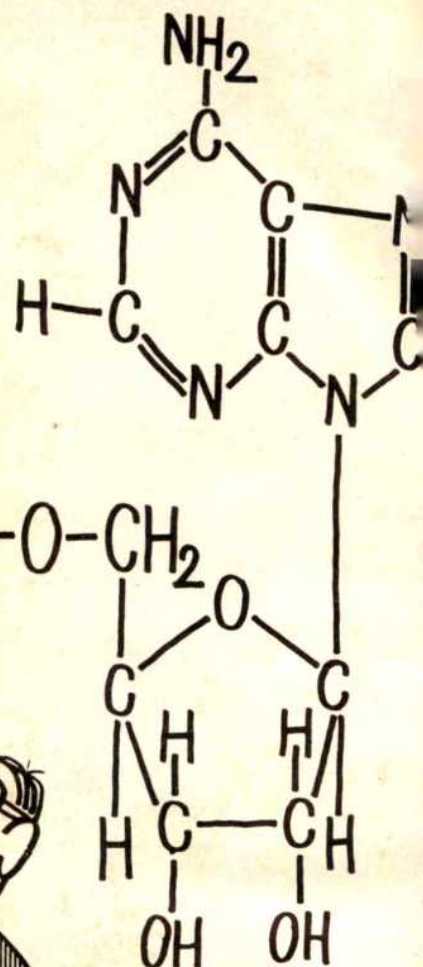
No 5623 1 September 1977 55p

No 5623 1 September 1977 55p

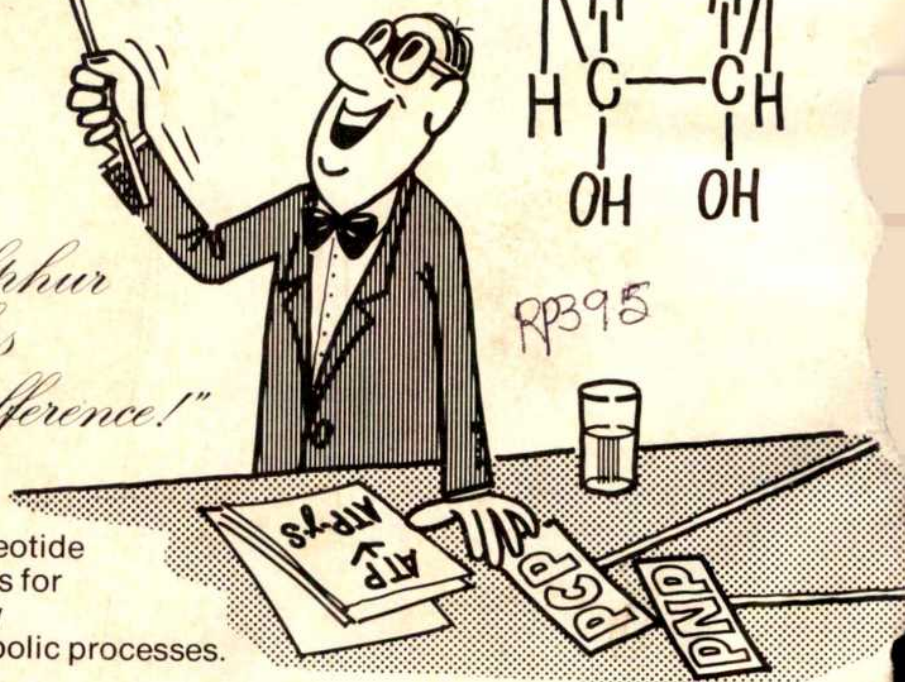


Decision making by Animals

ATP → ATP-γ-S



*"This little change,
ladies and
gentlemen - the
substitution of sulphur
for oxygen - makes
all the difference!"*



Boehringer Mannheim's nucleotide analogs are the preferred tools for investigation of the regulatory mechanisms governing metabolic processes.

We offer in excellent quality:

ATP-γ-S	Cat. No. 102 342	AMP-S	Cat. No. 161 268	AMP-PNP	Cat. No. 102 547	GMP-PNP	Cat. No. 106 402
ADP-β-S	Cat. No. 200 174	GTP-γ-S	Cat. No. 220 647	AMP-PCP	Cat. No. 102 555	GMP-PCP	Cat. No. 106 429

For further details
please contact
your local
representative or



Boehringer Mannheim GmbH
Biochemica
P.O. Box 310120
D-6800 Mannheim
W. Germany

- 4 Miller, H. L. *J. exp. anal. Behav.* **26**, 335–347 (1976).
 5 Baerends, G. P. & Kruijt, J. P. in *Constraints on Learning* (eds Hinde, R. A. & Stevenson-Hinde, J.) (Academic, New York & London, 1973).
 6 Houston, A. & McFarland, D. J. *Anim. Behav.* **24**, 459–475 (1976).
 7 McFarland, D. & Sibly, R. *Phil. Trans. R. Soc. Ser. B* **270**, 265–293 (1975).
 8 McFarland, D. & Sibly, R. *Anim. Behav.* **20**, 548–563 (1972).
 9 McFarland, D. in *Growing Points in Ethology* (eds Bateson, P. P. G. & Hinde, R. A.) (Cambridge University Press, London, 1976).
 10 Halliday, T. R. & Sweatman, H. P. A. *Anim. Behav.* **24**, 551–561 (1976).
 11 Halliday, T. R. *Anim. Behav.* **25**, 39–45 (1977).
 12 Halliday, T. R. *Anim. Behav.* **24**, 398–414 (1976).
 13 Houston, A. I., Halliday, T. & McFarland, D. *Med. Biol. Engng* **15**, 49–61 (1977).
 14 Dempster, M. A. H. in *Animal Economics* (eds Dempster, M. A. H. & McFarland, D. J.) (Academic, London & New York, in the press).
 15 Halliday, T. R. *Anim. Behav.* **23**, 291–322 (1975).
 16 Halliday, T. R. *Zool. J. Linn. Soc.* **56**, 291–300 (1975).
 17 Sibly, R. & McFarland, D. *Amer. Nat.* **110**, 601–617 (1976).
 18 Dempster, M. A. H. & McFarland, D. J. (eds) *Animal Economics* (Academic, London & New York, in the press).
 19 McFarland, D. J. *New Scientist* **72**, 376–379 (1976).

articles

On the optical identifications of five X-ray sources

H. V. Bradt, K. M. V. Apparao*, G. W. Clark, R. Dower, R. Doxsey, D. R. Hearn, J. G. Jernigan, P. C. Joss, W. Mayer, J. McClintock & F. Walter†

Department of Physics and Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The data from a recently completed survey of the galactic plane with the SAS-3 modulation collimators provide precise (20"–60") celestial positions of galactic X-ray sources. Preliminary positions of 60" precision are reported for five sources. One of these led to the identification of the star, γ Cas, as an X-ray source, and the others lend substantial confidence to previously proposed optical identifications: 3U0352+30 = X Per, 3U1145–61 = HEN 715, GX301–2 = WRA977, and GX304–1 = MMV star. These identifications seem to establish the existence of a previously suggested class of De-star X-ray emitters.

THE SAS-3 X-ray astronomy observatory carries rotation modulation collimators (RMC) with which the positions of X-ray sources in the energy range 2–11 keV can be measured^{1,2} with a precision of 20" for the brighter sources³. In uncrowded regions, the detection threshold of a typical observation (1–2 d) is about 3 μ Jy (see footnote to Table 1). A programme of observations of the entire galactic plane ($|b^{\text{II}}| \lesssim 10^\circ$) has now been completed and the analysis is in progress.

The objective of this programme is the identification of radio, infrared and optical counterparts of the X-ray sources. At present there are about 10 well established identifications of stellar optical objects with hard (≥ 2 keV) X-ray sources. Most of these identifications are compelling because of positional coincidences coupled with coincident temporal phenomena such as identical orbital periods at both optical and X-ray wavelengths. A comparable number of additional identifications with varying degrees of confidence have also been proposed.

We describe here the survey of the galactic plane and present the results of five position measurements derived from it. One of these led to the identification of γ Cas as an X-ray source, and the others substantially increase our confidence in previously proposed identifications. These results are derived from the quick-look data and an early version of the analysis system which limits the positional precision to 1.0' (90% confidence). They have been announced earlier in preliminary forms^{5–8}.

Other early SAS-3 RMC positions have been reported for the

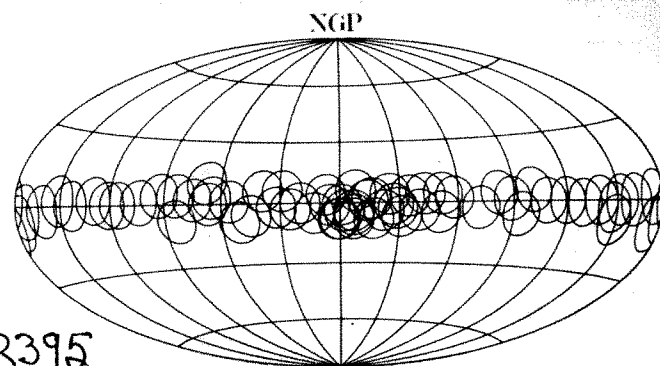
following galactic X-ray sources: Aql X-1, Ser X-1, GX9+9, and GX17+2 (ref. 9); 3U0143+61 and 3U1956+11 (ref. 10); Algol (ref. 2); MX1553–54 (ref. 11); the transients MX1803–24 (ref. 12), A1524–61 (ref. 13), and A0620–00 (ref. 1); the steady counterparts to two burst sources, 3U1733–27 (ref. 14) and A1905+00 (ref. 15); and the mid-galactic latitude source, 3U0044+32 (ref. 16). These and the positions reported herein constitute the first SAS-3 list ('1S') of galactic source positions. Subsequent papers will present '2S' positions as they become available. The latter will be derived from larger samples of data analysed with an improved system which yields the higher precision quoted above.

The galactic survey

The two SAS-3 modulation collimators have periodic triangular response functions of 2.3' and 4.5' FWHM respectively and $12^\circ \times 12^\circ$ FWHM fields of view. The celestial locations of the fields of view for the survey observations (Fig. 1) were chosen to yield two to three independent measurements of most sources and to provide substantial exposure to galactic latitudes $\pm 12^\circ$ in the region $-60^\circ < \ell^{\text{II}} < +40^\circ$ and $\pm 8^\circ$ elsewhere. The durations of the observations ranged from 0.5 to 2 d. Simultaneous X-ray data and optical aspect data which can be analysed for X-ray positions were obtained for about 30% of this time. Thus the useful duration of each observation ranged from $\sim 1 \times 10^4$ s to $\sim 5 \times 10^4$ s.

The celestial positions of the X-ray sources are derived from a cross-correlation analysis¹⁷ and a fast mapping algorithm. The

Fig. 1 Fields of view of the SAS-3 rotating modulation collimator during the survey of the galactic plane in galactic coordinates. The galactic centre is at the centre of the figure and the north galactic pole (NGP) is at the top.



*Permanent address: Tata Institute of Fundamental Research, Bombay, India.

†Present address: Department of Astronomy, University of California, Berkeley, California 94720.

Table 1 X-ray sources

SAS-3 designation	Other designations*	α	Position (1950) δ	\mathcal{L}^{II} b^{II}	Error radius ($\sim 90\%$)	Flux density* \dagger (2–6 keV)	X-ray* periods	Luminosity \ddagger (2–6 keV)
1S0053 + 604 (γ Cas)	MX0053 + 60 MX0049 + 59(?)	00 h 53 min 41.3 s 13.4221°	60°26' 57" 60.4492°	123.6° –2.1°	1.0'	20 μ Jy		2×10^{33} erg s $^{-1}$
1S0352 + 308 (X Per)	3U0352 + 30	03 h 52 min 14.4 s 58.0600°	30°53' 42" 30.8950°	163.1 –17.1°	1.0'	35 μ Jy	13.9 min 22 h? 581 d?	5×10^{33} erg s $^{-1}$
1S1145 – 619 (HEN 715)	3U1145 – 61	11 h 45 min 30.6 s 176.3775°	–61°55' 40" –61.9278°	295.6 –0.2°	1.0'	120 μ Jy		3×10^{35} erg s $^{-1}$
1S1223 – 624 (WRA 977)	GX301 – 2 3U1223 – 62	12 h 23 min 50.2 s 185.9592°	–62°29' 35" –62.4931°	300.1 –0.0°	1.0'	50 μ Jy (< 10 –200; 25 keV)	11.6 min	4×10^{36} erg s $^{-1}$ (2–30 keV)
1S1258 – 613 (MMV star)	GX304 – 1 3U1258 – 61	12 h 58 min 14.3 s 194.5596°	–61°20' 01" –61.3336°	304.1 1.2°	1.0'	80 μ Jy (< 8 –60; 25 keV)	4.5 min	

*See text for refs.

 $\dagger \mu\text{Jy} \equiv 1.000 \times 10^{-29}$ erg s $^{-1}$ cm $^{-2}$ Hz $^{-1}$ $= 2.418 \times 10^{-12}$ erg s $^{-1}$ cm $^{-2}$ keV $^{-1}$ $= 1.509 \times 10^{-3}$ keV s $^{-1}$ cm $^{-2}$ keV $^{-1}$ For a power-law spectrum of slope $\alpha = 1.08$ (for example, the Crab nebula⁴):1.0 μ Jy at 3.6 keV corresponds to an integrated flux of 0.96×10^{-11} erg s $^{-1}$ cm $^{-2}$ (2–6 keV) or ~ 0.6 Uhuru counts.1.0 μ Jy at 5.2 keV corresponds to 2.2×10^{-11} erg s $^{-1}$ cm $^{-2}$ (2–11 keV). I_{Crab} (3.6 keV) = 1,580 μ Jy; I_{Crab} (5.2 keV) = 1,060 μ Jy. \ddagger For the distances given in Table 2.

minimum intensity (counts cm $^{-2}$ s $^{-1}$) which is detectable in these cross-correlation 'maps' at the m standard deviation level can be shown to be $I_{\text{min}} = (m\pi^2/A) (B/8T)^{1/2}$ where B is the background rate (counts s $^{-1}$), A is the effective area at the peak of the response function (cm 2), and T is the effective observation time. An isolated source near the centre of the coarse field of view will be detected at the 7σ level ($m = 7$) in one of the two collimators ($A = 180$ cm 2 , $B = 30$ s $^{-1}$ and $T = 25,000$ s) if its intensity is

$$I_{\text{min}} = 4.7 \times 10^{-3} \text{ counts cm}^{-2} \text{ s}^{-1} \text{ (2–11 keV)}$$

$$\approx 2.3 \mu\text{Jy (2–11 keV)}$$

An intense source in the field of view will raise the effective background for the detection of a weak source. In the presence of a point-like source with the intensity of the Crab nebula at the centre of the field of view (380 counts s $^{-1}$, 2–11 keV), we have $\langle B \rangle = 30 + 380/2 = 220$ counts s $^{-1}$ and obtain $I_{\text{min}} = 6 \mu\text{Jy} (7\sigma)$. Furthermore, the bright source will exhibit a ring pattern which decreases in amplitude with radius from the source^{17,18}. This pattern can mask a nearby weak source, and therefore must be subtracted from the map¹⁷. This can be done with an uncertainty of $< 5\%$ of the bright source intensity. In our example, the residual rings from the 2.3' collimator, after subtraction of the Crab-like source, would have an amplitude of 5 μ Jy 1° from the source and 2 μ Jy at 5°.

Results

The position measurements reported here are summarised in Table 1. Characteristics of the optical candidates are summarised in Table 2 and finding charts are shown in Fig. 2. Of the five X-ray sources discussed here, one was identified in the course of this work (1S0053 + 604/ γ Cas), two were previously known and had convincing and well-studied optical counterparts (3U0352 + 30/X Per and GX301 – 2/WRA 977), and two have suggested counterparts which become credible with the present refined positions (3U1145 – 61/HEN 715 and GX304 – 1/MMV star).

Each of the positions was derived from at least four orbits of data and from both modulation collimators. Systematic errors in the positions dominate the statistical errors. We find the former to be no more than 30" to 40" based on observations of identified sources. Thus we report 60" error radii in this work as a conservative ($> 90\%$ confidence) estimate before the final analysis of the data. For each source, the multiple positions derived from individual orbits and collimators all lie well within this error circle.

The celestial areas defined by these circles are smaller by factors of 3–25 than the error regions obtained previously with the Uhuru¹⁹ and the Copernicus²⁰ satellites (see Fig. 2). In the case of 1S0053 + 604, there are no other position determinations of comparable precision. The proposed optical counterpart of each of the five sources lies within the SAS-3 error circle. The small error regions and the unusual character of these stars lend substantial confidence to the proposed identifications.

Table 2 Optical counterparts*

Star	Apparent magnitude	M_V	Type	Optical periods	Estimated distance	Angular distance from X-ray position
γ Cas (1S0053 + 604)	$V \sim 1.6$ –3.0	~ -4.5	B0.5(II–V)e	0.7 d?	0.30 kpc	12"
X Per (3U0352 + 30)	$V \sim 6.0$ –6.7	~ -3.6	O9.5(III–V)e	13.9 min? 584?	0.35 kpc	22"
HEN 715 (3U1145 – 61)	$V = 9.0$	~ -3.5	B1Vne		1.5 kpc	21"
WRA 977 (GX301 – 2)	$V = 10.8$	~ -6	B1.5Ia	23 d 11.6 min?	2.0 kpc	6"
MMV star (GX304 – 1)	$V = 14.7$					27"

*See text for refs

The five sources

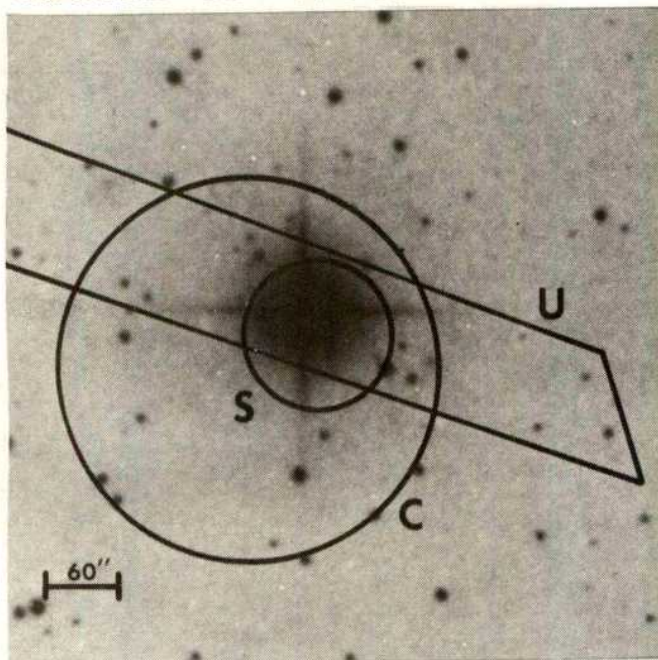
X-ray emission from **IS0053+604**/ γ Cas was detected by SAS-3 during 30–31 December 1975 with both modulation collimators and the source was designated MX0053+60 (ref. 5). This source was the highest point ($\sim 15 \mu\text{Jy}$, 2–11 keV) in the $10^\circ \times 10^\circ$ field of the cross-correlation maps which were constructed from seven orbits of data (1.3×10^4 s). For Table 1, we assume the spectral shape of the Crab nebula and quote a 2–6 keV flux density of

$20 \mu\text{Jy}$. The X-ray position lies $10''$ from γ Cas which we identify as the optical counterpart.

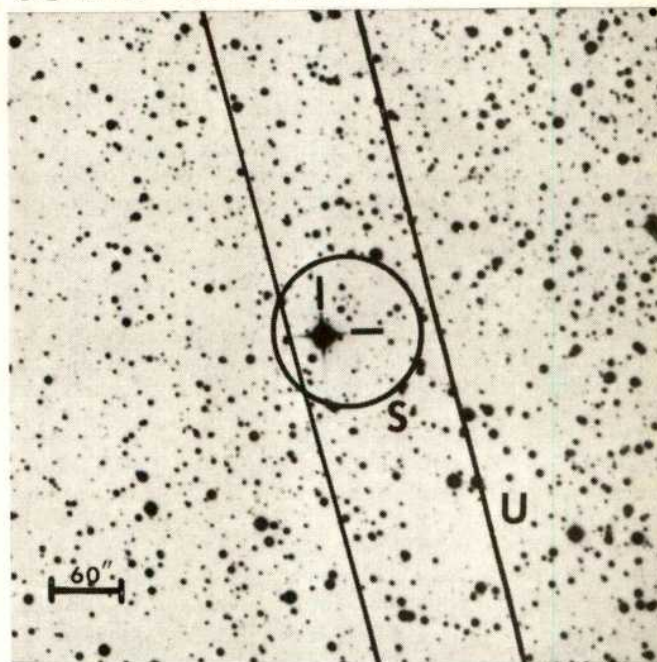
An X-ray source, MX0049+59, was detected earlier with OSO-7, 1.3° or about three standard deviations from γ Cas (ref. 21). Independent X-ray observations of this region in December 1975 and January 1976 with the Copernicus satellite²² yielded a positive signal from the region of γ Cas with a 3° uncertainty in celestial position. The source intensities derived from these several

Fig. 2 The X-ray error regions (U = Uhuru, C = Copernicus, S = SAS-3) and the proposed optical counterparts for four of the five sources. X Per is the very bright star in the field; the other candidates are marked. The photograph for the X Per region is from the blue Palomar Sky Survey plate (National Geographic Society); for HEN 715 and WRA 977, from the ESO quick blue survey; for the Mason, Murdin, and Visvanathan (MMV) star²⁴, from a plate taken by Canizares and Hiltner with the 4-m telescope (V filter) at CTIO.

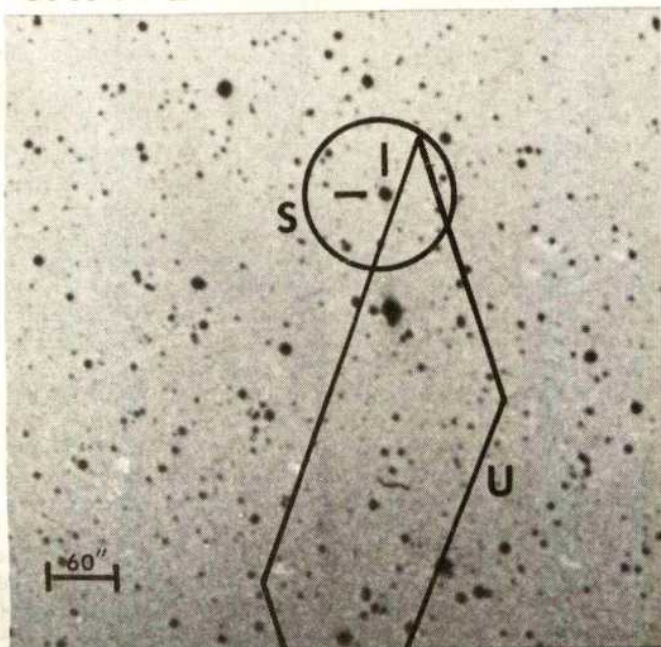
3U0352+30 / X Per



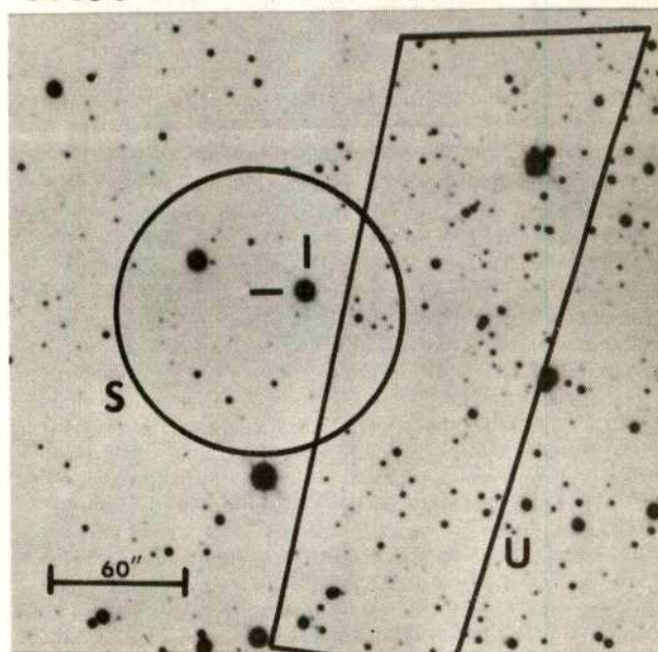
3U1145-61 / HEN 715



GX301-2 / WRA 977



GX304-1 / MMV star



observations are comparable, within about 50%. This supports the view that γ Cas is the only source in this region. The quoted intensities and the absence of an Uhuru sighting¹⁹ suggest that the intensity is variable.

γ Cas is a B0.5 (ii-V)e star with variable intensity ($V = 1.6$ – 3.0), variable line profiles, $M_V \sim -4.5$, and distance ~ 300 pc (see summaries in refs 23, 24). The spectral features indicate that the star is rotating rapidly, and variations of the emission line profiles suggest a 0.7-d period²⁵. It is an exceptionally variable Be star and in this respect is similar to X Per (ref. 23).

At the distance of 300 pc the 2–6 keV X-ray luminosity is $\sim 2 \times 10^{33}$ erg s⁻¹. A search for 44 Å emission by SAS-3 yielded an upper limit of ~ 130 μ Jy ($\sim 1/50$ the Cygnus Loop flux) in the energy ranges 0.10–0.28 keV and 0.4–0.8 keV. In the absence of a substantial interstellar cut off at ~ 0.6 keV, this limit and the 2–11-keV flux indicate a temperature $kT \gtrsim 1.5$ keV for a thermal spectrum, or a spectral index $\alpha \lesssim 1.0$ for a power law energy spectrum.

X rays from the 3U0352+30/X Per system were first detected with Uhuru at a 2–6-keV flux density of 35 μ Jy (ref. 19) and later with Ariel V at a comparable level²⁶. A 13.9-min X-ray period with peak-to-peak amplitude of 40% has been discovered²⁷. Also possible 22-h (or 11-h, ref. 27) and 581-d (ref. 28) periods have been reported. The X-ray source also exhibits irregular variability on time scales of minutes²⁹.

X Per, by far the brightest star in an early Uhuru error box, was proposed as the optical counterpart by several groups^{30–32}. A $V = 12$ star, ADS 2859B, which lies 22.5" from X Per has been suggested as the counterpart²³. The present results cannot exclude it. Its colours and spectrum indicate, however, that it is a normal M III star with no peculiarities which would suggest that it is the X-ray source³³.

X Per is rotating rapidly (~ 400 km s⁻¹, ref. 32) and is classified²³ as a 09.5 (III–V)e star with $M_V \sim -3.6$. Its intensity varies slowly on time scales of months or years from $V = 6.0$ to $V = 6.7$ (refs. 34, 29). Spectral studies yield evidence for a 584-d period³⁵, and narrow band photometry at 4,686 Å provides an indication of episodes of 13.9 min variability (ref. 36 and Canizares, personal communication). Searches for this episodic phenomenon on other occasions and by other observers have not yet confirmed it³⁷. Brucato and Kristian³² place X Per in the Perseus 2 association at a distance of 350 pc, which implies an X-ray luminosity of 5×10^{33} erg s⁻¹.

The 3U1145–61/HEN 715 system has a maximum X-ray flux density of 120 μ Jy (2–6 keV) which is variable by a factor of 5 (ref. 19). The B-emission star HEN 715 (SAO 251595) has been suggested previously as a candidate on the basis of the Uhuru position^{38–40}. It is relatively bright ($V = 8.95$) and blue ($B - V = 0.2$, $U - B = -0.85$) and exhibits fluctuations in brightness ($\Delta B \approx 0.02$, ref. 39). Its spectral classification is B1 Vne (ref. 41). We estimate the distance of this star to be 1.5 kpc if its luminosity is that of a B1 main-sequence star. This leads to a maximum luminosity of 3×10^{35} erg s⁻¹.

The GX301–2/WRA 977 system was first detected by Lewin *et al.*⁴² and McClintock *et al.*⁴³ during balloon observations which showed the source to be bright and variable in intensity by a factor of > 20 at high X-ray energies (< 10 – 200 μ Jy, 18–36 keV, ref. 44). At 2–6 keV, it is relatively weak (50 μ Jy) and variable by a factor of 3 (ref. 19). More recently, it has been found to exhibit an X-ray periodicity of 11.6 min (ref. 45) and to feature a strong iron absorption edge in its spectrum⁴⁶.

The emission line star WRA 977 ($V = 10.84$) has been proposed by Vidal⁴⁷ and Jones *et al.*³⁹ to be the optical counterpart. The star is heavily reddened ($E_{B-V} = 1.8$). The B1.51a classification suggests an absolute magnitude $M_V = -6$ and a distance of 2 kpc (ref. 47). This yields a 2–30-keV maximum X-ray luminosity of $\sim 4 \times 10^{36}$ erg s⁻¹.

At optical wavelengths, a tidal distortion light curve indicates a binary period of 23 ± 1 d (ref. 48). This has been confirmed by Petro (personal communication) who analysed a 3-yr compilation of photometric data provided by many observers. Finally, Mauder⁴⁹ reports that photometric data provide weak evidence

for the 11.6-min X-ray period. Thus, the established optical binary period of 23 d in WRA 977 has not been detected at X-ray wavelengths and the 11.6-min X-ray pulse period in GX301–2 has not been detected convincingly at optical wavelengths. It is our view, however, that the SAS-3 position removes any residual doubt about the identification of WRA 977 with GX301–2.

The GX304–1/MMV star system was first detected at X-ray wavelengths from balloons by Lewin *et al.*^{50, 51}. It is another of the small class of X-ray sources which have been detected at energies up to ~ 50 keV. Like GX301–2, it is highly variable in intensity at all energies. At 2–6 keV, its flux density is 80 μ Jy with a variability factor of 5 (ref. 19) and at 20–40 keV the factor is more than 7 (< 8 – 60 μ Jy, refs 43, 44). It has recently been found to be a slow X-ray pulsar with a period of 4.5 min (refs 52, 53).

The celestial region of this source is shown on a deep plate (Fig. 2) taken by Canizares and Hiltner at CT10 with the 4-m telescope. The marked star ($V = 14.72$ and $B - V = 1.80$) has been proposed by Mason, Murdin, and Visvanathan (MMV) as the optical counterpart⁵⁴. They report that this star exhibits H α emission with a FWHM of 700 km s⁻¹ and an equivalent width of 12 Å and that the absorption spectrum suggests a spectral type between B2 and A0. We do not attempt to estimate the distance to the star because it lies in a heavily obscured region of the sky (the Coalsack). It is unlikely that the bright star 1.5' south of this star is the counterpart as proposed earlier by Huckle *et al.*⁵³. It lies outside the conservative SAS-3 error circle and its colours do not suggest an early-type star (W.A. Hiltner, personal communication).

Discussion

These results seem to establish the existence of the class of accreting binary X-ray sources, suggested by Maraschi, Treves, and van den Heuvel⁴⁰, wherein the source of accreting material is a rapidly rotating main-sequence Be star. These authors propose that such stars with masses as low as 8 – $10 M_\odot$ could become X-ray sources through the accretion of circumstellar material on to a companion neutron star. They argue that the existence of such binary systems is reasonable from an evolutionary point of view.

The γ Cas, X Per and HEN 715 systems apparently belong to this class of X-ray emitters. The optical members are emission line, 09.5-B1, stars with luminosity classifications which are consistent with main-sequence membership. Two of these, γ Cas and X Per, are known to be rapid rotators^{25, 32}, with angular velocities that may be sufficient to yield approximately zero effective gravity in the equatorial regions^{25, 40}.

The spinup rate of the 13.9-min X-ray pulsar in the X Per system indicates that the compact accreting companion is a neutron star and not a white dwarf²⁸, although the latter would be consistent with the low X-ray luminosity^{55, 56}. The similarity between the X Per and γ Cas systems in X-ray luminosity, absolute magnitude, and in their unusually large optical variability²³ suggests that γ Cas also has a compact companion undergoing accretion. The nature of the compact member would be clarified if the system is found to exhibit periodic X-ray pulsations. Optical studies of these two stars do not exclude the existence of low-mass ($< 2 M_\odot$) secondaries^{24, 57}.

The data indicate that this type of system can give rise to an unusually low luminosity ($L_x \sim 10^{33}$ – 10^{34} erg s⁻¹) compared with that of other hard (> 2 keV) galactic X-ray sources (10^{35} – 10^{39} erg s⁻¹). The only other known example of such a low luminosity is Algol (ref. 2) with $L_x \sim 1.6 \times 10^{31}$ erg s⁻¹. This radio flaring object^{58, 59} is probably a very different type of system⁶⁰.

In contrast to the Be main-sequence stars, WRA 977 is a supergiant. Its X-ray partner is a slow pulsator with a luminosity which is quite high ($\sim 4 \times 10^{36}$ erg s⁻¹) and variable. This system is similar to the well known pulsating X-ray binary systems, 3U0900–40 and SMC X-1. It is well established that the X-ray emission from these objects arises from accretion on to a rotating magnetised neutron star (see refs 61, 62). Both a stellar wind and overflow of material from a critical potential lobe may be involved^{63, 64}. The extremely hard and variable X-ray spectrum of GX301–2 is unusual even among these sources^{65, 66}.

The X-ray source GX304–1 is similar to GX301–2 in that it has

a hard spectrum, a variable intensity at all energies and is also a slow X-ray pulsar. The tentative classification of its optical partner as B2 to A0 with a broadened H α emission line⁵⁴ suggests, however, that this might be another example of the Be-star systems discussed above.

We thank C. Canizares, J. Delvaille, A. Epstein, W. A. Hiltner, W. Lewin, L. Petro, S. Rappaport, and H. Schnopper for discussions and the staffs of the Center for Space Research at MIT and of Goddard Space Flight Center of NASA for their support. This work was supported in part by the US NASA under contact NAS5-1140. P.C.J. is an Alfred P. Sloan research fellow. This article is the first in a series on positions of galactic X-ray sources obtained with SAS-3.

Received 5 July; accepted 2 August 1977.

- ¹ Doxsey, R. *et al. Astrophys. J. Lett.* **203**, L9-L12 (1976).
- ² Schnopper, H. W. *et al. Astrophys. J. Lett.* **210**, L75-L77 (1976).
- ³ Doxsey, R. E., Apparo, K. M. V., Bradt, H. V., Dower, R. G. & Jernigan, J. G. *Nature* (in the press).
- ⁴ Toor, A. & Seward, F. *Astron. J.* **79**, 995-999 (1974).
- ⁵ Jernigan, J. G. *IAU Circ. No.* 2900 (1976).
- ⁶ Bradt, H. talk presented at the January meeting of High Energy Astrophysics Division of AAS, Cambridge, Massachusetts (1976).
- ⁷ Bradt, H. *IAU Circ. No.* 2957 (1976).
- ⁸ Bradt, H. & Apparo, K. M. V. *IAU Circ. No.* 3054 (1977).
- ⁹ Doxsey, R. *IAU Circ. No.* 2820 (1975).
- ¹⁰ Bradt, H., Doxsey, R., Jernigan, J. G. & Spada, G. *Bull. Am. phys. Soc.* **11-21**, 544 (1976).
- ¹¹ Walter, F. *IAU Circ. No.* 2959 (1976).
- ¹² Jernigan, J. G. *IAU Circ. No.* 2957 (1976).
- ¹³ Jernigan, J. G. *IAU Circ. No.* 2817 (1975).
- ¹⁴ Hoffman, J. A. *et al. Astrophys. J. Lett.* **210**, L13-L17 (1976).
- ¹⁵ Doxsey, R. *IAU Circ. No.* 2983 (1976).
- ¹⁶ Rappaport, S. *et al. Nature* **268**, 704-706 (1977).
- ¹⁷ Schnopper, H. *et al. Astrophys. J.* **161**, L161-L167 (1970).
- ¹⁸ Cruise, A. M. & Willmore, A. P. *Mon. Not. R. astr. Soc.* **170**, 165-175 (1975).
- ¹⁹ Giacconi, R. *et al. Astrophys. J. Suppl.* **27**, 37-64 (1974).
- ²⁰ Hawkins, F. J., Mason, K. O. & Sanford, P. W. *Astrophys. Lett.* **16**, 19-22 (1975).
- ²¹ Markert, T. *et al. Astrophys. J.* (in the press).
- ²² Mason, K. O., White, N. E. & Sanford, P. W. *Nature* **260**, 690-691 (1976).
- ²³ Moffat, A. F. J., Haupt, W. & Schmidt-Kaler, Th. *Astron. Astrophys.* **23**, 433-439 (1973).
- ²⁴ Cowley, A., Rogers, L. & Hutchings, J. *Pub. Astr. Soc. Pac.* **88**, 911-916 (1976).
- ²⁵ Hutchings, J. B. *Mon. Not. R. astr. Soc.* **150**, 55-66 (1970).
- ²⁶ Cooke, B. A. *et al. Mon. Not. R. astr. Soc.* (1977).
- ²⁷ White, N. E., Mason, K. O., Sanford, P. W. & Murdin, P. *Mon. Not. R. astr. Soc.* **176**, 201-215 (1976).
- ²⁸ White, N. E., Mason, K. O. & Sanford, P. W. *Nature* **267**, 229-230 (1977).
- ²⁹ Margon, B., Bowyer, S. & Penegor, G. *Mon. Not. R. astr. Soc.* **176**, 217-223 (1976).
- ³⁰ Braes, L. L. E. & Miley, G. K. *Nature* **235**, 273 (1972).
- ³¹ van den Bergh, S. *Nature* **235**, 273-274 (1972).
- ³² Brucato, R. & Kristian, J. *Astrophys. J. Lett.* **173**, L105-L107 (1972).
- ³³ Penston, M. V., Murdin, P., Penston, M. J., Bingham, R. G. & Sinclair, J. E. *Mon. Not. R. astr. Soc.* **176**, 237-239 (1976).
- ³⁴ Mook, D. E., Boley, F. L., Foltz, C. B. & Westpfahl, D. *Pub. Astr. Soc. Pac.* **86**, 894-898 (1974).
- ³⁵ Hutchings, J. B., Cowley, A. P., Crampton, D. & Redman, R. O. *Astrophys. J. Lett.* **191**, L101-L104 (1974).
- ³⁶ Liller, W. *IAU Circ. No.* 2888 (1975).
- ³⁷ Margon, B. *et al. Astrophys. J.* (in the press).
- ³⁸ Sofia, S. *Astrophys. J. Lett.* **188**, L45-L46 (1974).
- ³⁹ Jones, C. A., Chetin, T. & Liller, W. *Astrophys. J. Lett.* **190**, L1-L3 (1974).
- ⁴⁰ Maraschi, L., Treves, A. & van den Heuvel, E. P. J. *Nature* **259**, 292-293 (1976).
- ⁴¹ Feast, M. W., Stoy, R. H., Thackeray, A. D. & Wesselink, A. J. *Mon. Not. R. astr. Soc.* **122**, 239-253 (1961).
- ⁴² Lewin, W. H. G., McClintock, J. E., Ryckman, S. G. & Smith, W. B. *Astrophys. J. Lett.* **166**, L69-L72 (1971).
- ⁴³ McClintock, J. E., Ricker, G. R. & Lewin, W. H. G. *Astrophys. J. Lett.* **166**, L73-L76 (1971).
- ⁴⁴ Ricker, G. R., McClintock, J. E., Gerassimenko, M. & Lewin, W. H. G. *Astrophys. J.* **184**, 237-243 (1973).
- ⁴⁵ White, N. E. *et al. IAU Circ. No.* 2870 (1975).
- ⁴⁶ Swank, J. H. *Astrophys. J. Lett.* **209**, L57-L60 (1976).
- ⁴⁷ Vidal, N. V. *Astrophys. J. Lett.* **186**, L81-L83 (1973).
- ⁴⁸ Hammerslag-Hensberge, G., Zuiderwijk, E. J. & van den Heuvel, E. P. J. *Astr. Astrophys.* **99**, 321-323 (1976).
- ⁴⁹ Mauder, H. *IAU Circ. No.* 2946 (1976).
- ⁵⁰ Lewin, W. H. G., Clark, G. W. & Smith, W. B. *Astrophys. J. Lett.* **152**, L49-L53 (1968).
- ⁵¹ Lewin, W. H. G., Clark, G. W. & Smith, W. B. *Nature* **219**, 1235-1236 (1968).
- ⁵² McClintock, J. E., Rappaport, S. A., Nugent, J. N. & Li, F. K. *Astrophys. J. Lett.* **216**, L15-L18 (1977).
- ⁵³ Huckle, H. E., Mason, K. O., White, N. E. & Sanford, P. W., submitted to *Mon. Not. R. astr. Soc.* (1977).
- ⁵⁴ Mason, K. O., Murdin, P. G. & Visvanathan, N. *IAU Circ. No.* 3054 (1977).
- ⁵⁵ Fabian, A., Pringle, J. & Rees, M. *Mon. Not. R. astr. Soc.* **175**, 43-60 (1975).
- ⁵⁶ Katz, J. I. *Astrophys. J.* **215**, 265-275 (1977).
- ⁵⁷ Milgrom, M. *Astr. Astrophys.* **53**, 321-323 (1976).
- ⁵⁸ Wade, C. M. & Hjellming, R. M. *Nature* **235**, 270-271 (1972).
- ⁵⁹ Hjellming, R. M., Wade, C. M. & Webster, E. *Nature phys. Sci.* **236**, 43-46 (1972).
- ⁶⁰ Apparo, K. M. V. *Nature* **266**, 430-431 (1977).
- ⁶¹ Rappaport, S. & Joss, P. C. *Nature* **266**, 683-685 (1977).
- ⁶² Mason, K. O. *Mon. Not. R. astr. Soc.* **178**, 81-85 (1977).
- ⁶³ Basko, M., Hatchett, S., McCray, R. & Sunyaev, R. A. *Astrophys. J.* (in the press).
- ⁶⁴ Conti, P. S. *Astr. Astrophys.* (in the press).
- ⁶⁵ Rappaport, S. *et al.*, submitted to *Astrophys. J. Lett.*
- ⁶⁶ Jones, C. *Astrophys. J.* **214**, 856-873 (1977).

Generation of Icelandic rhyolites by melting of plagiogranites in the oceanic layer

Haraldur Sigurdsson

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island 02881

Trondhjemite and quartz diorite xenoliths ejected in recent Icelandic eruptions are believed to be derived from plagiogranites within layer 3, formed by fractional crystallisation of basaltic magmas in the lower crust. Evidence indicates that rift jumping to older crust can lead to partial melting of plagiogranites at depth and their eruption as rhyolitic magmas.

THE volume of rhyolites exposed within the lava pile in Iceland is disproportionately large (10%), with individual rhyolite complexes covering areas up to 450 km² (refs 1, 2). The pronounced bimodal compositional distribution of basic and acid extrusives (Fig. 1) suggests, furthermore, that a process of shallow-level fractional crystallisation in subvolcanic magma chambers cannot alone account for the origin of these acid magmas. The ⁸⁷Sr/⁸⁶Sr and Pb-isotopic evidence³⁻⁵, however, precludes the possibility of generation of the Icelandic rhyolites by fusion of old continental crustal material at depth. An alternative model has recently been proposed⁶⁻¹⁰ to account for the origin of the Icelandic rhyolites by hydrous partial melting of basalts within the crust. This hypothesis is inspired by the experimental work of Kushiro^{11,12} on the synthetic system Di-Fo-SiO₂-H₂O at 20 kbar, where a silica-rich liquid was generated at 960 °C. There are three principal obstacles to deriving Icelandic rhyolites by hydrous anatexis of basalts within the crust.

(1) The process dictates that the resulting acid melts be H₂O saturated, contrary to the evidence of Icelandic rhyolites which are often extruded as dry obsidian lavas or solidify at shallow levels as pyroxene-bearing granophyres, lacking hydrous phases. Many rhyolitic eruptions in Iceland are, of course, violently explosive events, clearly associated with a water-rich vapour phase. The oxygen isotope evidence is consistent with incorporation of some low-¹⁸O meteoric water into these magmas. Oxygen-isotope geochemistry of the acid rocks^{10,13} shows that the tephra products of acid explosive eruptions have $\delta^{18}\text{O}$ in the 0.4-2.5‰ range, whereas obsidian and rhyolite lavas have $\delta^{18}\text{O}$ values of 4-6 ‰. Thus the data are in agreement with the idea that the water-propellant of acid explosive eruptions in Iceland is largely meteoric water which has diffused into acid magma in subvolcanic magma chambers¹⁴.

(2) Anatexis of basalt in the presence of H₂O at pressures comparable with the lower Icelandic crust (5 kbar) generates andesitic and dacitic melts analogous to the calc-alkaline suite of island-arcs¹⁵ and unlike Icelandic rhyolites (Fig. 2).

(3) No field evidence has been presented so far for the existence of amphibolites or other suitable metabasite starting material under Iceland, nor have xenoliths of possible refractory phases of this process been erupted.

An alternative model proposed here for the origin of Icelandic rhyolites is the melting of plagiogranites within the oceanic layer. By analogy with ophiolite complexes¹⁷⁻²⁴

and petrological models of the oceanic crust^{20,21,25}, it is proposed that layer 3 under Iceland is composed of layered (cumulate) mafic rocks at the base, overlain by gabbros which grade upwards into leucocratic differentiates: trondjemites and quartz diorites. This model of the oceanic layer under Iceland is supported by the ejection of plagiogranite xenoliths in several recent eruptions and the occurrence of plagiogranites in ophiolite sequences. The model is also consistent with the geochemical evidence, such as the bimodal compositional distribution of Icelandic volcanics and the dry character of the rhyolites.

Icelandic plagiogranite xenoliths

Fragments of leucocratic rocks have been ejected in a number of recent eruptions in Iceland, notably the 1963–67 eruption of Surtsey and the 1875 plinian eruption of Askja. Most finds are in basaltic scoria cones in the eastern and Snaefellsnes zones, that is, outside the western volcanic zone²⁶. Most Surtsey xenoliths have undergone extensive partial fusion and assimilation of the acid liquid into the basaltic melt, leaving a refractory residue of anorthitic plagioclase, tridymite and more rarely cordierite and mulite. Study of leucocratic xenoliths from Surtsey has shown

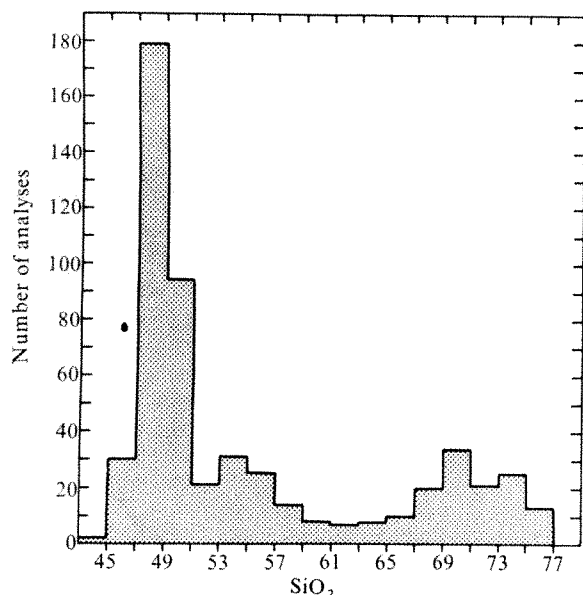


Fig. 1 Bimodal distribution of SiO_2 in 542 chemical analyses of volcanic rocks from Iceland.

that a complete gradation exists between anorthite-tridymite refractory assemblages and xenoliths of trondjemite and quartz diorite relatively unaffected by fusion²⁶. The texture of Icelandic plagiogranites is distinct from that of granophyres in Tertiary and Quaternary intrusive complexes exposed in eastern and western Iceland. The majority of the plagiogranite xenoliths contain 55–85% plagioclase, 10–35% quartz, minor clinopyroxene, K-feldspar, accessory zircon and variable amounts of intergranular pale brown to colourless glass. Plagioclase ranges from An_{26} to An_{44} and typical plagioclase composition in Surtsey xenoliths is $\text{An}_{35}\text{Ab}_{64}\text{Or}_1$. Plagioclase in Askja xenoliths is comparable but more potassic, with average composition of $\text{An}_{43}\text{Ab}_{55}\text{Or}_2$. Rounded grains of magnetite and augite ($\text{Wo}_{42}\text{En}_{34}\text{Fs}_{24}$) are the sole remnants of ferromagnesian minerals in the Surtsey xenoliths. Xenoliths from Snaefellsnes, and rare fragments from Surtsey, are granodiorites, with up to 25% K-feldspar in the mode, as reflected in their higher K_2O (ref. 28). The Icelandic

plagiogranite xenoliths are in varying stages of fusion, ranging from trondjemites with minor intergranular glassy films, to white frothy blocks consisting largely of very vesicular glass with minor relict quartz and plagioclase. Microprobe analyses of intergranular glasses in xenoliths ejected in the Surtsey 1963 eruption and Askja 1875 eruption (Table 1) show the general similarity between these partial melts and Icelandic rhyolites. The similarity in composition of partial melt in the 1875 Askja plagiogranite xenoliths and the rhyolitic tephra which brought these fragments to the surface (Table 1) is particularly persuasive evidence of an origin of the Askja acid magma by fusion of plagiogranites at depth.

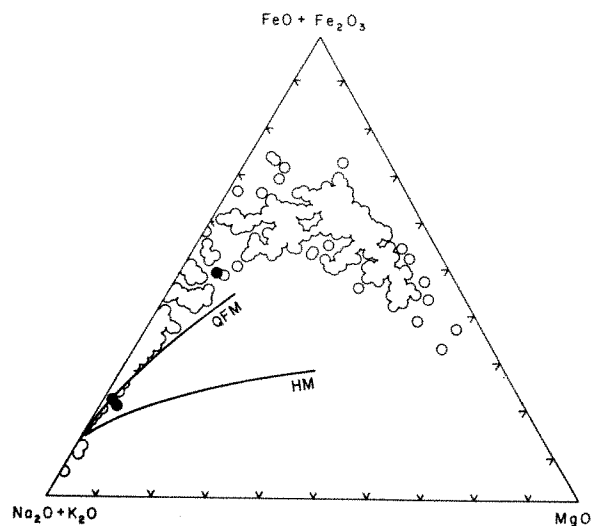


Fig. 2 Chemical variation in Icelandic volcanic rocks. Solid lines are the compositional trends of partial melts generated by anatexis of Hawaiian basalt at 5 kbar water pressure at the QFM and HM buffers¹⁶. Solid symbols mark the compositions of partial melts in plagiogranite xenoliths from Iceland (Table 1).

In Table 1 the composition of Icelandic plagiogranite xenoliths is compared with diorites from the Mid-Atlantic Ridge²⁷ and plagiogranites from ophiolite complexes¹⁹. It was suggested above that plagiogranites were formed during fractional crystallisation in magma chambers within the oceanic layer under Iceland. Direct evidence in support of this hypothesis is lacking, but it is, however, consistent with the high geothermal gradient²⁸, the ejection of cumulate gabbro nodules in Iceland^{20–31}, the geophysical structure of the lower crust, and the evidence from ophiolite complexes.

Plagiogranites in ophiolites

Most workers agree that ophiolites represent fragments of ancient lithosphere and thus provide important clues to the understanding of younger crust at modern spreading centres. Plagiogranites containing predominant zoned sodic plagioclase and quartz, with minor ferromagnesian minerals and rare K-feldspar, are a subordinate but persistent component of ophiolite sequences, occurring as discrete intrusive bodies of diffuse facies in upper part of layer 3 (refs 17, 18, 19, 22, 24, 25, 32). In the Vourinos ophiolite complex, for example, layered gabbros of the mafic zone grade upwards by increase in hornblende and quartz and decrease in pyroxenes into a 1 km thick massive layer of quartz diorite and hornblende diorite, making up over 6% of the complex¹⁹. Similar plagiogranites and other leucocratic rocks have also been dredged from the Mid-Atlantic Ridge^{27,33,34}, most notably 96 km west of the

Table 1 Composition of Icelandic plagiogranite xenoliths and their partial melts, and plagiogranites from ophiolites and the Mid-Atlantic Ridge

	1	2	3	4	5	6	7	8	9	10	11
SiO ₂	61.60	61.97	72.38	69.94	72.47	73.70	72.45	76.77	76.87	73.63	73.5
TiO ₂	0.22	0.94	0.45	0.25	0.33	0.38	0.40	0.52	0.42	0.81	0.31
Al ₂ O ₃	23.81	16.00	13.33	16.91	14.17	13.94	12.58	11.81	11.89	12.33	13.3
Fe ₂ O ₃	0.38	3.22	3.00	1.26	1.85						1.44
FeO	2.20	3.57	1.85	2.64	1.19	1.72*	4.88*	1.72*	1.62*	3.27*	1.81
MnO	0.02	0.09	0.04	0.09	0.08	0.03	0.01	0.03	0.01	0.01	0.08
MgO	0.25	2.43	0.38	0.94	1.39	1.18	0.73	0.51	0.35	0.66	0.33
CaO	6.00	3.24	2.95	2.46	1.48	2.75	3.42	0.91	0.60	2.33	1.64
Na ₂ O	5.90	5.55	3.77	3.14	5.55	4.30	4.70	3.90	3.90	4.18	4.46
K ₂ O	1.12	0.75	1.37	0.95	0.24	1.40	0.49	2.90	3.60	2.46	3.03
H ₂ O	0.46	1.28	0.30	1.20	0.90	0.70					
P ₂ O ₅	0.02	0.22	0.05	0.07	0.06	0.04	0.19	0.20		0.12	0.11
	99.98	99.26	99.87	99.85	99.71	100.14	99.85	99.27	99.25	99.80	100.01

1, Trondhjemite xenolith (1166) from Surtsey 1963 eruption²⁶. 2, Diorite dredged from Mid-Atlantic Ridge at 45°N (ref. 27). 3, Trondhjemite xenolith (1165) from Surtsey 1963 eruption²⁶. 4, Quartz diorite from the Vourinos ophiolite complex, Greece¹⁹. 5, Diorite dredged from Mid-Atlantic Ridge at 45°N (ref. 27). 6, Highly fused frothy xenolith from Surtsey 1963 eruption²⁶. 7, Partial melt of trondhjemite xenolith (1352) from Surtsey 1963 eruption. 8, Partial melt of quartz diorite xenolith (AS-26/1) from Askja 1875 eruption. 9, Partial melt of quartz diorite xenolith (AS-15) from 1875 Askja eruption. 10, Glass composition of rhyolitic tephra from the 1875 Askja eruption⁶⁰. 11, Average composition of 58 Icelandic acid rocks⁵¹. Values given are percentages.

*All iron reported as FeO.

ridge axis at 45°N. These quartz diorites and hornblende diorites are closely comparable with plagiogranites from ophiolites, both in chemistry and petrography (Table 1). The occurrence of plagiogranites in ophiolites and oceanic crust is best accounted for by a process of crystal fractionation of tholeiitic melts, operating in magma chambers within the oceanic layer^{18,19,27}. This concept is supported by the similarity in ⁸⁷Sr/⁸⁶Sr values of plagiogranites and associated gabbros^{32,33}. The negative Eu anomalies observed in plagiogranite REE patterns further show that plagioclase extraction has an important role in the differentiation process^{24,36}. The occurrence of plagioclase, olivine and clinopyroxene-cumulates or layered gabbros at the base of the mafic zone in ophiolite sequences is further evidence of crystal fractionation within layer 3.

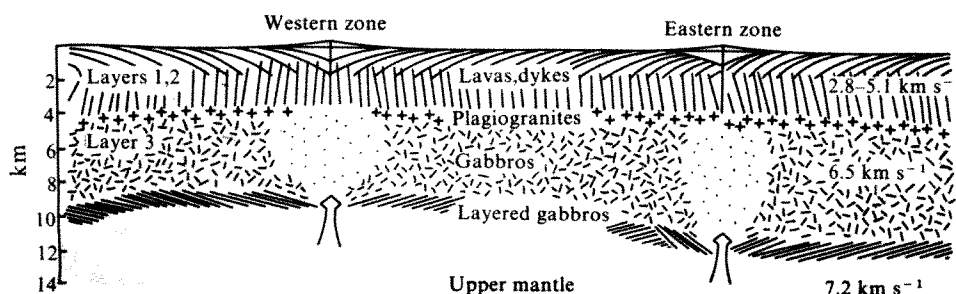
Petrological model of the Icelandic crust

The layered structure of the Icelandic crust is broadly similar to that of the ocean floors, but differs in two important details. The Icelandic crust varies from 8 to 15 km—approximately two to three times the thickness of normal oceanic crust³⁷. Much of this thickening is due to layer 2 and has been attributed to higher rate of lava production from the Icelandic mantle plume than on normal ocean ridges^{38,39}, combined with relatively slow spreading rate. Layer 3 under Iceland is also thicker than in normal oceanic crust, and differs significantly in having lower average P-wave velocity (6.5 km s⁻¹) than the oceanic layer (6.73 km s⁻¹)³⁷. This lower velocity can only partly be explained by higher temperatures and can also be attributed to higher proportion of differentiated rocks. The inverse relationship between thickness of the oceanic

layer and spreading rate²⁰ has been attributed to more efficient diffusion of interstitial melts in cumulates forming at the base of the crust, resulting in more extensive differentiation and secondary enlargement of the oceanic layer in slow-spreading regions such as Iceland³². Thus the differences in crustal structure between Iceland and normal ocean ridges may be a matter of degree rather than differences in character.

The proposed petrological model of the Icelandic crust is shown in Fig. 3. Layers 1 and 2 (2.8–5.1 km s⁻¹) are equated mainly with downwarped basalt lavas, dipping towards the long-lived western volcanic zone, grading into a lava-dyke complex with depth^{25,40}. Layer 3 is equated with gabbros, associated cumulate layered gabbros and leucocratic differentiates, along the lines suggested by Cann²⁵ for the structure of the oceanic layer. This part of the crust is envisaged as forming from crystallisation of basaltic magma in magma chambers within layer 3, principally under the rift zone flanks, where crystal settling in lateral parts of the magma chamber will give rise to layered gabbros at the base of the crust. During episodes of rifting and magma injection into the crust, such as the current episode in the north-eastern volcanic zone⁴¹, fractionation of the basaltic magma will be limited in extent. Between episodes of magma input, however, differentiation will proceed by crystal settling of calcic plagioclase and minor clinopyroxene and olivine in stagnant cooling lateral zones, distant from the axial zone of magma injection, resulting in the formation of differentiates solidifying as quartz-diorites, diorites and trondhjemites above gabbros in layer 3. Differentiates are unlikely to form in the active zone of basaltic magma injection immediately

Fig. 3 Diagrammatic model of the crust in southern Iceland, based on the seismic structure³⁷, geological evidence of the lava pile⁴⁰ and modifications of the oceanic crust model of Cann²⁵. The younger eastern rift zone has transected older crust, resulting locally in generation of rhyolite magmas from the partial melting of plagiogranites within layer 3 by ascending basaltic magmas.



under a rift, and are envisaged as forming rather within the upper part of layer 3, some kilometres away from the axial zone.

Generation of rhyolite magma

In Iceland the distribution of rhyolites is closely associated with central volcanoes, where they make up to 20% of the exposed volcanics in many cases⁸. As pointed out by Sigvaldason⁷, the great majority of rhyolite-producing central volcanoes of Late Quaternary to Recent age occur in volcanic zones which have dissected and are underlain by older crust, such as the eastern rift zone and the Snaefellsnes volcanic zone. Rhyolites are, on the other hand, altogether absent from the rift zone of Reykjanes peninsula¹², a region underlain by young crust. The distribution of plagiogranite xenoliths follows a similar pattern. They are most numerous in the Snaefellsnes zone, where recent volcanism has penetrated 5–13-Myr-old crust¹³, and in the eastern zone, from Surtsey to Askja. Saemundsson¹⁴ has inferred that the northern part of the eastern zone was established in its present position 4 Myr ago, following rift-jumping from the west, on to 8-Myr-old crust in the east.

It is proposed that the majority of rhyolites erupted in the Quaternary to Recent volcanic zones in Iceland are generated by melting of plagiogranite within layer 3 during episodes of injection of basaltic magma into a rift zone recently established within older crust. The availability of plagiogranites for this melting process is, however, limited to regions of older crust. Rhyolitic volcanism of this type is then restricted to regions of rift jumping, where a younger rifting episode transects older crust containing a differentiated gabbroic sequence with overlying plagiogranites. The structural and time relationships of individual rhyolitic centres are rarely known in sufficient detail to test this model. The well-studied Hallarmuli central volcano in western Iceland is a good case in point, however. Johannesson⁸ has shown that the earliest volcanics of this centre were acid ignimbrites erupted 6 Myr ago, on a 12.5-Myr basaltic crust. The model is also consistent with available geochemical evidence, such as measurable differences in ⁸⁷Sr/⁸⁶Sr ratio between rhyolites and field-associated basalts in the Torfajökull centre⁵. O'Nions and Gronvold⁵ pointed out that the present-day ⁸⁷Sr/⁸⁶Sr ratio of the rhyolites could be accounted for by postulating a 16-Myr-old source with initial Rb/Sr ratio of approximately 0.8. The observed range in Rb/Sr ratios of Icelandic plagiogranite xenoliths varies from 0.04 to 2.1 (ref. 26) and demonstrates the availability of such a source within older regions of the Icelandic crust. The large negative Eu anomaly in rare earth element (REE) patterns of Icelandic rhyolites⁵ is also comparable with the Eu anomaly in plagiogranites from ophiolite complexes^{24,32,36}, but the meagre data indicate lower overall REE abundances in the latter.

Implications of the proposed two-stage model for the origin of Icelandic rhyolites and petrological structure of the Icelandic crust are diverse. It is emphasised that the high volume of rhyolites erupted at the surface is not anomalous when considered in the framework of fractional crystallisation within layer 3. Furthermore, it is likely that the volume of rhyolites exposed at the surface in Iceland is not representative of their total volume in the crust as a whole, due to the possibility that the less dense rhyolitic melts will be preferentially concentrated at higher levels in the crust⁴⁵. If it is assumed, for purpose of illustration, that layers 1 and 2 are 4 km and layer 3 is 8 km thick, and that 10% of layers 1 and 2 are rhyolite, then formation of a salic differentiate residuum amounting to 5% of layer 3 would be required to form a plagiogranite source for the rhyolites. An important corollary of this model is that the basalt magmas which appear at the surface will in many cases have been modified by extensive fractional crystallisation², as the generation of leucocratic rocks within layer 3 is envisaged as a result of

differentiation of primary basaltic magma rising into the Icelandic crust. Also, it is likely that the heat required for partial melting of plagiogranite within older crustal regions is provided by extensive crystallisation of basaltic magmas in proximity with the leucocratic rocks. Thus basaltic lavas, associated with rhyolites which have originated in this way, are likely to be fractionation products of more primitive melts.

The mechanism envisaged for generation of Icelandic rhyolites is likely to be equally applicable to other oceanic hot-spots where rift-jumping has occurred. An appropriate example is the Easter Island region, where eastward jump of the Nazca-Pacific plate boundary has been documented⁴⁶. Taken together with the structural evidence, the marked chemical discontinuity between Easter Island rhyolites and associated basalts and the differences in ⁸⁷Sr/⁸⁶Sr ratio⁴⁷ suggest that Easter Island rhyolites may also owe their origin to fusion of plagiogranites within layer 3, as a consequence of rift-jumping. Similarly in the Azores region a marked bimodal compositional distribution exists between basalts and contemporaneous salic peralkaline lavas⁴⁸ where komendites have significantly higher ⁸⁷Sr/⁸⁶Sr ratios than associated basalts⁴⁹. Volcanism in the Azores islands is related to easterly trending fracture zones transecting older crust, generated at the Mid-Atlantic Ridge to the west. Thus the structural framework and geochemical evidence are also consistent with generation of the salic rocks in the Azores by remelting of plagiogranites at depth. Salic peralkaline lavas are volumetrically insignificant but widespread amongst oceanic islands. If their genesis can be interpreted in terms of partial fusion of plagiogranite within the oceanic layer, then is the cordierite–mullite refractory assemblage in the Icelandic xenoliths providing a clue to the origin of the peralkaline condition?

I thank R. S. J. Sparks for discussion. This research was supported by the US NSF.

Received 26 May; accepted 5 July 1977.

- 1 Saemundsson, K. *Naturfræðingurinn* **42**, 81 (1972).
- 2 Walker, G. P. L. in *Geodynamics of Iceland and the North Atlantic Area* (ed. Kristjánsson, L.) 189 (Reidel, Amsterdam, 1974).
- 3 Moorbath, S. & Walker, G. P. L. *Nature* **207**, 837 (1965).
- 4 Welke, H. et al. *Earth planet. Sci. Lett.* **4**, 221 (1968).
- 5 O'Nions, R. K. & Gronvold, K. *Earth planet. Sci. Lett.* **19**, 397 (1973).
- 6 Gronvold, K. thesis, Univ. Oxford (1972).
- 7 Sigvaldason, G. E. *The Petrology of Hekla and Origin of Silicic Rocks in Iceland*. Hekla Series V (Soc. Sci. Islandica, 1974).
- 8 Johannesson, H. thesis, Univ. Durham (1975).
- 9 Gibson, I. L. *Lithos* **2**, 343 (1969).
- 10 Muehlenbachs, K. *Yb. Carnegie Instn Wash.* **72**, 593 (1973).
- 11 Kushiro, I. *Am. J. Sci.* **267A**, 269 (1969).
- 12 Kushiro, I. *J. Petrol.* **13**, 311 (1972).
- 13 Muehlenbachs, K. et al. *Geochim. cosmochim. Acta* **38**, 577 (1974).
- 14 Kennedy, G. C. *Spec. Pap. Geol. Soc. Am.* **62** (1955).
- 15 Holloway, J. R. & Burnham, C. W. *J. Petrol.* **13**, 1 (1972).
- 16 Helz, R. T. *J. Petrol.* **17**, 139 (1976).
- 17 Dewey, J. F. & Bird, H. M. *J. geophys. Res.* **76**, 3179 (1971).
- 18 Coleman, R. G. *J. geophys. Res.* **76**, 1212 (1971).
- 19 Moores, E. M. *Spec. Pap. Geol. Soc. Am.* **118** (1969).
- 20 Moores, E. M. & Jackson, E. D. *Nature* **250**, 136 (1974).
- 21 Christensen, N. I. & Salisbury, M. H. *Rev. Geophys. Space Phys.* **13**, 57 (1975).
- 22 Moores, E. M. & Vine, F. J. *Phil. Trans. R. Soc. A268*, 443 (1971).
- 23 Vine, F. J. & Moores, E. M. *Geol. Soc. Am. Mem.* **132**, (1972).
- 24 Allegre, C. J. et al. *Bull. Soc. Geol. France* **7**, 461 (1973).
- 25 Cann, J. R. *Geophys. J. R. astr. Soc.* **39**, 169 (1974).
- 26 Sigurdsson, H. *Geol. Mag.* **105**, 440 (1968).
- 27 Aumento, F. *Science* **165**, 1112 (1969).
- 28 Hernandez, J. F. & Grillo, L. R. *Phys. Earth planet. Int.* **8**, 1 (1974).
- 29 Tryggvason, T. *Bull. Geol. Inst. Univ. Uppsala* **38**, 1 (1957).
- 30 Jonsson, J. *Naturfræðingurinn* **33**, 9 (1963).
- 31 Jakobsson, S. *Acta Naturalia Islandica* **2** (1966).
- 32 Coleman, R. G. & Peterman, Z. E. *J. geophys. Res.* **80**, 1099 (1975).
- 33 Aumento, F. et al. *Phil. Trans. R. Soc. A268*, 623 (1971).
- 34 Miyashiro, A. et al. *Earth planet. Sci. Lett.* **7**, 361 (1970).
- 35 Peterman, Z. E. et al. *U.S. Geol. Surv. Prof. Pap.* **750-D**, D157 (1971).
- 36 Kay, R. W. & Senechal, R. G. *J. geophys. Res.* **81**, 964 (1976).
- 37 Palmason, G. *Rit* **40** (Soc. Sci. Islandica) (1971).
- 38 Schilling, J.-G. *Nature* **242**, 565 (1973).
- 39 Vogt, P. R. in *Geodynamics of Iceland and the North Atlantic Area* (ed. Kristjánsson, L.) 105 (Reidel, Amsterdam, 1974).
- 40 Bodvarsson, G. & Walker, G. P. L. *Geophys. J. R. astr. Soc.* **8**, 285 (1964).
- 41 Björnsson, A. et al. *Nature* **266**, 318 (1977).
- 42 Palmason, G. & Saemundsson, K. *Am. Rev. Earth planet. Sci. Lett.* **2**, 24 (1974).
- 43 Moorbath, S. et al. *Earth planet. Sci. Lett.* **4**, 197 (1968).
- 44 Saemundsson, K. *Geol. Soc. Am. Bull.* **85**, 495 (1974).
- 45 Sigurdsson, H. *Greinar* **4** (Soc. Sci. Islandica) 53 (1966).
- 46 Handschumacher, D. et al. *Geol. Soc. Am. Abstr.* **8**, 901 (1976).
- 47 Baker, P. E. et al. *Contrib. Mineral. Petrol.* **44**, 85 (1974).
- 48 Self, S. & Gum, B. M. *Contrib. Mineral. Petrol.* **54**, 293 (1976).
- 49 White, W. M. thesis, Univ. Rhode Island (1977).
- 50 Sparks, R. S. J., Sigurdsson, H. & Wilson, L. *Nature* **267**, 315–318 (1977).
- 51 Walker, G. P. L. *Bull. Volc.* **29**, 375 (1966).

Structure of nucleosome core particles of chromatin

J. T. Finch, L. C. Lutter, D. Rhodes, R. S. Brown, B. Rushton, M. Levitt & A. Klug

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

Crystals have been obtained of nucleosome cores and analysed by X-ray diffraction and electron microscopy. The core is a flat particle of dimensions about $110 \times 110 \times 57$ Å, somewhat wedge shaped, and strongly divided into two 'layers', consistent with the DNA being wound into about $1\frac{1}{2}$ turns of a flat super-helix of a pitch about 28 Å. The organisation of the DNA can be correlated with the results of enzyme digestion studies. A change in the screw of the DNA double helix on nucleosome formation can be deduced.

THERE is a good deal of evidence that, as proposed by Kornberg¹, chromatin consists of repeating units, termed nucleosomes, containing a fairly well defined length of DNA² (200 base pairs in rat liver³) associated with an octamer aggregate of the histones containing pairs of each of the four main types (for review see ref. 4). The fifth histone, H1, is also associated with the nucleosome, but in a manner which is not clear. Enzyme digestion studies on nuclei, using micrococcal nuclease, have shown that while the DNA content of nucleosomes is often about 200 base pairs, quite large variations are found according to the species or tissue investigated. But it has been found that further nuclease digestion produces a 'core' particle⁵⁻⁹ containing the same number, ~140 of base pairs in all cell types so far investigated. The rest of the DNA in the repeat length not included in the core is thought of as a linker between core particles. We report here the crystallisation of nucleosome core particles, and X-ray and electron microscopic studies on the crystals which have led to a low-resolution map of the structure. By combining this result with data from enzyme digestion studies on the DNA in nucleosomes and general considerations on the bending of DNA, we can obtain a picture of the organisation of DNA in a nucleosome core and a rationale for this organisation.

The work began following the discovery in this laboratory by Noll and Kornberg in 1974-75 that, on more prolonged digestion with micrococcal nuclease, the repeating unit of rat liver chromatin is broken down progressively from 200 base pairs of DNA. They found a pause in the digestion at the 160-base-pair stage and a longer one at 140 base pairs, between which stages histone H1 is released⁹. Early experiments on crystallising particles at the 160-140-base-pair stage were not successful, probably due to the presence of residual H1. It was not until the development of a method for the preparation of homogeneous nucleosome cores on a large scale for the purpose of biochemical studies (L.C.L., in preparation) that crystals could be regularly and reproducibly grown. Crystallisation of nucleosome cores has been reported previously⁷ but no analysis has been made.

Intact nucleosome cores have been crystallised and studied but we found that the best crystals so far obtained, which diffract to spacings of about 20 Å, come from particles which have had some of their histones cleaved by proteolysis. For the reasons given later we think that the core particles have not in consequence suffered

any significant change in structure, and that the results obtained are generally valid. The unit cell is large and contains three particles in the asymmetric unit, which then has a molecular weight of about 600,000, so that the solution of the structure by X-ray methods is a formidable task. For this reason the X-ray analysis has been carried out in conjunction with electron microscope studies on the crystals, and related aggregates, using methods of image reconstruction developed in this laboratory¹⁰. We have solved the three centro-symmetric projections of the crystal structure, and because one of them is down an axis looking through the width of only one particle, a fairly clear picture of the overall structure of the nucleosome core has emerged.

Preparation and characterisation of nucleosome cores

A detailed description of the preparation will be given elsewhere (L.C.L., in preparation). Essentially it involves preparation of nuclei from rat liver² and production of long chromatin from these nuclei by digestion with micrococcal nuclease¹¹. The long chromatin is then passed over a Sepharose 4B column equilibrated with 0.45 M NaCl to remove histone H1 as well as non-histone proteins, redigested with micrococcal nuclease and chromatographed on Sepharose 4B to produce a preparation of monomer core particles. The DNA of these cores is nominally 140 base pairs in length⁹. Polyacrylamide gel electrophoresis of the DNA from this preparation shows a single band, as seen in Fig. 1. When compared with the DNA from a DNase I digestion of chromatin, which contains a series of bands which differ by 10 bases¹², it can be seen that the monomer DNA has length distribution similar to that of a single DNase I-produced band; that is, less than ± 3 bases. The monomer preparation contains no histone H1 and usually contains normal amounts of histones H2A, H2B, H3 and H4 (Fig. 2).

These intact nucleosome cores almost invariably gave microcrystals as described below. But, the largest single crystals (Fig. 3) were obtained on occasions when the histones in the core particles showed extensive proteolysis (Fig. 2c, d). In most of these preparations the H3 and H2B bands have almost completely disappeared and new low molecular products are present. When this proteolysis occurred an endpoint seemed to be reached, as judged by the gel patterns. We have not investigated the nature of the products, but we believe that these partially proteolysed nucleosome cores are not much changed in their gross structure from the intact ones. First, the sedimentation constant of the proteolysed particle in 100 mM NaCl is hardly changed from the intact material (10.3S against 10.5S: we thank Dr P. J. G. Butler for these measurements). Second, brief DNase I digestion shows a pattern of bands every 10 bases (Fig. 1) which is very similar in its intensity distribution to that from intact particles, except that the rate of digestion of the proteolysed particles is higher. Moreover the relative frequencies of cutting at the individual sites, as determined by end-labelling experiments (see below), remain very similar for the proteolysed particles. Third, the crystal structures of the

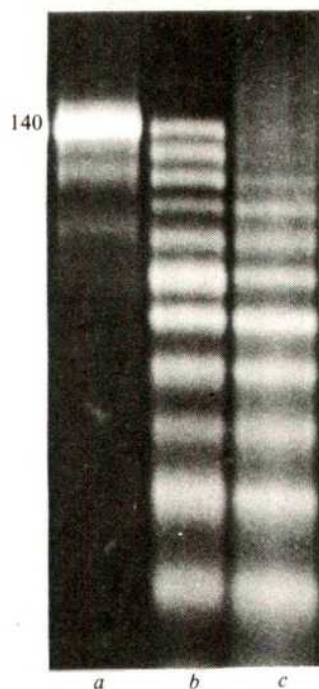


Fig. 1 Polyacrylamide gel analysis of DNA in single stranded form from *a*, 140-base-pair nucleosome cores which gave the large crystals used for X-ray work (and in which some of the histones had been proteolytically cleaved, Fig. 2c); *b*, preparation (*a*) digested with DNase I; *c*, rat liver nuclei digested with DNase I. The DNase I digestion and gel electrophoresis were carried out as described in refs 3 and 9.

two are closely related. Furthermore, various preparations showing somewhat different patterns of proteolysis (for example, Fig. 2c, *d*) crystallised in identical forms, but with occasional differences in habit. For these reasons it is possible that the smaller fragments of the proteolysed histones (~1,000 to 3,000 molecular weight) which can be seen on the gel patterns are still present in the particles which have crystallised. If so, then the histone molecules on the core particle may not be much changed in tertiary structure, although some covalent linkages have been cleaved. This question is under investigation.

Crystallisation methods

The general methods used were similar to those described for crystallisation of various transfer RNAs in this laboratory^{13,14}. The 'monomer' peak from the Sepharose column was concentrated to about 3–6 mg ml⁻¹, and this solution was dialysed against a buffer containing 10mM Tris-HCl, pH 7.4, 0.2 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride and 0.3 mM azide. Crystals of the nucleosome cores have been obtained at both 4 °C and room temperature using a variety of components in the crystallisation mixture, but not enough experiments have been done so far to provide a phase diagram as complete as that for tRNA (ref. 14). The final concentration of the nucleosomes in the crystallisation mixture ranged from 0.5 to 2 mg ml⁻¹. Microcrystals and small single crystals were grown in the presence of 0.4–0.6 mM spermine tetrahydrochloride, or 3–4 mM MgCl₂ plus 0.2 mM spermine or else at 10 mM MgCl₂, 1.5 mM spermine and NaCl up to concentrations of 100 mM in 10% (w/v) hexan-1,6-diol. Small crystals were also grown in the presence of polyethyleneglycol 6000 (5–10%, w/v) which had the effect of reducing somewhat the concentration of Mg²⁺ and spermine required.

The crystals were grown either in drops in glass cavity slides, equilibrated against the organic solvents or in small



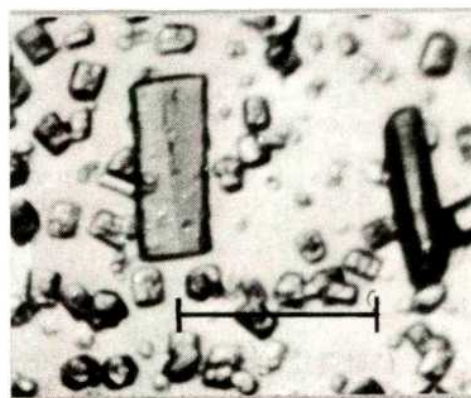
Fig. 2 Histone composition of *a*, rat liver nuclei; *b*, crystals of nucleosome cores with intact histones; *c* and *d*, crystals of two preparations of proteolytically cleaved nucleosome cores. The sodium dodecyl sulphate-polyacrylamide gel analyses were carried out as in ref. 42.

stopped glass tubes containing the appropriate mixtures. In both the vapour diffusion and direct addition methods, crystallisation occurred in 1–4 d.

Crystal packing

No large single crystals of intact material have yet been grown, but in the conditions described above microcrystals were obtained. X-ray diffraction 'powder' diagrams from these show three clear rings of spacings at 96, 55 and 37 Å and a fainter ring at about 29 Å. The first three spacings

Fig. 3 Crystals of nucleosome cores. The two larger ones are typical of those used for the single crystal X-ray diffraction work. Scale bar, 0.2 mm.



index on a two-dimensional hexagonal cell of side 110 Å, and correlate well with electron microscopic observations on the crystals which show extensive areas of a hexagonal arrangement of particles with this same cell side (Fig. 4). Such close packing is natural for particles with a circular shape. Although the micrograph showed these crystals to be several layers deep with the particles in register, so that the nucleosomes formed columns hexagonally packed, no obvious extra lines were found in the powder diagrams to indicate the inter-layer spacing. In view of the results described below, however, it seems likely that the inter-layer spacing is 57 Å—and so unresolved from the 55 Å ring for the basal hexagonal cell—and that the observed ring at 29 Å is its second order, since it definitely does not index on the hexagonal cell.

X-ray powder diagrams from small crystals of cleaved material show rings with the same spacings as the intact material but in addition, rings at spacings, 168, 72 and 50 Å, and other weaker unresolved rings. The significance of these became clear when crystals grew sufficiently large to be studied singly. To anticipate the results, these spacings reflect the fact that the cleaved core particles do not stack over each other in register but are displaced sideways to produce 'wavy' instead of straight, hexagonally packed columns. The largest crystals grew to about $200 \times 50 \times 20 \mu\text{m}$ in size and X-ray precession photographs were obtained. The X-ray patterns extend to spacings of about 20 Å and the patterns in the directions of the cell axes are shown in Fig. 5. At this stage we cannot tell whether the limit to the resolution is set by disorder in the crystals or by the combination of small crystal size and large unit cell. The crystals are orthorhombic of unit cell sides $a=110$ Å, $b=192$ Å and $c=340$ Å. The absence of odd index reflections along the directions of the reciprocal cell sides suggests that, at least to this resolution, the space group is $P2_12_12_1$. Most of the X-ray data out to spacings of 25 Å have been collected and a three-dimensional Patterson function calculated, the features of which are consistent with this space group.

The ratio of the cell sides $b:a$ is 1.74, very close to $\sqrt{3}=1.73$, suggesting a pseudo-hexagonal packing in the (001) plane, probably of columns spaced 110 Å apart. The high intensity of the 006 and 0012 reflections indicate strong subdivisions along the c axis of 57 and 28.5 Å, but there is no indication of a 110-Å subdivision in this direction in either the X-ray pattern or the Patterson. We therefore conclude that the units packed along a column are spaced 57 Å apart, and have a strong bipartite character in this direction. On this basis there would be 12 of these units per unit cell and these can be identified as nucleosome cores, since density measurements, although difficult with such small crystals, set

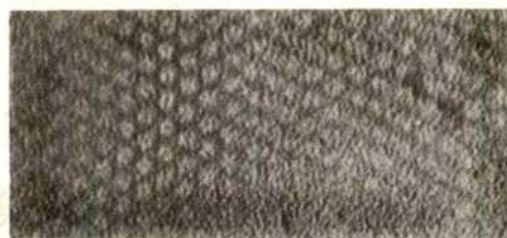


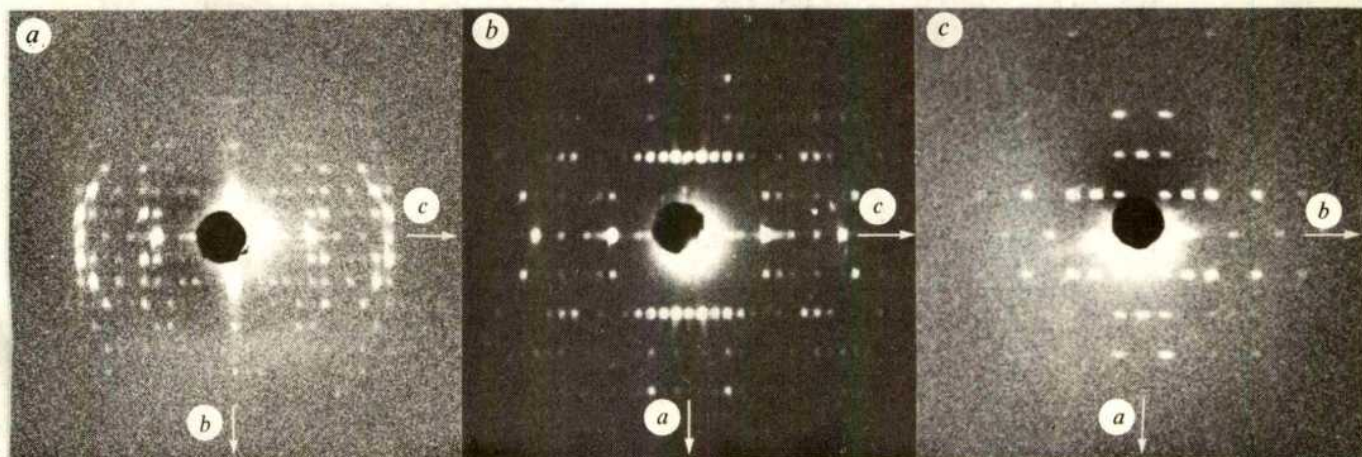
Fig. 4 Electron micrograph of crystal of nucleosome cores with intact histones, negatively stained with uranyl acetate. The cores are hexagonally packed with an interparticle distance of 110 Å. The dark centres indicate a lower density there than at higher radii. ($\times 300,000$).

limits of 9 and 14 for the number of particles in the cell. Twelve particles per unit cell means 3 in the asymmetric unit, which then has a molecular weight of 600,000.

Both the [100] and [010] X-ray photographs show strong reflections in the neighbourhood of the 006 and 0012 reflections, which, together with other features of the patterns, suggest that the columns of nucleosomes are not straight. The simplest interpretation¹³, confirmed later by electron microscopy, is that in the [010] projection, the columns of nucleosomes appear as approximate sine waves of period $c=340$ Å and amplitude about 14 Å, adjacent waves being approximately in phase. In the [100] projection, the columns are also sinusoidal with a period of 340 Å and amplitude about 8 Å, but adjacent waves are out of phase. Together the two projections imply that in three dimensions the line of particles in the z direction follows a sine wave lying in a plane rather than a helix. The projections of the sinusoidal columns down the z axis are elongated and there are two different directions of elongation in the unit cell. Hence although the centres of the projected columns lie on a hexagonal lattice, the packing is only pseudo-hexagonal, accounting for the presence in the [001] X-ray photograph of reflections with $h+k$ odd.

The chief differences in the 'powder' patterns from the intact and cleaved material can now be explained. In the crystals of the intact cores, the particles stack in register in the c direction, leading to straight rather than wavy columns (hence the absence of the 011 reflection at 168 Å). These columns are cylindrical when projected along the c axis, hence at low resolution the packing appears indistinguishable from hexagonal, whereas the wavy columns of the cleaved particles give an elliptical projection and hence stronger departures from hexagonality, exemplified by the 120 reflection at 72 Å. The straight columns of the intact material are divided into

Fig. 5 X-ray precession photographs of crystals of nucleosome cores taken in the directions of the unit cell sides a , [100]; b , [010]; c , [100].



57 Å and 28 Å spacings in the same way as is the cleaved, corresponding to the nucleosome spacing and its halving. This has been confirmed by recent X-ray photographs of single crystals of intact core particles which show the strong 00/ reflections corresponding to the thickness and bipartite nature of the nucleosome core. The *c*-axis repeat is now only 110 Å, corresponding to two nucleosomes 55 Å 'high', while the *a* and *b* axes remain the same.

Electron density map

The packing deduced from the X-ray data has been confirmed by electron microscopy. Patterns which can be identified with views of the crystal in the direction of the cell sides have been found in electron micrographs of fragments of crystals. Although there is considerable shrinkage particularly in the 340-Å direction (vacuum dried crystals visibly shrink some 10%–20% in this direction) the distribution of intensity in optical diffraction patterns made from the micrographs agree well with the low-angle part of the corresponding X-ray patterns. We have found images corresponding to the three principal projections, selected and processed the best of them using optical and computer methods for image analysis¹⁰. We have used the phases (signs in this case) of the reflections determined by computing the Fourier transforms of the images, with the amplitudes derived from the X-ray patterns to calculate electron density maps of the principal projections of the crystal structure. This analysis did not give the signs of some of the outermost strong reflections and we therefore assigned signs to make the three crystallographically independent particles in the unit cell as alike as possible. This use of 'non-crystallo-

graphic symmetry' is a compensating advantage of working with a large unit cell. A fuller account will be published elsewhere¹⁵.

In the [100] and [001] projections there is considerable overlap of units at different depths in the unit cell although in the former the sinusoidal columnar nature of the packing is evident and in the latter the pseudo hexagonal packing of the columns. The least overlap occurs in the [010] projection, the map of which is shown in Fig. 6. Wavy columns of particles, that is nucleosome cores, about 57 Å apart, are clearly visible. The density in each particle is strongly divided into two but the two halves are not always parallel to each other, so that the particle itself looks wedge shaped. Indeed the run of particles in an arc of the sine wave is reminiscent of the arrangement of voussoirs in an arch, but the map gives the impression that the three crystallographically independent cores do not all have quite the same rotational setting about the *c* direction.

The electron micrographs we have obtained of the crystals do not directly show much detail beyond the arrangement of the core particles. We have therefore searched for specimens which would show more details of the internal structure and so be compared with the map shown in Fig. 6. To this end we have set up specimens for crystallisation, examined them in the electron microscope before visible crystals appeared and found wavy columns of particles. Figure 7 shows some nested columns, similar to the packing which would exist in a section of the crystal perpendicular to the *y* axis and one layer thick. Along the columns, associated pairs of 27-Å striations are often clearly evident, but the nature of the

Fig. 6 Electron density map of the projection of the crystal of nucleosome cores in the direction of the *a* axis of the unit cell. The nine strongest amplitudes in the [100] X-ray diagram (Fig. 5a) out to spacings of 25 Å were included. The signs of seven of these were taken from the calculated Fourier transform of an electron micrograph of a crystal in the corresponding orientation. The signs of the remaining two amplitudes were chosen to make the three crystallographically independent particles in the asymmetric unit most nearly equivalent.

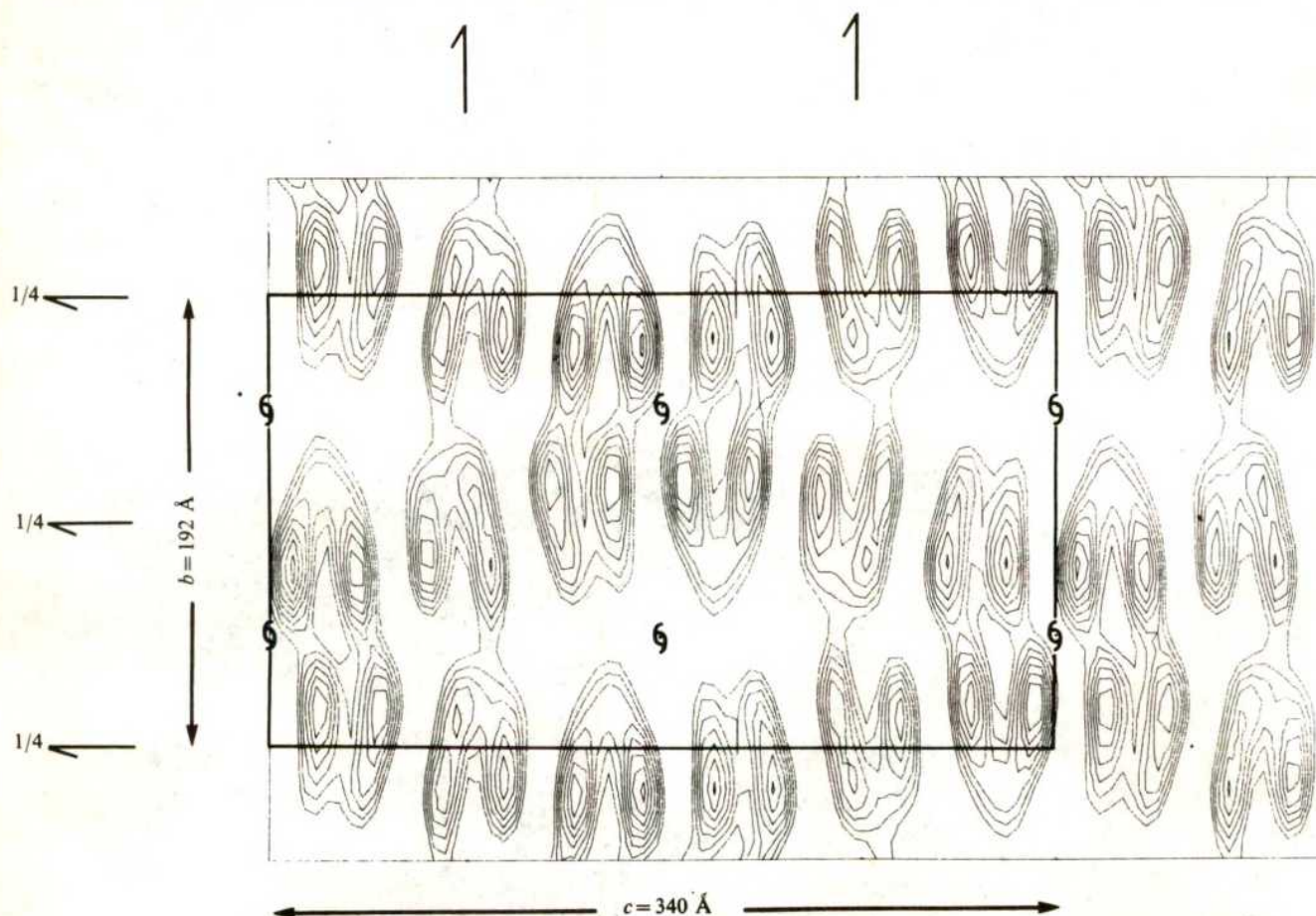




Fig. 7 Electron micrograph of columns of nucleosome cores formed after a short time in the conditions of crystallisation, negatively stained with uranyl acetate ($\times 300,000$). The wavy columns correspond to those along the *c* axis in the crystal (Fig. 6). They are about 100 Å in diameter and consist of stacks of bipartite nucleosome cores spaced about 55 Å apart. The two halves of the particles do not always appear parallel and are often V-shaped especially at bends in the columns. The fine details along the two halves of a particle are often related by mirror symmetry consistent with views of a particle with several radially directed pseudo-dyad axes.

pairing varies along the column. Sometimes the associated pair of striations are parallel but often they are not, giving a wedge-shaped appearance to the bipartite unit. In all of these cases, however, there is a strong tendency for the two halves to be related by approximate mirror symmetry, consistent with views at right angles to a twofold axis, or pseudo twofold axes, such as might arise from the arrangement of eight similar histone molecules about a true dyad.

Relation to small-angle scattering studies in solution

The flat shape seen for the nucleosome core is consistent with the determination of the volume and surface area of (intact) core particles by a model-independent analysis of small angle X-ray scattering data in solution (A. Tardieu and L. Sperling, in preparation). Whereas the radius of gyration (determined from the very low-angle data) would correspond to a uniform sphere of radius 57.6 Å, the surface area (determined from analysing the scattering curve to a resolution of 20 Å) would correspond to a sphere of radius 72 ± 5 Å, suggesting a shape departing markedly from the spherical.

There have been earlier proposals for a disk shape for the nucleosome core^{16,17}. The model calculations of the Searle group¹⁸ to explain their low angle X-ray and neutron scattering data in solution (to a resolution of about 30 Å) show that a flat particle fits the data well; and they have proposed a model for the core of the type we have found, although scattering in solution which yields spherical averages of intensities, can never prove a model. Only three dimensional data can resolve superposed intensities and give direction to dominant spacings observed.

Thus it can be seen that the 38-Å and 27-Å bands observed in X-ray scattering from nucleosome cores in solution (A. Tardieu and L. Sperling, in preparation), and also of course in whole chromatin, come from directions at right angles to each other. It is very likely that the 38-Å reflection which occurs in the plane of disk has a contribution from the second maximum of a Bessel function corresponding to the radius of the DNA, the first maximum being at 70 Å, which in the X-ray data appears as a broad shoulder superposed on the steeply falling part of the 'shape' scattering curve. But the 70-Å

maximum shows up clearly in the spherically averaged 'internal' scattering function deduced from neutron scattering using contrast variation¹⁹. The 27-Å band comes predominantly from internal structure along the axis of the short cylinder, corresponding to the spacing of the turns of the DNA superhelix. Again, this fluctuation of density is evident in the 'internal' structure function¹⁹. There is therefore no gross difference in structure between nucleosomes in solution and in the crystal.

There are also other obvious implications for interpreting the X-ray patterns from whole chromatin. Thus the crystal data shows that the well known '55-Å reflection' of chromatin in fact occurs in two directions at right angles (in the neighbourhood of the reflections 200 and 006) and has a quite different origin in the two cases, the first as a second order of the side to side spacing of the cores, and the second as the distance of contact between their flat faces. The first order of the side to side spacing at 110 Å is cancelled by the packing in the crystal but presumably corresponds to the '110-Å reflection' of chromatin.

Structure of nucleosome core

The most informative of the three projections we have obtained is that down the *a* axis (Fig. 6) because one is looking only through a single layer of particles with a little overlap at the edges. The nucleosome core is clearly a flat particle of dimensions $57 \times 110 \times 110$ Å but seems to have a wedge shape consistent with its being formed of something less than two superhelical turns of DNA wound on a flat histone core. Because of its shape we call it a platysome. Again, as described above for the electron micrographs, the two halves of a platysome in the map are related by approximate mirror symmetry, consistent with a dyad or pseudo-dyads in the plane of projection. This is consistent with a model in which two turns of DNA (strictly parts of two turns) run along the outside of a histone core which may itself consist of two layers or turns. At the present resolution, and also because one is dealing with a projection, one cannot distinguish DNA and protein, but the map is not consistent with a structure in which the DNA lies in a deep groove formed by the histones. Rather, the DNA is fitted on to the periphery of the histone octamer rather like a helical tyre. The location of the DNA on the outside of the nucleosome was originally suggested by Kornberg¹, and it has been confirmed by low-angle neutron scattering in solution that the bulk of the DNA does lie at an outer radius^{19,20}.

It is not possible at the present stage to follow the path of the DNA in the nucleosome core but the simplest assumption is that it follows a more or less regular helix. Whatever the details, the DNA is wound in a flat superhelix of pitch about 28 Å and average diameter about 90 Å (Fig. 8a). The value for the pitch comes from the side view of a particle in the electron density map (Fig. 6). The pitch of the superhelix is small enough to allow interactions between the two turns, mediated by cations and/or histone salt bridges. The diameter is fixed by the fact that the very outside diameter of a nucleosome core can at most be only 5 Å or so greater than the shortest centre-to-centre distance, which is not less than about 100 Å. The reason is that platysomes in neighbouring columns parallel to the *c* axis are staggered by only about 10 Å (Fig. 6) so there is not much interlocking which would have led to a larger diameter for the DNA. Assuming a diameter for the DNA of 22 Å the diameter for the DNA coil is about 90 Å, and if the DNA is assumed to be in the B form, we estimate there are 75–82 bases per superhelical turn.

Little can be concluded about the arrangement of histones. The dimensions of the histone core, deduced by

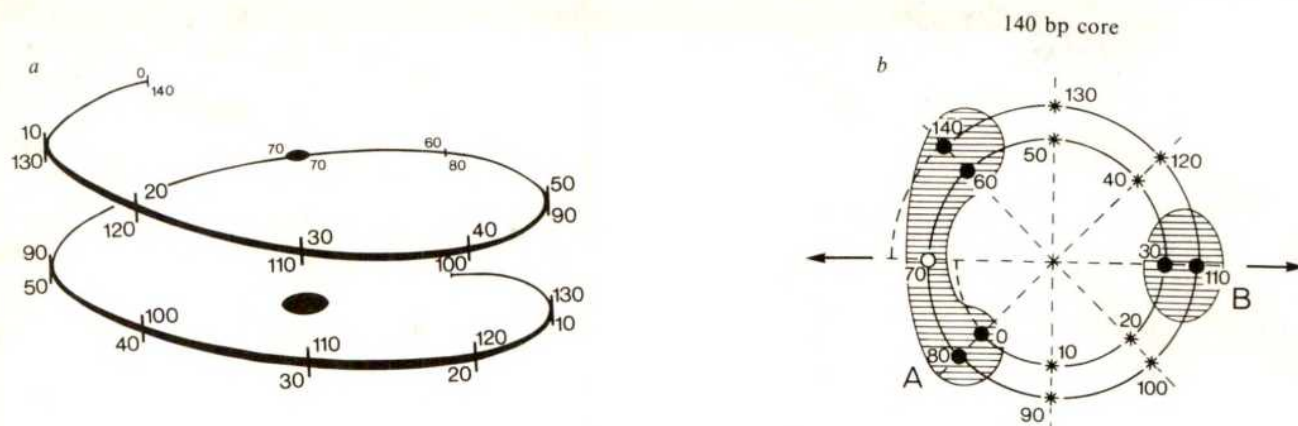


Fig. 8 *a* Diagram, drawn roughly to scale, of the $1\frac{1}{2}$ turns of the DNA superhelix proposed for the 140-base-pair nucleosome core. The top set of numbers gives the distances in bases of the DNase I cutting sites from the 5' end of one strand, and the bottom set refers to the other strand, related to the first by the dyad shown. For definiteness, the number of bases per turn is shown as 80, but the exact number is not established. *b*, The $1\frac{1}{2}$ turns of one strand of the DNA superhelix represented as a spiral to show how the supercoiling brings sites 80 bases apart close together and groups the sites of low or medium cutting frequency by DNase I into the two diametrically opposite areas A and B, shown shaded. The arrow indicates the dyad. The numbers give the distance in bases from the 5' end. ★, High frequency DNase I cutting. ●, Low (or micrococcal) pauses. ○, Medium.

'subtracting' the DNA, show the octamer to be a compact particle, of packing volume similar to that found for other globular protein aggregates such as haemoglobin. It is, however, not possible to tell whether the histone molecules form a shallow helical ramp of two turns or whether there are simply two layers which are distorted or have projections on them to provide such a helical ramp. There is a striking similarity between some of the dimensions which occur in the columns of nucleosomes found in the crystals and the spacings observed in optical diffraction patterns of electron micrographs of certain histone fibres assembled without DNA²¹. In the columns in the crystal the histone cores of adjacent platysomes must be in contact and the packing may be similar to that in the histone fibres. If this is so, the 28-Å group of reflections in the crystal (compare Fig. 5a) would have a contribution from the histone cores, which would thus also tend to be divided into two.

Relation to enzyme digestion studies

We now turn to the question of the organisation of the DNA in the nucleosome core, and show how our structural results can be correlated with the results of enzyme digestion studies. The action of DNase I on chromatin or nucleosome cores is to produce a set of DNA fragments differing in size by 10 bases¹², and this was interpreted as reflecting the fact that the enzyme either cuts at points on smoothly bent DNA which are 'maximally exposed' (that is, lie furthest from the centre of the particle) or at 'kinks' in the DNA where the structure is distorted. Whatever the explanation, the important fact is that periodicity along the DNA helix is 10 bases or very close to 10. It is clear, however, that the cuts are not equally frequent every 10 bases and the assumption is that frequency of cutting reflects differential protection of different tracts of the DNA by the histone interactions. Several studies have been made of the frequency distribution by radioactively labelling an end of the DNA in the 140 base-pair core, then digesting with DNase I, and then analysing the distribution of end-label amongst the single-stranded fragments produced (refs 22 and 23, and M. Noll in preparation). Numbering in bases from the 5' end, it is found that the sites at 10, 20, 40, 50, 90, 100, 120 and 130 are readily attacked, so that cuts tend to occur at 30–40 or 80–90 bases apart. There are quantitative differences in the results of the different investigators but the local rank order among the cuts is the same.

Now as stated earlier the diameter of the DNA coil is best fitted by about 80 bases per turn, so that points on the DNA double helix 80 bases apart are closest together on the superhelix, only about 30 Å apart, and can therefore be protected, or otherwise, by neighbouring patches of histones (Fig. 8b). The positions of the most frequent cuts are consistent with this, but would not in themselves rule out a periodicity of 90 bases. The decision in favour of 80 is, however, unambiguously made by considering the least frequently cut sites, which are at positions 30 and 110 and are flanked on both sides by sites of high frequency cutting. This reasoning can be extended to include the results of micrococcal digestion studies, which show a 'pause' at the 160 base-pair stage before the more stable 140 base pair particle is produced. If the 160-base-pair particle corresponded to a structure with two complete turns, (which can be recreated by extending the DNA of Fig. 8 from the points 0 and 140 to –10 and 150), then the sites at 0 and 140 reflect places of relatively high protection of the DNA. These two positions can be correlated with the weak DNase I cuts at 80 and 60 respectively, which are 80 bases away. Such a 160 base pair particle would have its two ends 'in line' with each other and with the midpoint of the DNA. Through the midpoint would pass the putative dyad of the structure. The other end of the dyad would pass between the turns of the DNA, that is, between sites 30 and 110 (Fig. 8a).

The DNase I cutting pattern is consistent with the assumption that the nucleosome core possesses a dyad and the matter is discussed in detail elsewhere²². If there is a dyad as shown in Fig. 8a, then if a cut occurs on one strand, it is equally likely that a cut occurs at the equivalent position on the other strand, that is at the same distance from the 5' end. But since what is measured is the average of the frequency of cutting on the two strands, it is not possible to tell whether the cutting is equally apportioned between the two. Crick has pointed out that the strongest evidence for a dyad comes from the fact that there are weak (or zero) cuts, as here cuts at equivalent positions on both strands must be both zero or small and hence equal. Apart from the histone composition there are other biochemical results suggesting the presence of a dyad. It has been shown²⁴ that DNase II can cut down the DNA of a 200-base-pair nucleosome into two equal halves without disrupting the particle physically and there is electron microscopic evidence that in

appropriate conditions a nucleosome can open up into two separate half-nucleosomes²⁵.

Supercoiling of the DNA

If DNA is closed into a circle after interaction with histones in the presence of a 'DNA relaxing' enzyme, the DNA is seen to supercoil when the histones are removed²⁶. Several workers have estimated that the 'number of supercoils' is between -1 and $-1\frac{1}{2}$ per nucleosome²⁶⁻²⁸ (the minus sign indicates left-handed supercoiling). It was pointed out by Crick²⁹ that what these experiments measure is the change in linkage number (here the number of times one strand of the DNA double helix is wound around the other strand) caused by forming nucleosomes. It is not possible by the techniques used to distinguish between the separate contributions made to the change in linkage number by the coiling of the DNA into a superhelix and by any concomitant change in the local twist of the DNA double helix. Only the sum is conserved. The change in linkage number is the same as the number of superhelical turns only if the screw of the DNA double helix does not change in the local coordinate frame defined by the superhelix path.

Our results on the crystals show that as the maximum DNA radius in the core particle is about 45 Å, the 140 base pairs of DNA must make more than $-1\frac{1}{2}$ turns of superhelix. In fact we propose $1\frac{1}{2}$ superhelical turns corresponding to 80 base pairs per turn. The only way to reconcile these results with the linkage number change of $-1\frac{1}{2}$ is to conclude that the helical screw of the DNA double helix does indeed change on binding DNA free in solution on to the nucleosome. It would be possible to calculate this change in screw if we knew to what length of DNA the observed linkage number change applied. If the full 200 base pairs of DNA in the chromatin repeat are wound superhelically in the nucleosome giving $-2\frac{1}{2}$ turns, the average screw rotation angle between bases must decrease by $(-1\frac{1}{2} + 2\frac{1}{2})/200 \times 360$ degrees ≈ 2.3 degrees. If only 140 base pairs of DNA are wound superhelically and the remaining 60 base pairs are straight as in solution, the decrease in screw angle is $(-1\frac{1}{2} + 1\frac{1}{2})/140 \times 360$ degrees ≈ 1.3 degrees. Now the DNase I cutting pattern strongly suggests that the number of base pairs per turn of DNA double helix on the nucleosome is close to 10 (36° base rotation). If so, the number of base pairs of DNA free in solution must be either 10.7 or 10.4, for these two models respectively (33.75° or 34.7° base rotation).

Now while it is commonly accepted that DNA has 10 base pairs per turn, this value comes from X-ray diffraction work on fibre patterns where the DNA is in a rather dry state and the crystal packing tends to produce an integral number of base pairs per turn. There is no reason to believe that this number must apply exactly to free DNA in solution and indeed it has been suggested³⁰ that the screw of the DNA in solution was closer to 11 base pairs per turn. The arguments are based on rather limited evidence from the position of the 12-Å peak, an experimental result which has been confirmed³¹, but which could have other explanations.

Energy calculations (M. L., in preparation) on double helical DNA show that DNA can be smoothly bent into a superhelix of radius 45 Å. The superhelical structure has a local geometry very like that of straight DNA and an energy only slightly higher than that of the straight structure. Furthermore, the straight DNA has the lowest energy with 10.7 base pairs per turn, whereas the superhelical DNA has the lowest energy with 10.0 base pairs per turn. The base pairs overlap less in the superhelically bent form to enable the base pairs to twist and tilt to accommodate the bending strain. These calculated values for the screw of straight and bent DNA are in good agreement

with the values deduced above from the combination of the results of quite different types of observation.

General features of the DNA folding

The calculations mentioned above show that it is not necessary, on merely energetic grounds, to postulate that the folding of the DNA on to the nucleosome requires sharp kinks^{32,33} at periodic intervals. Indeed, one can see *post hoc* the advantages of the nucleosome structure proposed here for folding a long, stiff molecule like DNA. It has been known for some time that DNA in solution behaves like a stiff rod (with a persistence length of 625 Å; ref. 34) but the energy calculations show that DNA can bend smoothly down to a radius of curvature of about 40 Å without undue strain (<3 kcal per 10 base pairs). Because the strain energy on bending increases as the inverse square of the radius of curvature, the superhelix should have as large a diameter as possible. One may ask then why the 140 base pairs are not merely bent into a single turn, apart from any extended protein arrangement this might require. The advantage of having more than one turn of superhelix for the DNA is that this makes it possible to have stabilising interactions between DNA in adjacent turns. The most obvious source of stabilisation would come from bridging negatively charged phosphate groups on the DNA by means of cations or positively charged histone side chains. The strength of this lateral interaction will depend on the length over which the two turns interact, the separation of adjacent turns, and the relative positions of the phosphate backbone in each turn. In the structure proposed here there are about two superhelical turns per nucleosome, which gives a full turn for lateral interactions, but still keeps the radius of curvature large. These turns are separated by about 28 Å, an ideal distance for repeated interactions between phosphate groups of double helices that are in phase. When the number of base pairs per turn of the double helix is integral (10 here) then the same stabilising interactions between adjacent superhelix turns can occur repeatedly along the chain.

How do H3 and H4 alone 'organise' the DNA?

There is a growing body of evidence^{25,35-38} that the DNA can be folded into a body with many of the features of the nucleosome core by the arginine-rich histones H3 and H4 without the intervention of the other two histones. Indeed, it was part of Kornberg's original reasoning that the arginine-rich tetramer formed the basis of the nucleosome, and that the lysine-rich histones completed the structure. The first evidence was provided by Felsenfeld and his colleagues as judged by the protection provided against micrococcal and other nucleases^{35,36} and more recently it has been demonstrated by Chambon and his colleagues²⁵ that distinct subnucleosomal particles of diameter about 80 Å can be formed by reconstitution with only the histones H3 and H4 and that these particles contain about 130 base pairs which have about the same amount of supercoiling as a complete nucleosome core containing all four histones. It also seems likely that it is only a single tetramer which is involved rather than a pair of tetramers simulating an octamer (P. Chambon, private communication; see also ref. 37).

The simplest explanation³⁵ for this sub-nucleosomal particle is that the 140 base pairs of DNA condense around a central core or kernel formed by the H3-H4 tetramer, but other more open structures are possible which take into account the stiffness of the DNA and which moreover can be regarded as incomplete nucleosome cores leading to the basic structure described here.

This may be accomplished in two ways. The first is that the H3-H4 tetramer is asymmetrically located in the

nucleosome, say in the region marked A in Fig. 8, and that this tetramer clamps together two turns near the positions marked 0, 70 and 140; this would be sufficient to impose the supercoiling. A single tetramer would not, however, give protection against nucleases to the DNA in the region B and moreover it is difficult to see how the appropriate length of the DNA would be determined. A second, attractive explanation attaches significance to the fact that the two main regions of protection of DNA by the histones lie diametrically opposite each other (A and B in Fig. 8b). The H3-H4 tetramer could span the diameter of the nucleosome core to act as a spool around which two turns of DNA could be wound. Reconstitution with the tetramer would then resemble wrapping a garden hose around one's forearm. This would have the advantage of additional protective interactions and at the same time measuring out the appropriate length of DNA. H2A and H2B would then complete the nucleosome.

There is an interesting parallel between the packing of DNA in eukaryotic chromosomes and in the nucleoid of *Escherichia coli*. In both cases there seems to be about one 'supercoil' (or rather change in linkage number) per 200 base pairs^{39,40}. The folding of the DNA in *E. coli* seems to be produced by a small basic protein⁴¹, which moreover is apparently a dimer (Rouvière-Yaniv, personal communication). One could imagine that the dimer clamps together two superhelical turns at one point as described above. As eukaryotes developed, further basic proteins may have been introduced to produce a more regular, better protected particle—the nucleosome—capable of being organised into higher order structures.

We thank Dr F. H. C. Crick for valuable discussions, Mr R. Staden for measuring the X-ray intensities and Dr A. Jack for computing the Patterson function. L.C.L. was supported by a US NIH postdoctoral fellowship.

Received 27 June; accepted 29 July 1977.

- ¹ Kornberg, R. D. *Science* **184**, 868–871 (1974).
- ² Hewish, D. R. & Burgoyne, L. A. *Biochem. biophys. Res. Commun.* **52**, 504–510 (1973).
- ³ Noll, M. *Nature* **251**, 249–251 (1974).
- ⁴ Kornberg, R. D. *A. Rev. Biochem.* **46**, (in the press).
- ⁵ Sollner-Webb, B. & Felsenfeld, G. *Biochemistry* **14**, 2915–2920 (1975).
- ⁶ Axel, R. *Biochemistry* **14**, 2921–2925 (1975).
- ⁷ Bakayev, V. V., Melnickov, A. A., Osicka, V. D. & Varshavsky, A. J. *Nucleic Acids Res.* **2**, 1401–1419 (1975).
- ⁸ Shaw, B., Herman, T., Kovacic, R., Beaudreau, G. & Van Holde, K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 505–509 (1976).
- ⁹ Noll, M. & Kornberg, R. D. *J. molec. Biol.* **109**, 393–404 (1977).
- ¹⁰ Crowther, R. A. & Klug, A. *A. Rev. Biochem.* **44**, 161–182 (1975).
- ¹¹ Noll, M., Thomas, J. O. & Kornberg, R. D. *Science* **187**, 1203–1206 (1975).
- ¹² Noll, M. *Nucleic Acids Res.* **1**, 1573–1578 (1974).
- ¹³ Brown, R. S. *et al. Eur. J. Biochem.* **31**, 130–134 (1972).
- ¹⁴ Ladner, J. E., Finch, J. T., Klug, A. & Clark, B. F. C. *J. molec. Biol.* **72**, 99–101 (1972).
- ¹⁵ Finch, J. T. & Klug, A. *Cold Spring Harb. Symp. quant. Biol.* **42**, (in the press).
- ¹⁶ Langmore, J. P. & Wooley, J. C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2691–2695 (1975).
- ¹⁷ Langmore, J. P. thesis, Univ. Chicago (1975).
- ¹⁸ Richards, B., Pardon, J., Lilley, D., Cotter, R. & Wooley, J. *Cell Biology, Int. Rep.* **1**, 107–116 (1977).
- ¹⁹ Hjeltn, R. P., Kneale, G. G., Suau, P., Baldwin, J. P. & Bradbury, E. M. *Cell* **10**, 139–151 (1977).
- ²⁰ Pardon, J. F. *et al. Nucleic Acids Res.* **2**, 2163–2176 (1975).
- ²¹ Sperling, R. & Amos, L. A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ²² Lutter, L. C. *Cold Spring Harb. Symp. quant. Biol.* **42**, (in the press).
- ²³ Simpson, R. T. & Whitlock, J. P., Jr *Cell* **9**, 347–353 (1976).
- ²⁴ Altenburger, W., Hörz, W. & Zachau, H. G. *Nature* **264**, 517–522 (1976).
- ²⁵ Oudet, P., Germond, J. E., Bellard, M., Spadafora, C. & Chambon, P. *Phil. Trans. R. Soc. B* (in the press).
- ²⁶ Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1843–1847 (1975).
- ²⁷ Keller, W. & Wendel, I. *Cold Spring Harb. Symp. quant. Biol.* **39**, 198–208 (1974).
- ²⁸ Keller, W. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4876–4880 (1975).
- ²⁹ Crick, F. H. C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2639–2643 (1976).
- ³⁰ Bram, S. J. *J. molec. Biol.* **58**, 277–288 (1971).
- ³¹ Maniatis, T., Venable, J. H., Jr & Lerman, L. S. *J. molec. Biol.* **84**, 37–64 (1974).
- ³² Crick, F. H. C. & Klug, A. *Nature* **255**, 530–533 (1975).
- ³³ Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. & Sakore, T. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3068–3072 (1976).
- ³⁴ Hays, J. B., Magar, M. E. & Zimm, B. H. *Biopolymers* **8**, 531–536 (1969).
- ³⁵ Camerini-Otero, R. D., Sollner-Webb, B. & Felsenfeld, G. *Cell* **8**, 333–347 (1976).
- ³⁶ Sollner-Webb, B., Camerini-Otero, R. D. & Felsenfeld, G. *Cell* **9**, 179–193 (1976).
- ³⁷ Camerini-Otero, R. D. & Felsenfeld, G. *Nucleic Acids Res.* **4**, 1159–1181 (1977).
- ³⁸ Boseley, P. G., Bradbury, E. M., Butler-Brown, G. S., Carpenter, B. G. & Stephens, R. M. *Eur. J. Biochem.* **62**, 21–31 (1976).
- ³⁹ Worcel, A. & Burgi, E. J. *J. molec. Biol.* **71**, 127–147 (1972).
- ⁴⁰ Benyajati, C. & Worcel, A. *Cell* **9**, 393–407 (1976).
- ⁴¹ Rouvière-Yaniv, J. & Gros, F. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3428–3432 (1975).
- ⁴² Thomas, J. O. & Kornberg, R. D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2626–2630 (1975).

Immunological selection of tumour cells which have lost SV40 antigen expression

P. T. Mora, C. Chang, L. Couvillion, J. M. Kuster & V. W. McFarland

Macromolecular Biology Section, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

In an already tumorigenic spontaneously transformed mouse cell, after further transformation by SV40, the virus-specific antigenic function becomes dominant. By transplantation into syngeneic mice SV40 antigen negative revertant tumour cells can be selected out.

THE nature and function of early proteins of oncogenic viruses are receiving considerable attention. For simian virus 40 (SV40) the early protein, coded by about half of the SV40 genome, has dual functions. One is apparently as a regulator protein with a crucial role in the induction of cell division, the initiation of viral DNA replication and in cell 'transformation'. The second is antigenic. In the transformed cells these two type of functions, paradoxically, may oppose each other *in vivo*. The transplantation antigen can be the cause of immunological recognition and rejection.

SV40 (virus), or cells transformed by SV40 in tissue culture are tumorigenic only in the newborn hamster and *Mastomys*. (*Rattus (Mastomys) natalensis* is an African rodent intermediate in size between the rat and the mouse.) Neither SV40 nor the cells transformed in culture by SV40 are as a rule tumorigenic in the mouse¹ or in the rat²,

or as far as it is known in any other species including the natural host of the virus the simian monkey. Tumours have been obtained in the mouse from SV40 transformed non producer cells, but only after considerable time and a number of tissue culture transfers (t.c.t. >50) followed the SV40 transformation step, and when relatively large number of cells (about 10⁶) were injected^{3–5}. Even then, most of the tumours were transitory, and were eventually rejected by the mice^{3–6}. If the immunological competence of the mouse is weak, like in the newborn, or is impaired (by, for example, irradiation, anti-lymphocyte serum, or neonatal thymectomy) SV40 T antigen-positive tumours may be produced more readily than in the immunologically competent syngeneic mouse^{4,7}. Such tumours then become retransplantable in the immunologically competent mouse.

In the AL/N mouse strain an SV40 transformed cell line SV AL/N, even after very large number (~275) of t.c.t. and at 10⁸ cell dose is rejected by the immunologically competent adult mice⁸. The recognition and rejection is due to SV40 specific surface and transplantation antigens^{9,10}. In irradiated mice tumours can be caused by injecting 10⁷ SV AL/N cells. The tumour cells re-established in culture retain SV40-specific nuclear (T) and cell surface antigens¹¹, and have increased tumorigenicity: 10⁴–10⁵ cells induce tumour in the immunologically competent mouse⁸.

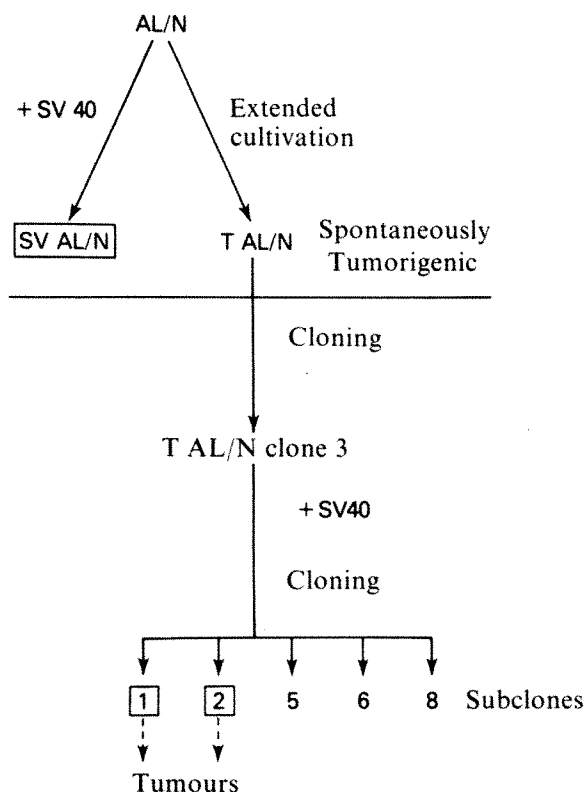


Fig. 1 Cell genealogy and cloning. The box indicates the SV40-positive cell lines. Above the horizontal lines are the mass cell lines. For cloning the T AL/N cells at t.c.t. 68 were well dispersed by trypsinisation, and 0.2 ml diluted aliquots in growth medium (reinforced Eagles with doubled amino acid and vitamins, with added antibiotics and 10% foetal calf serum) containing on the averages 0.4 cells were put into each of the 96 wells of a Falcon microtests plate. Each well was surveyed daily, and clones were picked only from wells in which there was only one cell at the onset, and the cell growth was from one focus. A single clonal line T AL/N C1 3 was selected. For infection 2.5×10^5 T AL/N clone 3 cells were exposed in 1 ml Eagles medium for 1 h at 37°C in a shaking water bath to 9×10^7 plaque forming units (PFU) SV40, small plaque forming strain 776. This ratio of PFU per cell (360) was chosen to obtain 30%–50% T antigen positive cells at 2 d after infection. The cells were then placed in growth medium in a 10-cm Falcon Petri dish and cultivated as before¹². After t.c.t. at 5 d into two dishes and a total of 10 d growth, there were $\sim 10^7$ cells, of which 30%–40% were T-antigen positive (T antigen tests by nuclear fluorescence were carried out as previously⁸ on separate aliquots). At this time the cells were recloned using the method described above. Five subclones, each clearly grown up from one cell, were obtained. Substantially all (> 95%) of the cells of the subclones designated 1 and 2 were strongly T antigen positive when tested repeatedly up to > 200 t.c.t., while all of the cells of the subclones 5, 6, 8 were T antigen negative. Arrows with broken line indicate the origin of the T-antigen negative (revertant) tumour cell lines.

From the same AL/N cell line from which the SV AL/N cells were obtained, a highly tumorigenic spontaneously transformed T AL/N cell appeared during tissue culture. After 40 t.c.t. 10^3 T AL/N cells gave progressively growing and lethal fibrosarcomas when injected intramuscularly or subcutaneously into the adult immunologically competent syngeneic mouse^{8,9,12}.

What would happen to the tumorigenicity of the T AL/N cells if they were infected and transformed also by SV40? The SV40 induced cell surface and transplantation antigens were expected to facilitate rejection in the syngeneic mouse, opposing the tumorigenicity of the T AL/N cells. This system allowed us to embark on a novel study on the balance between strong cellular tumorigenicity and a superimposed strong and well characterisable (SV40 specific) antigenic property.

Transformation by SV40 is an inefficient process, since

only a minor fraction of the infected cells have stable insertion and thus expression of SV40 DNA. The SV40 may infect and transform cells selectively which have greater, or lower tumorigenicity. To avoid such selection we first cloned the T AL/N cells. The resulting cells, T AL/N clone 3 had retained the high tumorigenicity. After infection by SV40 of the T AL/N clone 3 cells, both T antigen-positive and negative subclones were obtained and characterised (see Fig. 1). The SV40 T antigen positive subclones were about 100-fold less tumorigenic than the T antigen-negative sister subclones or the parent T AL/N clone 3 cells. When the tumours from the T antigen positive subclones were re-established in culture, we found that SV40 T, surface and transplantation antigen negative (revertant) tumour cells resulted.

We describe here the conditions when such *in vivo* selection reproducibly occurs, prove that the revertant tumour cells are true progenies of an SV40 transformed cell, and also describe some of the phenotypic properties of the various cells. We also show that the ability of cells to grow without anchorage in a viscous medium is not a general correlate of tumorigenicity in the syngeneic mouse.

AL/N mouse cell clones

The genealogy and certain properties of the cell lines are summarised in Fig. 1 and Table 1. The AL/N mouse

Fig. 2 Presence of SV40-specific surface antigen in the T antigen-positive cells. % Chromium release represents residual cytotoxicity of an antiserum prepared in the syngeneic mouse against SV AL/N cells after the absorption by the different cells. ●, Control (no cells were used for absorption); △, absorption by T AL/N clone 3 cells; □, absorption by the T antigen-negative subclone 5 cells; ○, absorption by the T antigen-positive subclone 1 cells. Similarly, there was no significant absorption by the cells of the T antigen negative subclone 6, but almost complete absorption of the cytotoxicity by cells of the T antigen-positive subclone 2 (data not shown). For absorption 10^6 cells were plated into 60-mm Falcon plastic tissue culture Petri dishes. After 24 h growth the near confluent cell layer ($\sim 2 \times 10^6$ cells) was washed twice with the growth medium, and 400- μl aliquots of antisera (diluted 1:200 with growth medium) were incubated (30 min, 37°C) sequentially with three such dishes containing 2×10^6 cells. The absorbed antisera were centrifuged at 500g for 5 min, and further dilutions were tested for residual cytotoxicity on SV AL/N target cells, by a method described before^{18,19}.

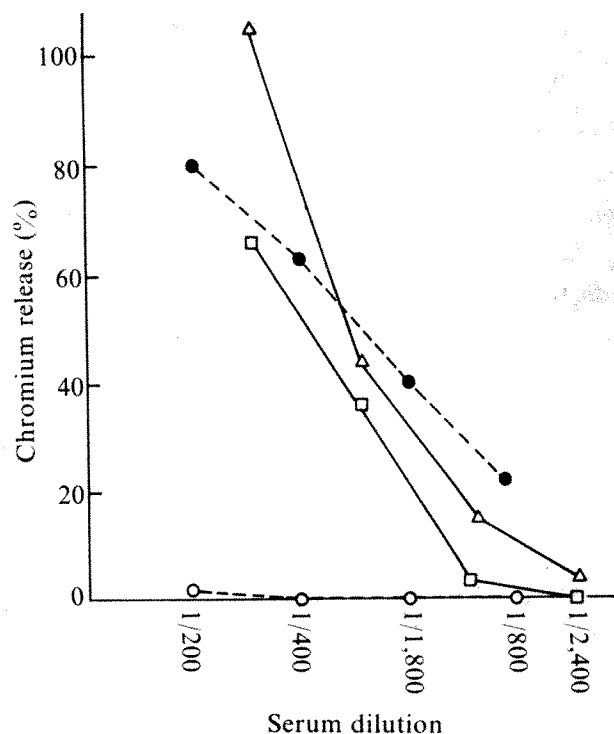


Table 1 Summary of the biological properties of the cell lines

Cell line	SV40 T antigen	Tumorigenicity TD ₅₀ * (Cells per mouse)	Time to death† (weeks)	Tissue culture growth property Saturation‡ density (cells cm ⁻² × 10 ⁵)	Colony formation in viscous Methocell suspension‡ (%)
T AL/N mass line	—	< 10 ²	9	5.5	2
T AL/N clone 3	—	10 ^{1.7}	14	3.0	0.007
SV40 infected					
Subclone 1	+	10 ^{3.8}	13	1.0§	20
2	+	10 ^{3.9}	14	0.6§	10
5	—	10 ^{1.5}	17	2.4	< 0.0001
6	—	< 10 ¹	21	3.5	0.06
8	—	10 ^{2.5}	16	3.1	0.03
SV AL/N mass line	+	> 10 ⁸	—	5.3	10

*Tumorigenicity was tested by intramuscular (i.m.) injection of cells at least in three dilutions each differing 10-fold, into groups of 6–8-week-old AL/N mice, 10 mice in each group. TD₅₀ is the cell dose which caused in 50% of the animals large (min. 1 cm Ø), and eventually lethal, tumours. It was calculated as follows: $TD = 10^{d+x}$, where d is the (\log_{10}) number of cells which caused tumour in just below 50% of the mice, and $x = (a-50)/(a-b)$ where a is the % of mice with tumours within that group of 10 mice which had nearest to but above 50% tumours, and where b is the % nearest to but below 50%. The TD₅₀ values were reproducible within $\pm 0.5 \log_{10}$ at 10² cell dose, and $\pm 0.2 \log_{10}$ at 10⁴ cell dose.

†Time when substantially all the animals, injected (i.m.) with a cell dose rounded up to the nearest integer of the exponential of TD₅₀, die off due to progressive tumour growth. There were no regressing tumours.

‡Determined in the presence of 10% foetal calf serum⁸.

§Cells after reaching confluency tend to detach from the substratum.

embryo cell line—parent line to all AL/N cells reported here—at t.c.t. 28 had a median tumour cell dose, TD₅₀ of 10³–10⁵ (ref. 12); and at t.c.t. 40, TD₅₀ < 10² (ref. 8). This T AL/N cell was characterised again at 65 t.c.t. (Table 1), and cloned as described in the legend to Fig. 1.

The SV AL/N cells were rejected. The spontaneously transformed T AL/N cells and the derivative T AL/N C1 3 cells were strongly tumorigenic (TD₅₀ ~ 10³), as were the SV40 T antigen-negative subclones 5, 6, and 9. But, the T antigen-positive subclones 1 and 2 were about 100 fold less tumorigenic (TD₅₀ ~ 10⁴), than the parent T AL/N C1 3 cells, or the sister subclones 5, 6, and 8 (Table 1).

The SV40 T, transplanted, and cell surface antigens probably represent modifications of products of the same SV40 cistron^{14,15}. The antigens co-purify^{10,14}, and mutants in *tsA* coordinately affect expression of all three antigens¹³. Experiment 1 in Table 2 suggests the presence of SV40 specific transplanted antigen(s) in whole subclone 1 and 2 cells; this then may be the cause of the reduced tumorigenicity of these cells. Experiments 2 and 3 in Table 2 demonstrate the presence of SV40-specific transplanted antigens in extracts of subclone 1 cells, but the absence in extracts of the T antigen-negative cells, such as subclone 5, T AL/N clone 3 cells, and a tumour cell line (number 124) obtained from subclone 1 cells (see below). Similarly, SV40-specific surface antigen was shown to be present in the T antigen-positive cells, but not in the corresponding T antigen-negative cells, when tested by an SV40-specific serum mediated cytotoxicity microassay (Fig. 2).

Cells of both of the SV40 antigen-positive subclones 1 and 2 carry in a rescuable form²⁰ the SV40 virus used for the transformation: by co-cultivation with permissive (CV-1 monkey) cells and fusion with ultraviolet light-inactivated Sendai virus, from the heterokaryons formed, infectious SV40 small plaque-forming virus was recovered: 7×10^6 plaque-forming units (PFU) per ml from subclone 1, 2×10^5 PFU per ml from subclone 2.

Tumour cell lines

Tumours were induced repeatedly by injecting 10⁴ or 10⁵ cells of subclones 1 and 2, into the hind leg of syngeneic adult AL/N mice, and from the tumours cell lines were established as before⁸. Chronologically at first, four tumour cell lines were obtained: first tumour line from 10⁴ subclone 1 cells at t.c.t. 17 (after the SV40 infection) designated cell

line 113; second from 10⁵ subclone 1 cells at t.c.t. 25; third from 10⁴ subclone 2 cells at t.c.t. 21; fourth from 10⁵ subclone 2 at t.c.t. 22. All four tumours were hard, encapsulated, and were identified histopathologically as fibrosarcomas, similar⁸ to those induced by the T AL/N or the T AL/N C1 3 cells. All cells from all four tumours were found completely devoid of SV40 T antigen by the nuclear immunofluorescence test⁸ in primary culture, or after additional and prolonged culturing (>30 t.c.t.). The SV40 T antigen-positive cells apparently were rejected by the immunologically competent mice, and tumours grow up only from SV40 T antigen-negative cells.

Nude mice—congenitally athymic thus deficient in thymus-dependent immunity—would not be expected to reject the SV40 T, surface and transplanted antigen-positive cells. To test this, *nude* mice (random +/nu × nu/nu, from NIH Laboratory Animal Genetic Center) were injected with 2×10^5 subclone 1 cells, and tumours were obtained within 4 weeks in most animals (11 of 14 mice). Cells of these tumours (4 tested) were all positive for SV40 T antigen by both nuclear fluorescence and the quantitative complement fixation test, both in

Fig. 3 Tests for SV40-specific surface antigen in various cell lines. The % cell lysis is the same as % chromium release in Fig. 2, and represent residual cytotoxicity against the SV AL/N target cells, after absorption by the various cell lines. Control (no cells used for absorption): —▽—; absorption by subclone 5 cells: —□—; absorption by subclone 1 tumour cells from a syngeneic mouse (cell line 113): —△—; absorption by subclone 1 cells: —○—; absorption by subclone 1 tumour cell from a nude mouse: —□—. Absorption and assay conditions as in Fig. 2.

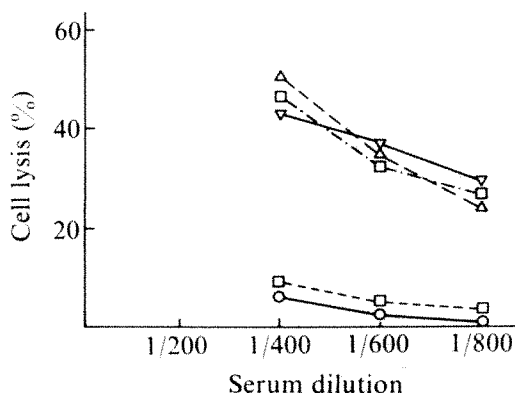


Table 2 Tests for SV40-specific transplantation antigens

Immunisation		Challenge		Tumour	<i>P</i>
Cell or extract	Dose number or mg	Cell type	Number	death/inoculated	
Experiment 1					
Subclone 1 cell	10 ³ (×4)	'M' SV AL/N T ²	10 ⁴	0/10	<0.05
	10 ³ (×4)		10 ⁵	0/10	<0.05
Subclone 2 cell	10 ³ (×4)		10 ⁴	0/10	<0.05
	10 ³ (×4)		10 ⁵	0/10	<0.05
Control	—		10 ⁴	4/10	
	—		10 ⁵	5/10	
Experiment 2a					
Subclone 1 extract	1	mKSA-ASC	10 ⁴	0/7	<0.005
	1		10 ⁵	1/8	<0.005
Subclone 5 extract	1		10 ⁴	4/7	NS
	1		10 ⁵	7/8	NS
T AL/N Clone 3 extract	1		10 ⁴	3/7	NS
	1		10 ⁵	7/8	NS
Control	—		10 ⁴	9/11	
	—		10 ⁵	20/20	
Experiment 2b					
Subclone-1 extract	1	Meth-A	10 ⁴	0/7	<0.005
	0.2		10 ⁴	2/7	<0.005
Control	—		10 ⁴	10/10	
Subclone 1 extract	1		10 ⁴	5/6	NS
	0.2		10 ⁴	5/7	NS
Control	—		10 ⁴	7/7	
Experiment 3					
Subclone 1 tumour 124 extract	1	mKSA-ASC	10 ⁴	5/5	
	0.5		10 ⁴	5/5	
	0.25		10 ⁴	5/5	
Control	—		10 ⁴	8/8	

In experiment 1 syngeneic AL/N mice were immunised by i.m. injection ×4 weekly apart (into right hind leg) of the given number of subclone 1 or 2 cells. These mice were challenged 1 week after the last immunisation by SV40-positive tumorigenic cells 'M' SV AL/N T², obtained and characterised previously⁸, by injection into their left hind leg. The number of progressively growing tumours in the left hind leg is given. In experiments 2a and b BALB/c mice were immunised by extracts (mg protein) of the various cells obtained with 0.5% Triton X-100 (refs 10, 16). The mice were challenged by an SV40-transformed BALB/c mouse kidney cell mKSA (ref. 3) which after repeated transplantation in the BALB/c mice⁹ was established in ascites form: mKSA-ASC¹⁷; it was shown before that cells in both forms contain SV40-specific transplantation antigen^{3,17}. For specificity control the challenge was by a chemically transformed Meth A BALB/c mouse embryo cell. Tumour deaths occurred by ascites or by fibrosarcoma at the site of challenge after about 1 month, groups were observed for 3 months. Experiment 3 shows the absence of SV40-specific transplantation antigen in the extract of tumour cells 124 (see text); these experiments were done similar to, and simultaneously with experiment 2. Control mice were injected with TBS. P values were calculated as before¹⁴; NS, not significant.

primary culture and also after various numbers (~10) of tissue culture transfers.

When tested for SV40-specific surface antigen, all tumour cell lines obtained from the syngeneic animals were devoid, but the tumour cells from the nude mice possessed this surface antigen. Examples, with controls, are given in Fig. 3; clearly, subclone 1 tumour cells (compare line 113) do not possess SV40-specific surface antigen. The subclone 1 tumour cell lines (for example 124, see below) were devoid of SV40-specific transplantation antigen (Table 2, experiment 3).

There are two possibilities to explain the *in vivo* selection of SV40 antigen negative tumour cells. The first possibility is that a T antigen-positive subclone, say subclone 1, is not a true subclone, but is consistently contaminated with a low number of (about 10⁻²) of untransformed T AL/N clone 3 cells (which cannot be detected even by the T antigen nuclear staining) and which can grow out as tumours; while the majority of the cells which are the SV40-specific T, surface and transplantation antigen-positive cells are all rejected by the immunocompetent mouse. The second possibility is that subclone 1 was indeed a good clone, free of T AL/N clone 3 cells, but a certain small (~1–0.1%) fraction or number of subclone 1 cells loses (the ability to express) SV40 antigens, and these revertant cells are then selected out to grow as tumours, while the SV40 T, surface, and transplantation antigen-positive cells, which are in great (about 10²–10³) excess, are all rejected.

Recloning of subclone 1 cells gave only T antigen-positive cells (six reclones tested). Co-cultivation experiments of known number of subclone 1 and T AL/N clone 3 cells

indicated no preferential growth of subclone 1 cells (data not shown). Both of these made it unlikely that subclone 1 had originated from the two type of cells. It took, however, a fortunate finding of characteristic marker chromosome translocation to virtually eliminate the first possibility. When chromosomes were banded from subclone 1 cells and from the derivative tumour cells 113, three common marker chromosomes, number 1, 2 and 3 in Fig. 4, were found clearly in each metaphase of these two cell lines. Metaphases were chosen at random and 17 metaphases from subclone 1, and 14 metaphases in the resulting tumour cells 113 were compared. However, when banding the T AL/N clone 3 cells (at t.c.t. 21, chromosome modality 38), in 20 randomly chosen metaphases none was found to possess the easily identifiable chromosomal marker 1, but clearly all were found to possess the easily identifiable chromosomal markers (classified as Robertsonian translocations) proving that the subclone 1 cells but not the T AL/N clone 3 cells are the direct parents of the tumour cells.

Being assured that subclone 1 is indeed a true clone, we used the subclone 1 cells to check the reproducibility of selecting SV40 antigen negative tumour cell lines by *in vivo* passage through the immunologically competent syngeneic mouse. At t.c.t. 12, 10⁴ subclone 1 cells repeatedly induced T and surface antigen negative tumour cell lines (see Table 3, tumour cell lines 127 and 128), as did 10⁵ subclone 1 cells (cell line 124). Six cloned lines were obtained from tumour cell line 124, these were designated as cell lines 129–134, and all were found to be T antigen negative. The tumorigenicity of tumour cells 124 was high (TD₅₀ = 10³), similar to other T antigen negative cells;

however, its clonal derivatives 129–134 had various TD_{50} values between 10^2 and $>10^4$, indicating that cloning may select for differences in tumorigenicity. These and other phenotypic data are summarised in Table 3.

Thus, the true clonal subclone 1 cells give reproducibly SV40 antigen negative tumours when passed through the immunologically competent mice. These tumours can be obtained after injection of as few as 10^4 or 10^5 cells. The tumour cells can possess high tumorigenicity ($TD_{50} \sim 10^2$) and the resulting fibrosarcomas never regress: the tumour cells in this respect are similar to the T AL/N C1 3 parent cells or to the T antigen-negative sister subclones. Apparently subclone 1 cells are prone to revert or segregate at a high frequency to a stably SV40 antigen negative cell line. Current studies by DNA reassociation kinetics indicate that subclone 1 cells contain approximately 1 genome equivalent of SV40 DNA, while the tumour lines or tumour clones tested contain about 0.5 copies per diploid complement of DNA which seem to be depleted in some of the early half of the SV40 genome, and which are not transcribed to RNA. These observations (J.M.K., P.T.M., M. Brown and G. Khoury, in the press) are consistent with the biological and chromosome findings.

The tumorigenicity in the syngeneic host of the cell lines investigated does not correlate with anchorage-dependent growth in viscous medium. There were highly tumorigenic cells with negligible or no observable growth in

Fig. 4 Marker chromosomes. Partial karyotypes of four subclone 1 cells (banded at t.c.t. 22) and four resulting tumour cell 113 metaphases (banded after 12 t.c.t. following the re-establishment) are compared pairwise in rows a, b, c, d. The three marker chromosomes present in these cells are arranged columns 1, 2 and 3. Chromosome(s) from the subclone 1 cells appear in left side of the columns. Chromosome(s) from the resulting tumour cells 113 appear in right side of column. Slides were stained with quinacrine-mustard for Q banding²¹, and photographed with a Zeiss photomicroscope III equipped for epi-fluorescent microscopy. After photomicrographic enlargements of the chromosomes, karyotypes were numbered and arranged according to size²². The subclone 1 cells were essential sub-diploid (chromosome modality 36) at t.c.t. 16, but some cells became polyploid when banded (t.c.t. 22) which explains why some of the marker chromosomes are present more than once. Tumour cells 113 were essentially sub-diploid (chromosome modality 33) at t.c.t. 8 and 12.

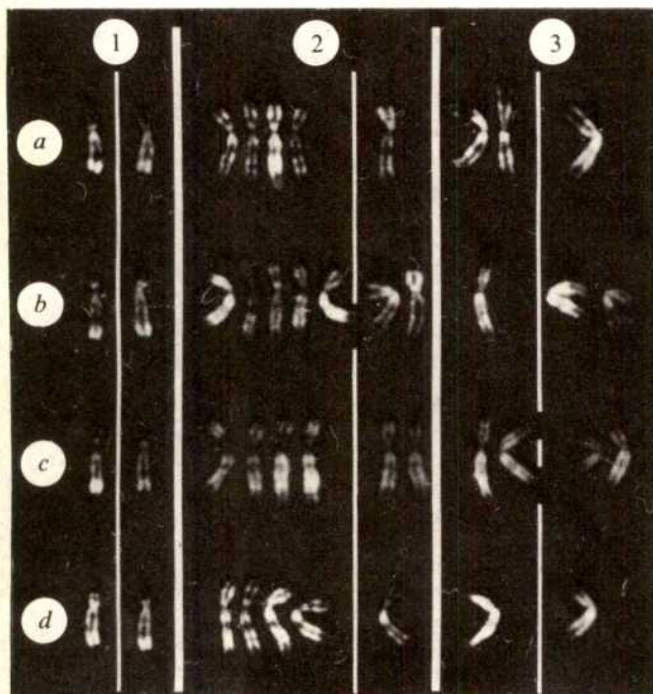


Table 3 Properties of the subclone 1 tumour cells and of clones of tumour line 124

Tumour cell line	Tumorigenicity (TD_{50})	Colony formation in viscous Methocell suspension (%)
113	NT	10
124	10^2	0.18
127	NT	0
128	NT	0.01
Clones of 124		
129	10^2	0.01
130	10^2	0.28
131	10^3	0
132	$>10^4$	1.5
133	10^3	0.12
134	$>10^4$	0

All tumour cells were SV40 T and surface antigen negative; all the clones of 124 were tested only for SV40 T antigen, they were all negative. The tumour line 124 was cloned by a single cell technique. Tumorigenicity and colony formation in viscous Methocell were determined as in Table 1. NT, not tested.

Methocell (see Tables 1, 5). We have similar observations on other BALB/c and AL/N highly tumorigenic cloned cells (data not shown). Neither did the presence and functioning of the SV40 early gene correlated absolutely with anchorage-independent growth: the tumour cell line 113 is SV40 antigen negative, but grows about as well in Methocell as the SV40 antigen-positive subclones 1 and 2. A recent publication appeared with partially contrasting claims in other less well matched cell systems²³.

The above experiments open up new kinds of genetic, immunochemical and biochemical studies using the strong *in vivo* antigen activity endowed to a mammalian tumour cell by transformation with SV40 to cause rejection. It also gives a novel perspective on biological consequences of SV40 transformation of cells, probably in the majority of the mammalian species. We emphasise, however, that the above experiments show that SV40 endows immunogenicity to, and allows recognition and rejection of, only those tumorigenic cells which were transformed by SV40, and may not alter the tumour-specific transplantation antigen associated with the spontaneously occurring or otherwise induced malignant transformation.

We thank Miss Cecily Sun for chromosomal banding, Drs O. J. and D. A. Miller for review of the marker chromosomes, and Dr L. W. Law for the ascites cell line.

Note added in proof: The revertant tumour cells (cf. clone 130) can be re-transformed by SV40, and on transplantation of a T antigen-positive clone T antigen-negative revertant tumour cell line can be obtained again.

Received 16 June; accepted 5 July 1977.

- Black, P. H. & Rowe, W. P. *Proc. Soc. exp. Bio. Med.* **114**, 721–727 (1963).
- Altstein, A. D., Vassiljeva, N. N. & Sarycheva, O. F. *Nature* **213**, 931–932 (1967).
- Kit, S., Kurimura, T. & Dubbs, D. R. *Int. J. Cancer* **4**, 384–392 (1969).
- Wesslen, T. *Acta path. microbiol. scand.* **B78**, 479–487 (1970).
- Teveshia, S. S. & McMillan, V. L. *Intervirology* **3**, 269–276 (1974).
- Aaronson, S. A. & Todaro, G. J. *Science* **162**, 1024–1026 (1968).
- Wright, P. W., Smith, H. S. & McCoy, J. J. *natn. Cancer Inst.* **51**, 951–959 (1973).
- McFarland, V. W., Mora, P. T., Schultz, A. & Pancake, S. J. *cell Physiol.* **85**, 101–111 (1975).
- Smith, R. W., Morganroth, J. & Mora, P. T. *Nature* **227**, 141–145 (1970).
- Chang, C., Pancake, S. J., Luborsky, S. W. & Mora, P. T. *Int. J. Cancer* **19**, 258–266 (1977).
- Pancake, S. J. & Mora, P. T. *Virology* **59**, 323–327 (1974).
- Mora, P. T., Brady, R. O., Bradley, R. M. & McFarland, V. W. *Proc. natn. Acad. Sci. U.S.A.* **63**, 1290–1296 (1969).
- Anderson, J. L., Chang, C., Mora, P. T. & Martin, R. G. *J. Virology* **21**, 459–467 (1977).
- Anderson, J. L., Martin, R. G., Chang, C., Mora, P. T. & Livingston, D. M. *Virology* **76**, 420–425 (1977).
- Deppert, W. & Walter, G. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2505–2509 (1976).
- Luborsky, S. W., Chang, C., Pancake, S. J. & Mora, P. T. *Biochem. biophys. Res. Commun.* **71**, 990–996 (1976).
- Drapkin, M. S., Appella, E. & Law, L. W. *J. natn. Cancer Inst.* **52**, 259–264 (1974).
- Smith, R. W. & Mora, P. T. *Virology* **50**, 233–246 (1972).
- Pancake, S. J. & Mora, P. T. *Cancer Res.* **36**, 88–94 (1976).
- Takemoto, K. K., Todaro, G. J. & Habel, K. *Virology* **35**, 1–8 (1968).
- Casperson, T., Zech, L., Johansson, C. & Modest, E. J. *Chromosoma* **30**, 215–227 (1970).
- Committee on Standardized Genetic Nomenclature for Mice. *Standard karyotype of the mouse, Mus musculus*. *J. Hered.* **63**, 69 (1972).
- Wright, T. C., Ukena, T. E., Campbell, R. & Karnovsky, M. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 258–262 (1977).

letters to nature

Upper limits for the radio pulse emission rate from exploding black holes

IT has been suggested¹ that the explosion of a primordial black hole (pbh) might produce a coherent radio pulse detectable at a distance of $\sim 10^4$ pc with a simple antenna. Several searches²⁻⁵ for radio pulses arriving from random directions in space, such as might be produced by supernovae⁵⁻⁷, have already been carried out using wide beam (all-sky) antennas, and the negative results obtained are used here to place limits on the pbh radio pulse emission rate per unit volume. An indication has already been given⁸ of the limits which some previous radio searches impose, and of the influence of antenna aperture and beamwidth on achievable limits. This paper presents a more general analysis of limit criteria, including consideration of the effects on the pulse timescale of the interstellar medium, and examines the factors which determine the optimum search strategy for pbh radio pulses. It is reported that the best radio limit⁴ known to the author, could be improved by at least times 10^6 using existing techniques. In addition, examination of this existing limit⁴ shows that it is already ~ 80 times better (99.5% confidence level) than a limit which it has been suggested⁸ might be attainable in the optical region, and is $\sim 10^6$ times better than pbh explosion rate limits obtained from γ -ray observations^{9,10}, although the γ -ray limits do not involve the generation of a coherent electromagnetic pulse.

We assume that the pbh's are concentrated in galactic haloes (radii ≈ 20 kpc)⁹ and that the pulse energy, E_t , in J (ref. 1) is given by

$$E_t \approx 1.5 \times 10^{28} \nu^{-0.33} \quad (1)$$

where ν is the characteristic frequency (Hz) of emission. The value of the characteristic frequency is highly uncertain¹, and so the situation is considered where ν is equal to the frequency of observation. The attainable limit, L (volume⁻³ time⁻¹), established by a negative search at the 99.5% confidence level is given by

$$L \approx 5 (VS)^{-1} \quad (2)$$

where S is the duration of the search. V , the volume of space observed above the threshold sensitivity, is given by

$$V \approx (\Omega/3) R_M^3 \quad (3)$$

where Ω is the antenna beam solid angle and R_M is the maximum distance at which detection is possible. We adopt a detection threshold of 5σ signal-to-noise ratio and examine the factors which determine R_M . For an antenna of gain g (relative to an isotropic radiator), and receiver bandwidth $\Delta\nu$, the pulse energy collected (E_{col}) is at least

$$E_{\text{col}} \approx \frac{1}{4} (E_t/4\pi R_M^2) (\Delta\nu/\nu) (gc^2/4\pi\nu^2) \quad (4)$$

$$= 2 \times 10^{42} \nu^{-3.33} \Delta\nu R_M^{-2} g \quad \text{J} \quad (5)$$

where c is the velocity of light and R_M is in metres. (The factor of $\frac{1}{4}$ allows for the source being up to -3 dB away from the beam axis ($\frac{1}{2}$) and for only one polarisation being observed ($\frac{1}{2}$)).

The minimum pulse energy, E_{min} , which the radiometer can detect, is

$$E_{\text{min}} \approx 5kT(\Delta\nu\tau)^{0.5} \quad (6)$$

(where k is Boltzmann's constant, T is the system temperature, and τ is the integration time), provided that the pulse duration at the integrator input, $t_p \leq \tau$. The intrinsic timescale of the pulse is $\sim 1/\nu$ (ref. 1), so that t_p is principally determined by the radiometer response time $\approx 1/\Delta\nu$, by interstellar dispersion broadening over the band $\Delta\nu$, and by interstellar scattering. If $\Delta\nu$ is gradually increased, the signal-to-noise ratio will improve until the pulse time scale is dominated by dispersion. Once this stage is reached ($\Delta\nu \approx 10^4 - 10^5$ Hz for typical dispersion measures) little further improvement in signal-to-noise ratio is obtainable by further increase in $\Delta\nu$ (see equation (9) below), unless de-dispersion is used. The timescale, t_p , in s due to dispersion is given by

$$t_D = 2.8 \times 10^{-7} \nu^{-3} \Delta\nu DM \quad (7)$$

where DM is the dispersion measure in m^{-2} . Setting $t = t_D = \tau$ in equation (6) above gives

$$E_{\text{min}} \approx 4 \times 10^{-26} T \nu^{-1.5} \Delta\nu (DM)^{0.5} \quad \text{J} \quad (8)$$

where T is in Kelvins. Equating E_{col} and E_{min} , gives

$$R_M \approx 8 \times 10^{33} \nu^{-0.92} T^{-0.5} g^{0.5} (DM)^{-0.25} \quad \text{m} \quad (9)$$

$\Delta\nu$ has been eliminated from this expression.

In general, the system temperature, T , is a function of both sky noise and receiver noise. To minimise T (and DM), observations should be directed towards the regions of the galactic poles, where (for the north galactic pole (NGP)) the sky temperature, T_{sky} , is given by¹¹⁻¹³

$$T_{\text{sky}} \approx 2 \times 10^{24} \nu^{-2.7} \quad (10)$$

This gives $T_{\text{sky}} \approx 80$ K at 200 MHz. Above this frequency, receiver noise will begin to dominate the system noise, so that further increase in ν will tend to reduce sensitivity. Therefore, for observations near the NGP at $\nu \approx 200$ MHz with a sky noise limited system, equation (9) becomes

$$R_M \approx 5 \times 10^{21} \nu^{0.43} g^{0.5} (DM)^{-0.25} \quad (11)$$

To estimate DM , a model is adopted¹⁴ in which the electron distribution is uniformly stratified parallel to the galactic plane and has an exponential distribution perpendicular to the plane

with a scale height of 600 pc (refs 14, 15) and a density at the plane of $3.3 \times 10^4 \text{ m}^{-3}$. Thus, DM is given in m^{-2} by

$$DM \simeq 6 \times 10^{23} (1 - \exp(-R_M \sin b / 1.8 \times 10^{19})) / \sin b \quad (12)$$

where b is the galactic latitude. If $g \geq 10$, the antenna beam limits (-3 dB) will be less than 30° from the NGP so that to a good approximation, $\sin b \simeq 1$. For $g = 10$ (a small, simple antenna) and $\nu = 200 \text{ MHz}$, equations (11) and (12) give $R_M \simeq 2.2 \text{ kpc}$. Putting the expression for R_M given by equation (11) into equation (3) gives as the volume of space observed

$$V \simeq 7 \times 10^{65} \nu^{1.3} g^{0.5} (DM)^{-0.75} \text{ m}^3 \quad (13)$$

which, for $g = 10$ and $\nu = 200 \text{ MHz}$, is $6 \times 10^9 \text{ pc}^3$. Thus, observation for one year (assuming no pulses were detected) could yield a 99.5% confidence limit of $\sim 10^{-9} \text{ pc}^{-3} \text{ yr}^{-1}$. Use of an antenna 10 times larger in diameter ($\sim 15 \text{ m}$ at 200 MHz) for the same observation period might improve this limit 10-fold and would observe to the edge of the halo. The long observation times required for further limit improvement may rule out the use of still larger antennas. An Arecibo-type antenna, however, using existing de-dispersion facilities might just reach out to $\sim 700 \text{ kpc}$ in the approximate direction of the NGP, but to be worthwhile, the increased volume would have to contain an external galaxy. A more effective way of improving the limit would be to use a multiply-phased array. A 10×10 element array at 200 MHz could observe to the halo edge, and still maintain a $\sim 1\text{-sr}$ solid angle, thereby offering a potential 1,000-fold increase in the volume of space observed, relative to a single element ($g = 10$), and so attain a limit of $\sim 10^{-12} \text{ pc}^{-3} \text{ yr}^{-1}$ in 1 yr observation.

The above results for R_M and V are obtained assuming a rather idealised situation in which the characteristic emission frequency is $200 \text{ MHz} \simeq$ observation frequency, and observations directed towards the NGP are carried out with a sky noise limited system and correctly optimised integration time. For this situation, and with $g \geq 10$, $R_M \geq 4 \times$ scale height of the electron distribution, so that to a good approximation DM is independent of R_M and from equation (13)

$$V \propto g^{0.5} \quad (14)$$

Previous all-sky searches, however, included observations both near to and far from the NGP, and were generally not sky noise limited and did not have optimised integration times. The effect was that these searches were usually confined within the galactic plasma, so that DM was no longer approximately independent of R_M (equation (12)) and the proportionality in equation (14) was not necessarily valid. For any particular search

$$R_M = (E_t / (4\pi W \nu t_p))^{0.5} \quad (15)$$

where W is the search flux threshold per unit bandwidth, and the output pulse timescale, t_p , is determined by the dispersion, scattering, bandwidth and integration time which the pulse encounters. In all previous searches, $t_p \simeq t_D$, so that when $\tau \gg t_p$, $t_p \simeq \tau$, and putting the expression for R_M given by equation (15) into equation (3) shows that proportionality (14) is applicable in this case. When $\tau \leq t_D$, V must be obtained, however, by solving equations (12) and (15) for R_M and then substituting the expression obtained into equation (3). The result is that when $\tau \leq t_D$, V is a weaker function of g than it is in equation (14) and in the extreme case where approximately $DM \propto R_M$ (for example, when observing

along the galactic plane) and $\tau \ll t_D$, V is independent of g . Thus, it is important to observe well out of the galactic plane and have $\tau \simeq t_D$.

Table 1 gives the best pbh radio pulse rate limits I know (99.5% confidence level) within the frequency decades 10–100 MHz, 100 MHz–1 GHz, and 1–10 GHz, and the actual frequencies at which they were obtained. In each case, it is assumed that the characteristic emission frequency equals the observation frequency. Also shown are the corresponding values of R_M for each

Table 1 Primordial black hole radio pulse rate limits

Reference	Frequency range (MHz)	Rate limit ($\text{pc}^{-3} \text{ yr}^{-1}$)	Frequency (MHz)	Distance, R_M (pc)
*	10–100	1.5×10^{-6}	45	370
4	100–1,000	5×10^{-7}	270	975
5	1,000–10,000	4×10^{-5}	1,400	525

* Unpublished observations at 45 MHz, December 1970 to March 1971, carried out by R. W. P. Drever and W. P. S. Meikle (Glasgow), G. A. Baird, T. Delaney and B. G. Lawless (Dublin), R. A. Porter and R. E. Spencer (Jodrell Bank).

search. The searches at 45 MHz and 1.4 GHz had $\tau < t_D$, and the 270-MHz search had $\tau > t_D$. These limits are 10^4 – 10^6 times better than the pbh explosion rate limits achieved from γ -ray studies^{9,10}.

I thank Professor R. W. P. Drever for many valuable comments and suggestions, and also Professor S. A. Colgate, Dr J. Hough and Dr R. D. Joseph for helpful discussions.

W. P. S. MEIKLE

Astronomy Group,
Physics Department,
Imperial College of Science and Technology,
London, UK

Received 1 June; accepted 28 June 1977.

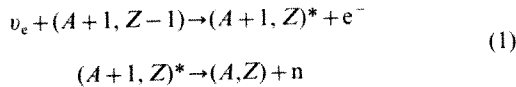
- ¹ Rees, M. J. *Nature* **266**, 333–334 (1977).
- ² Charman, W. N. *et al.* *Nature* **228**, 346–348 (1970).
- ³ Troitskii, V. S. *et al.* *Radiophys. Quant. Electron.* **16**, 239–252 (1975). *Trans. Radiofiz.* **16**, 323–341 (1973).
- ⁴ Huguenin, G. R. & Moore, E. L. *Astrophys. J. Lett.* **187**, L57–L58 (1974).
- ⁵ Colgate, S. A., McKee, C. R. & Blevins, B. *Astrophys. J. Lett.* **173**, L87–L91 (1972).
- ⁶ Jelley, J. V., Baird, G. A. & O'Mongain, E. *Nature* **267**, 499–500 (1977).
- ⁷ Page, D. N. & Hawking, S. W. *Astrophys. J.* **206**, 1–7 (1976).
- ⁸ Porter, N. A. & Weekes, T. C. *Astrophys. J.* **212**, 224–226 (1977).
- ⁹ Sironi, G. *Mon. Not. R. astron. Soc.* **166**, 345–353 (1974).
- ¹⁰ Webster, A. S. *Mon. Not. R. astron. Soc.* **166**, 355–371 (1974).
- ¹¹ Daniel, R. R. & Stephens, S. A. *Space Sci. Rev.* **17**, 45–158 (1975).
- ¹² Readhead, A. C. S. & Duffett-Smith, P. J. *Astron. Astrophys.* **42**, 151–153 (1975). *Mon. Not. R. astron. Soc.* **174**, 7–17 (1976).
- ¹³ Condon, J. J. & Backer, D. C. *Astrophys. J.* **197**, 31–38 (1975).

Neutrino-induced nucleosynthesis and deuterium

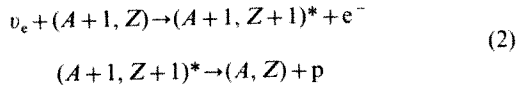
DOMOGATSKY and Nadyozhin¹ have suggested that the neutrino flux from a collapsing stellar core might induce nuclear transformation in the mantle and envelope of the surrounding pre-supernova star. They further suggest that such weak interactions might be responsible for producing the so called 'p-process' nuclei which are the rare heavy elements bypassed by the neutron capture chains responsible for synthesising most nuclei heavier than iron. This letter points out a weakness in their model as a mechanism for p-nucleosynthesis and concurrently suggests a rather novel method for the synthesis of deuterium that may have been operative during an early generation of supermassive stars ($M \sim 10^6$ – $10^8 M_\odot$).

First, consider the conditions requisite for producing p-process nuclei from the more abundant neighbouring r- and s-process isotopes (see ref. 2 for an explanation of this nomenclature).

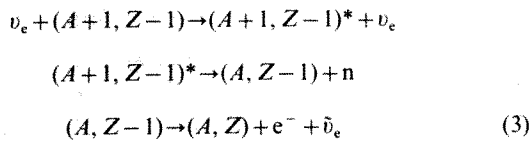
One might invoke the charged-current processes suggested by Domogatsky and Nadozhin for producing the nucleus (A, Z)



where $(A+1, Z)^*$ is a neutron unbound state of the nucleus having atomic mass $A+1$ and Z protons or by



In addition to the charged-current processes that they suggest, one might also add the neutral current process



and others that come to mind. The cross section, however, for each of these processes is very small, Domogatsky and Nadozhin estimate a value $\sigma_\nu \sim 10^{-41} \text{ cm}^2$ as typical. According to Cameron³ the relative abundance of p -nuclei to neighbouring nuclei produced by neutron capture is about 1%. Therefore, the production factor

$$P = \phi_\nu \sigma_\nu \gtrsim 0.01 \quad (4)$$

where

$$\phi_\nu = \frac{E_{\nu(\text{tot})}}{4\pi R^2 E_\nu} \quad (5)$$

R is the radius from the collapsing, neutrino-emitting core to the mass shell where the neutrino-induced nucleosynthesis occurs, and E_ν is the mean energy of the emitted neutrinos. An upper bound to the total energy emitted in neutrinos is the binding energy of a typical neutron star, 10^{53} erg . Actually a smaller number, say 10^{52} erg , is probably more appropriate⁴. Using $E_\nu = 10^{52} \text{ erg}$ and $E_\nu = 15 \text{ MeV}$ one finds from equation (4) that $R \lesssim 2 \times 10^8 \text{ cm}$.

Problems arise when the conditions extant at $R = 2 \times 10^8 \text{ cm}$ in a realistic pre-supernova star^{5,6,7} are examined. For most massive pre-supernova stars of interest (say $M \gtrsim 15 M_\odot$) this region lies interior to the neon burning shell and probably interior to the oxygen burning shell and is characterised by temperatures in excess of $2 \times 10^9 \text{ K}$. At such high temperatures, all heavy seed nuclei will be rapidly photodisintegrated to iron-group nuclei. It can be estimated, using rates given by Woosley and Howard⁸, that at $T = 2 \times 10^9 \text{ K}$ all seed nuclei heavier than $A = 150$ will be destroyed by photodisintegration reactions in a time of only a few thousand seconds. This is considerably less than the time occupied by oxygen and silicon burning in the core of the star, hence seed nuclei with $A \geq 150$ are not present at $R = 2 \times 10^8 \text{ cm}$ when the star blows up. Actually the problem is even more severe than so far described. The number 0.01 used in equation (4) is applicable only if all the r - and s -process nuclei ever produced have been exposed to precisely the conditions described. Since in reality the fraction of seed nuclei so exposed has been very small the required production ratio must be correspondingly larger, $P = 0.1$, for example, if only

10% has been irradiated. Thus the required exposure radius is smaller, the temperature higher, and the likelihood of seed survival smaller. The p -process nuclei can, on the other hand, be produced by more traditional strong and electromagnetic processes that occur farther out in the star as has been recently shown by Woosley and Howard⁸.

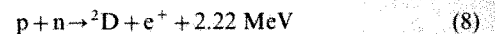
Consider now another possibility for neutrino-induced nucleosynthesis. The reaction



can produce a neutron flux in the hydrogen envelope surrounding a strong anti-neutrino source. It is important to note that anti-neutrinos are required for this process. Neutrinos, as result from electron capture and positron decay, for example, will not work. If the density of the hydrogen exceeds roughly

$$\gtrsim 10^{-8} \left(\frac{1 \text{ mb}}{\sigma_{n\gamma}} \right) \left(\frac{10^8 \text{ cm s}^{-1}}{v_T} \right) \text{ g cm}^{-3} \quad (7)$$

these neutrons will be captured before decaying. Here v_T is the mean velocity of the neutron and $\sigma_{n\gamma}$ is the radiative capture cross section in millibarns. It is assumed here that inelastic scattering will reduce the neutron energy to some relatively low value ($\sim \text{keV}$) before capture occurs. Even though heavy elements such as ^{56}Fe have substantially larger neutron capture cross sections than hydrogen it is assumed that the numerical preponderance of H-nuclei will make them more likely candidates for capturing the neutrons produced by equation (6). Thus deuterium is formed by



The viability of this mechanism for deuterium production depends upon (1) the existence of a sufficient anti-neutrino flux to produce D/H of at least 2×10^{-5} (ref. 9), the currently accepted interstellar value, and (2) the material irradiated being cool enough so that the deuterium formed is not destroyed by ${}^2\text{D}(p, \gamma){}^3\text{He}$ before ejection. First I shall show that supernova probably cannot satisfy these requirements.

The anti-neutrino flux from a supernova explosion is thermally produced and is not as large as the neutrino flux which receives a big contribution from electron capture. Assume the collapsing core emits 10^{52} erg of 15 MeV anti-neutrinos (this would be an upper bound). The cross section for the process in equation (6) at $E_\nu = 15 \text{ MeV}$ can be calculated¹⁰ to be roughly $2 \times 10^{-41} \text{ cm}^2$. From equations (4) and (5) the production ratio at a radius R will then be

$$P = 7 \times 10^{-6} (10^{10} \text{ cm}/R)^2$$

Since the radius of the hydrogen burning shell in a highly evolved massive star⁷ exceeds $2 \times 10^{10} \text{ cm}$ one cannot produce sufficient quantities of deuterium to account for present abundance determinations. This argument is strengthened by the fact that the hydrogen temperature does not drop below 10^7 K until $R \gtrsim 10^{11} \text{ cm}$ thus deuterium formed interior to this radius would be destroyed in less than 1,000 s (ref. 11) (less than the speed of sound communication time to the core).

I will end this letter, however, with a speculation on a site where equation (6) might function with greater efficiency. Some models for active galactic nuclei and quasars suggest the existence early in a galaxy's lifetime of supermassive stars and/or black holes¹². The explosion of a $10^8 M_\odot$ star might produce 10^{60} erg (ref. 13). The formation of a $10^6 M_\odot$ black hole would emit a similar quantity of

energy. If the collapse of these objects produces matter at very high temperature ($T \gtrsim 10^{10}$ K) a substantial fraction of this energy might be emitted as anti-neutrinos. If one takes 10^{59} erg, $E_\nu = 15$ MeV, and $R = 10^{12}$ cm, for example, a production ratio $D/H \sim 7 \times 10^{-3}$ could be attained. Even if a small fraction of all the hydrogen in a galaxy were processed through these conditions the present abundance of deuterium could be understood.

This research has been supported in part by the National Science Foundation (grant Number AST76-10933).

S. E. WOOSLEY

Lick Observatory,
Board of Studies in Astronomy and Astrophysics,
University of California, Santa Cruz 95064

Received 31 May; accepted 28 June 1977.

- ¹ Domogatsky, G. V. & Nadyozhin, D. K. *Mon. Not. R. astron. Soc.* **178**, 33 (1977).
- ² Clayton, D. D. (ed.) *Principles of Stellar Evolution and Nucleosynthesis* (McGraw-Hill, New York, 1968).
- ³ Cameron, A. G. W. in *Explosive Nucleosynthesis* (eds Schramm, D. N. & Arnett, W. D.) (University of Texas Press, Austin, 1972).
- ⁴ Wilson, J. R. *Phys. Rev. Lett.* **32**, 849 (1974).
- ⁵ Arnett, W. D. *Astrophys. J.* **194**, 373 (1974).
- ⁶ Enrico Fermi Institute Preprint 77-6, (submitted to *Astrophys. J.*, 1977).
- ⁷ Weaver, T. A. & Woosley, S. E. paper, presented at American Astronomical Society meeting, Honolulu, 1977.
- ⁸ Woosley, S. E. & Howard, W. M. Lawrence Livermore Lab. Preprint UCRL-78933.
- ⁹ Rogerson, J. B. & York, D. G. *Astrophys. J. Lett.* **186**, L95 (1973).
- ¹⁰ Tubbs, D. L. & Schramm, D. N. *Astrophys. J.* **201**, 467 (1975).
- ¹¹ Fowler, W. A., Caughlan, G. R. & Zimmerman, B. A. *Ann. Rev. Astron. Astrophys.* **13**, 69 (1975).
- ¹² Thorne, K. S. & Braginsky, V. B. *Astrophys. J. Lett.* **204**, L1 (1976).
- ¹³ Fricke, K. J. *Astrophys. J.* **189**, 535 (1974).

Support for the astronomical theory of climatic change

AMONG recent papers supporting the astronomical theory of climatic change¹⁻⁵, the work of Hays *et al.*⁵ is of particular importance in providing detailed quasi-periods from deep-sea cores. I wish to draw attention here to the fact that the periods found are very close to the periods predicted by Berger^{6,7} in the latest and most accurate calculation of the variations of the various 'Milankovitch' parameters. The roughly 24,000- and 19,500-yr periods from the core samples are not significantly different from the periods associated with the largest amplitude terms in the series expansion of the precessional parameter $e \sin \tilde{\omega}$; the periods around 42,000 yr are essentially identical with the most important term in the expansion of the obliquity ε ; and the peaks in the range of 106,000 yr containing most of the variance, might be regarded as either a contribution from the eccentricity, where a weighted mean of the main amplitude terms has a period of 110,753 yr, or as a beat effect of precessional periods, as has been noted by Wigley⁸.

The astronomical theory of climatic change, originating from Croll⁹ and developed by Milankovitch¹⁰ has been widely discussed and needs no elaboration here. To establish the validity of

that theory, the following three criteria must be met. (1) Are the quasi-periodicities of the Earth's orbital elements significantly present in the geological record?¹¹ (2) Is there any significant correlation between insolation curves and geological data?¹² (3) Can these insolation changes be correlated with climatic changes^{13,14}?

I have previously^{6,7} demonstrated the accuracy of a new astronomical solution for the long-term variations of the Earth's orbital elements. From theoretical formulae given in refs 6 and 7 it is possible to write, in an easily applicable form, the series expansion of astronomical elements required in the computation of the insolation, that is, the eccentricity e of the Earth's orbit, the precessional term $e \sin \tilde{\omega}$ ($\tilde{\omega}$ being the longitude of the perihelion measured from the moving vernal equinox) and the obliquity ε (inclination of the equator on the ecliptic):

$$\begin{aligned} e &= e_0 + \sum E_i \cos(\lambda_i t + \phi_i) \\ e \sin \tilde{\omega} &= \sum P_i \sin(\alpha_i t + \beta_i) \\ \varepsilon &= \bar{\varepsilon} + \sum A_i \cos(\gamma_i t + \delta_i) \end{aligned}$$

where $e_0 = 0.028\,706\,9$, $\bar{\varepsilon} = 23^\circ.320\,556$ and time $t = 0$ refers to 1950 AD.

Trigonometric series of this type not only make e , $\tilde{\omega}$ and ε more easily computable, but also provide us directly with a power spectral analysis of their time variation. As in this paper, the main interest lies in the spectrum of the astronomical elements, the period and mean rate associated with the only main largest amplitude terms have been reproduced in Tables 1-3. The computation of e , $e \sin \tilde{\omega}$ and ε will be detailed, and all terms of the trigonometrical expansions will be listed elsewhere.

Table 2 Main terms in the series expansion of the obliquity ε

	Amplitude, A_i	Mean rate, γ_i (" per yr)	Period (yr)	Phase, δ_i (deg)
1	-2,462.2	31.609 974	41,000	251.9
2	-857.3	32.620 504	39,730	280.8
3	-629.3	24.172 203	53,615	128.3
4	-414.3	31.983 787	40,521	292.7
5	-311.8	44.828 336	28,910	15.4
6	308.9	30.973 257	41,843	263.8
7	-162.5	43.668 246	29,678	308.4
8	-116.1	32.246 691	40,190	240.0
9	101.1	30.599 444	42,354	223.0
10	-67.7	42.681 324	30,365	268.8

Mean period over 5 Myr, 41,000 yr.

Taking the precessional component, $e \sin \tilde{\omega}$, the four largest terms have periods of 23,716, 22,428, 18,976 and 19,155 yr. The first two and the last two clearly correspond respectively to the 24,000- and 19,500-yr periods found by Hays *et al.* in deep-sea cores, with the splitting of the theoretical mean period 21,700 yr in two peaks, depending on the resolution of their spectral analysis.

In the obliquity term, not only the dominant component has a period of 41,000 yr but also 5 of the next 10 largest components have periods very close to 41,000 yr. That explains quite well the steadiness of the quasi-periodicity observed in the long-term variations of ε : the mean period over the past 5 Myr is 41,000 yr and over the next 1 Myr it will be 41,040 yr.

For the eccentricity, the situation is still more complicated. As suggested in Berger⁷, the most important term has a periodicity of 412,085 yr—too long to be clear in the analysis of Hays *et al.*—and the convergence of the series is very slow. Because of this, while the weighted mean periodicity for terms 2 to 5 in the expansion is 111,000 yr, the average period from the computed values of e is 95,800 yr, during the past 5 Myr; the prediction for the next 1 Myr being a mean of 91,400 yr. If longer cores can be investigated by

Table 1 Main terms in the series expansion of the precession parameter $e \sin \tilde{\omega}$

	Amplitude, P_i	Mean rate, α_i (" per yr)	Period (yr)	Phase, β_i (deg)
1	0.018 608 0	54.646 478	23,716	32.0
2	0.016 275 2	57.785 364	22,428	197.2
3	-0.013 006 6	68.296 536	18,976	311.7
4	0.009 888 3	67.659 819	19,155	323.6
5	-0.003 367 0	67.286 006	19,261	282.8
6	0.003 330 8	55.638 352	23,293	90.6
7	-0.002 354 0	68.670 349	18,873	352.5
8	0.001 400 2	76.656 031	16,907	131.8
9	0.001 007 0	56.798 442	22,818	157.5

Mean period over the previous 5 Myr: 21,700 yr.

techniques allowing high resolution spectrum, I would expect the 100,000-yr peak to be split into peaks at 95,000 and 123,000 yr, with the main period of 413,000 yr becoming strongly displayed. These 95,000- and 123,000-yr periods are related either to a beat respectively between terms 1 and 3 and between terms 2 and 3 of the $e \sin \omega$ series expansion (Table 1), and to terms numbered 2 and 3 in series expansion of the eccentricity (Table 3).

Table 3 Main terms in the series expansion of the eccentricity e

	Amplitude, E_i	Mean rate, λ_i (" per yr)	Period (yr)	Phase, ϕ_i (deg)
1	0.011 029 40	3.138 886	412,885	165.2
2	-0.008 732 96	13.650 058	94,945	279.7
3	-0.007 492 55	10.511 172	123,297	114.5
4	0.006 723 94	13.013 341	99,590	291.6
5	0.005 812 29	9.874 455	131,248	126.4
6	-0.004 700 66	0.636 717	2,035,441	348.1
7	-0.002 544 64	12.639 528	102,535	250.8
8	0.002 314 85	0.991 874	1,306,618	58.6
9	-0.002 219 55	9.500 642	136,412	85.6
10	0.002 018 68	2.147 012	603,630	106.6
11	-0.001 723 71	0.373 813	3,466,974	40.8
12	-0.001 661 12	12.658 184	102,384	221.1
13	0.001 313 42	12.021 467	107,807	233.0

Mean period over the previous 5 Myr, 95,800 yr.

This prediction is in line with Table 4 of Hays *et al.*⁵ and also with the evidence from their Fig. 9 which suggests a positive correlation between summer temperature and eccentricity during the past 500,000 yr. Indeed, looking for the influence of e alone on the insolation, we can see that it acts only through the $(1-e^2)^{-1/2}$ factor in the total energy received by the Earth¹⁵. As e oscillated between 0.000483 and 0.060791 in the past 5 Myr, it is easily found that the total variation in solar radiation received by the Earth as compared with the present value, is no more than -0.17% or $+0.014\%$. These variations are small, but they are in the right direction according to the results of Hays *et al.*: they are positively correlated with the change in summer temperature and not in the opposite sense, as required by Milankovitch¹⁰, who stated that the influence of e was almost exclusively through the $e \sin \omega$ term. As a consequence, from an astronomical point of view, the 100,000-yr period seems to be closely related either to the nonlinear interaction between the two precession peaks and to the eccentricity period itself if Fig. 9 of ref. 5 can be confirmed.

Some important conclusions may be drawn from the fact that the obliquity peak in geological records is smaller than the $\sim 105,000$ -yr peak or, at least, less dominant than formerly thought¹⁶. Analysing the solar radiation available on the assumption of a perfectly transparent atmosphere¹⁷, it can be deduced that, on the one hand: (1) The insolation received at the equinoxes and the differences between the length of the summer and of the winter astronomical seasons, are functions only of the precessional parameter, $e \sin \omega$. And (2), at the solstices, this parameter has a larger influence than the obliquity. On the other hand: (1) During astronomical seasons, the insolation received at any latitude is a function of the obliquity e . And (2), at high latitudes, the deviations of the insolation from present day values for the caloric seasons are mostly functions of e (ref. 18).

Thus, as the 'eccentricity-precessional beat' period seems to have an important role in past climates as recorded in geological data, the seasonal contrast and the equinoctial or, better, monthly¹⁹ insolation values have to be considered in the modelling of the glacial-interglacial climate fluctuations in addition to or instead of the usual astronomical-caloric seasons frequently used.

Analysing the remaining terms in the trigonometrical expansion of $e \sin \omega$ and e , it seems that the next periods that could be found in geo-ecological records would be, for $e \sin \omega$ around 15,000 and 50,000 yr, for e around 21,000, 31,000 and 54,000 yr and, as far as a combined effect of both precession and obliquity is concerned,

59,000 and 64,000 yr. Discovery of these peaks would remove any remaining doubts about the validity of the astronomical theory of climatic change.

I thank Dr J. Gribbin for reading the manuscript.

A. L. BERGER

*Institut d'Astronomie et de Géophysique,
Université Catholique de Louvain,
Chemin du Cyclotron 2,
1348 Louvain-la-Neuve, Belgium*

Received 9 May; accepted 5 July 1977.

- Kukla, G. J. *Nature* **253**, 600-603 (1975).
- Weertman, J. *Nature* **261**, 17-20 (1976).
- Suarez, M. J. & Held, I. M. *Nature* **263**, 46-47 (1976).
- Johnson, R. G. & McClure, B. T. *Quat. Res.* **6**, 325-355 (1976).
- Hays, J. D., Imbrie, J. & Shackleton, N. J. *Science* **194**, 1121-1132 (1976).
- Berger, A. *Celestial Mech.* **15**, 53-74 (1977).
- Berger, A. *Astron. Astrophys.* **51**, 127-135 (1976).
- Wigley, T. M. L. *Nature* **264**, 629-631 (1976).
- Croll, J. *Theory of Secular Changes of the Earth Climate* (Appleton, New York, 1875).
- Milankovitch, M. *Kongl. Serb. Akad. Beograd Spec. Publ.* **132** (1941) (translated by the Israel Program for Scientific Translations, Jerusalem, 1969).
- Mitchell, J. M., Jr. *Quat. Res.* **6**, 481-493 (1976).
- Broecker, W. S. & van Donk, J. *Rev. Geophys. Space Phys.* **8**, 169-198 (1970).
- Berger, A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **21**, 227-235 (1977).
- Gates, W. L. *Science* **191**, 1138-1144 (1976).
- Berger, A. in *WMO/IAMAP Symposium on Long-Term Climatic Fluctuations*, *WMO* **421**, 65-72 (1975).
- Broecker, W. S. *Meteorological Monograph* **8**, 139-141 (1968).
- Berger, A. *Ann. Soc. scient. Bruxelles* **89**, 69-91 (1975).
- Bernard, E. *Acad. R. Sciences Outre-Mer. Sciences Naturelles Méd., nouv. sér.* **12** **1** (1962).
- Berger, A. *EOS Trans. Am. Geophys. Union* **57**, 254 (1976).

LaNi_{5-x}Al_x is a versatile alloy system for metal hydride applications

METAL alloys which reversibly absorb and desorb hydrogen are being used or considered for a variety of energy applications^{1,2}. Alloys of general composition AB₅ are prime candidates because of their good hydrogen absorption/desorption kinetics and large hydrogen storage capacity. The LaNi₅-H₂ system, in particular, has been extensively investigated. In many applications, however, materials are required whose plateau pressures are different from those of LaNi₅H_{6.7}. The desorption pressure of LaNi₅H_{6.7} may be modified by substitutions either of La or Ni with other elements. Previous work³ has shown that 20% substitutions of Ni by a variety of transition metals can lower plateau pressures by a factor of ~ 4 . We report here that Al can substitute for Ni in LaNi₅ with dramatic results in lowering decomposition pressures without impairing the kinetics or the hydrogen carrying capacities. The important new result is that Al substitutions allow a wide range of decomposition pressure to be spanned in continuous fashion. In the range 0-20% Al, the plateau pressures of the LaNi₅-LaNi₄Al hydride system are reduced by a factor of ~ 300 .

Alloys were prepared from metals of 99.9% purity by arc melting on a water-cooled copper hearth in an argon atmosphere. The alloys were homogenised at 800 °C. It was noted that diffraction patterns taken on as cast material were equally as sharp as those taken on homogenised alloys. X-ray diffraction patterns were taken with filtered FeK α radiation. Computer programs were used to verify indexing of the X-ray patterns.

Alloy samples, weighed to ± 0.0001 g, were placed in an all 316 stainless steel reactor fitted with a 1 μ m porous stainless steel filter disk. The reactor was connected to an all 316 stainless steel high pressure manifold with connections to a 0-2,000 lb inch⁻² Heise pressure gauge (accuracy $\pm 0.1\%$), a vacuum pump, and a high pressure hydrogen gas cylinder. Hydrogen was Matheson's pre-purified grade (99.95% min). Hoke valves rated to 3,000 lb inch⁻² were used. The reactor was immersed in a water bath whose temperature up to 80 °C was maintained to ± 0.2 °C by use of a

Thermistemp temperature controller. For higher temperatures a nichrome wound cylindrical furnace was employed. Equilibration was considered to have been achieved when the pressure remained constant for a period of 10–15 min. Equilibration times were generally from 30 min to 2 h. Samples were 'activated' by evacuating the reactor containing the alloy and exposing the sample to high pressure hydrogen (300–800 lb inch⁻²) for 1–2 h. Desorption measurements were performed by removing measured amounts of hydrogen and re-equilibrating the system.

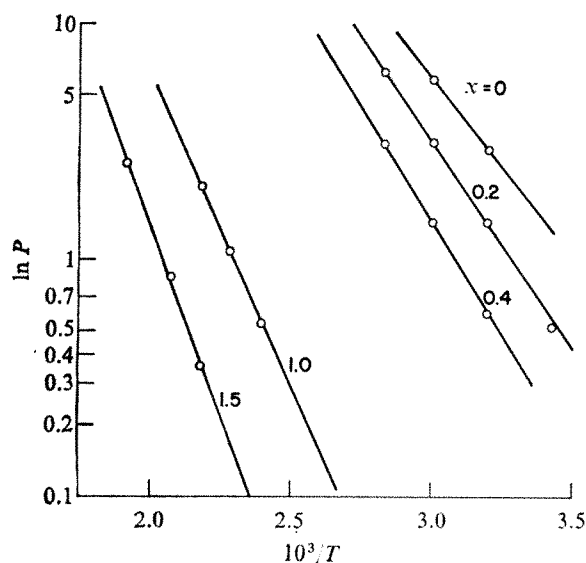
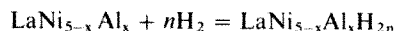


Fig. 1 Plot of $\ln P$ against $1/T$ for $\text{LaNi}_{5-x}\text{Al}_x$ hydrides.

Pressure–composition data have been obtained for alloys of the following composition: $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, LaNi_4Al , and $\text{LaNi}_{3.5}\text{Al}_{1.5}$. Preliminary data at several temperatures allow us to plot $\ln P$ against $1/T$ for the hydrides of these four compounds. From the slope and intercept of the van't Hoff plots (Fig. 1), ΔH and ΔS values for the reaction



are estimated and given in Table 1.

The increasing stability of the hydrides can also be illustrated by isobaric data as in the last column of Table 1.

X-ray diffraction measurements have been made on a number of $\text{LaNi}_{5-x}\text{Al}_x$ alloys. These results, showing the a_0 and c_0 lattice parameters and the crystal cell volumes as a function of the Al concentration, are illustrated in Fig. 2. The substitution of one Al atom into the 5c and g sites results in a sharp increase in c_0 , and a smaller increase in a_0 . Intensity calculations show that Al tends to enter the 3g sites in preference to the 2c sites. This finding is consistent with an earlier investigation of the $\text{YCo}_5\text{--YNi}_5$ system⁴. Further substitution of Al causes c_0 to level off but a_0 increases at a faster rate. The initial increase in c_0 must result from a lengthening of the bond between layers, while the subsequent levelling off in c_0 results from the beginning of a trend for the large La atom to collapse into the hexagonal ring of Ni(Al) atoms above and below it.

Table 1 Data from $\text{LaNi}_{5-x}\text{Al}_x + n\text{H}_2 = \text{LaNi}_{5-x}\text{Al}_x\text{H}_{2n}$ reaction

Compound	ΔH (kcal mol ⁻¹ H ₂)	ΔS (cal mol ⁻¹ H ₂ °C)	Temperature (°C) for $P = 2.0$ atm
LaNi_5	7.2 ± 0.1	26.1 ± 0.4	~ 25
$\text{LaNi}_{4.8}\text{Al}_{0.2}$	8.3 ± 0.1	27.3 ± 0.4	~ 50
$\text{LaNi}_{4.6}\text{Al}_{0.4}$	9.1 ± 0.2	28.1 ± 0.7	~ 70
LaNi_4Al	12.7 ± 0.3	29.2 ± 0.7	~ 180
$\text{LaNi}_{3.5}\text{Al}_{1.5}$	14.5 ± 0.6	29.6 ± 1.4	~ 240

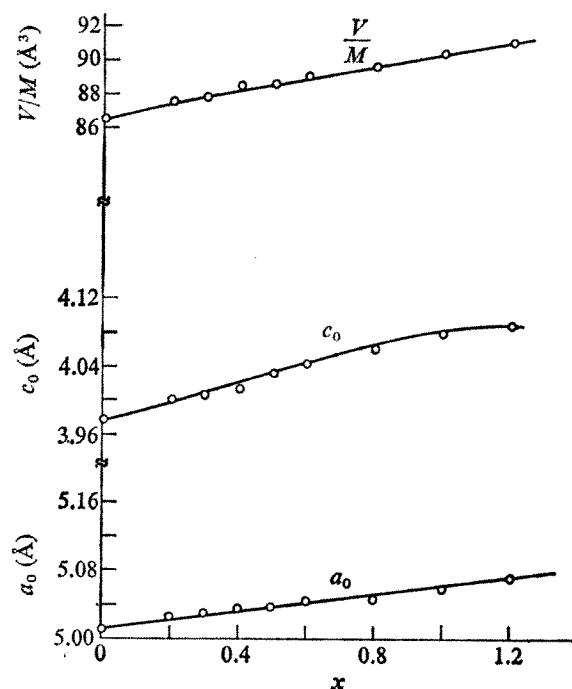
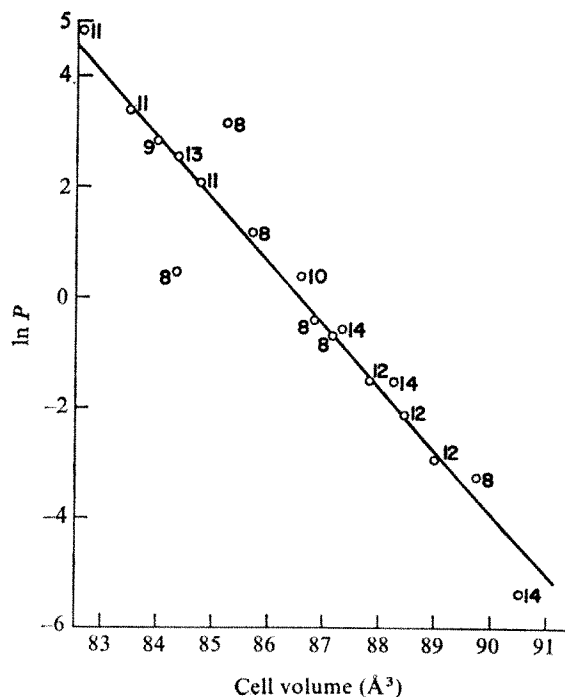


Fig. 2 Plot of a_0 , c_0 , and V/M against x in $\text{LaNi}_{5-x}\text{Al}_x$ alloys.

Several workers^{5,6,7} have pointed to the importance of crystal structure and geometrical considerations with regards to the stability of metal alloy–hydrogen systems. In Fig. 3, a direct linear correlation between $\ln P_{\text{plateau}}$ and the alloy cell volume is shown for the compounds: LaCo_5 , CeCo_5 , PrCo_5 , NdCo_5 , SmCo_5 , GdCo_5 , YCo_5 , LaNi_5 , PrNi_5 , SmNi_5 , GdNi_5 , LaCo_4Ni , LaCo_3Ni_2 , LaCo_2Ni_3 , NdNi_5 , $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, and LaNi_4Al . The observed trend of lower plateau pressures with increasing Al content is in agreement with the cell volume correlation observed for other AB_5 compounds.

Fig. 3 Plot of $\ln P$ against alloy cell volume for AB_5 type hydrides, with ref. nos. See ref. 8 for LaCo_5 , CeCo_5 , PrCo_5 , NdCo_5 , SmCo_5 , GdCo_5 ; ref. 9 for YCo_5 ; ref. 10 for LaNi_5 ; ref. 11 for PrNi_5 , SmNi_5 , GdNi_5 ; ref. 12 for LaCo_4Ni , LaCo_3Ni_2 ; LaCo_2Ni_3 ; ref. 13 NdNi_5 ; the present work (ref. 14 on Fig.) for $\text{LaNi}_{4.8}\text{Al}_{0.2}$, $\text{LaNi}_{4.6}\text{Al}_{0.4}$, LaNi_4Al .



A new ternary alloy system has been developed involving Al substitution of Ni in AB_3 compounds which permits control of hydrogen decomposition pressures over wide pressure and temperature ranges, while preserving the excellent hydrogen absorption-desorption kinetics and large hydrogen carrying capacity of $LaNi_5$.

This work was performed under the auspices of the Division of Physical Research of the U.S. Energy Research and Development Administration.

MARSHALL H. MENDELSON
DIETER M. GRUEN

Argonne National Laboratory,
Chemistry Division,
9700 South Cass Avenue,
Argonne, Illinois 60439

AUSTIN E. DWIGHT

Department of Physics,
Northern Illinois University,
DeKalb, Illinois 60115

Received 27 May; accepted 27 June 1977.

- ¹ Gruen, D. M. *et al.* *Proc. 11th IECEC* 681 (1976).
- ² Reilly, J. J. *et al.* *Proc. 26th Power Sources Symp.* 11 (1974).
- ³ van Mal, H. H., Buschow, K. H. J. & Miedema, A. R. *J. Less-Common Metal.* 35, 65 (1974).
- ⁴ Dwight, A. E. *J. Less-Common Metal.* 43, 117 (1975).
- ⁵ Buschow, K. H. J. & van Mal, H. H. *J. Less-Common Metal.* 29, 203 (1972).
- ⁶ Bechman, C. A., Goady, A., Takeshita, T., Wallace, W. E. & Craig, R. S. *Inorg. Chem.* 15, 2184 (1976).
- ⁷ Lundin, C. E. *et al.* *Proc. 11th IECEC* 961 (1976).
- ⁸ Kuipers, F. A. thesis, Technische Hogeschool, Delft (1973).
- ⁹ Takeshita, T., Wallace, W. E. & Craig, R. S. *Inorg. Chem.* 13, 2282 (1974).
- ¹⁰ Lundin, C. E. & Lynch, F. E. First Annual Report, No. AFOSR-F44620-74-000020 (Denver Research Institute, 1975).
- ¹¹ Anderson, J. L. *et al.* Los Alamos Report, LA-5320-MS (1973).
- ¹² van Mal, H. H., Buschow, K. H. J. & Kuipers, F. A. *J. Less-Common Metal.* 32, 289 (1973).
- ¹³ Gruen, D. M., Mendelsohn, M. H. & Sheft, I. *Proc. Symp. Electrode Materials and Processes for Energy Conversion and Storage* (in the press).

Ni-Sn interaction in temper embrittled steel detected by Mössbauer spectroscopy

PREREQUISITES for reversible temper embrittlement of steel¹ are the presence of a trace impurity² (Sb, Sn, As or P) and a major alloying element^{3,4} (Ni, Cr or Mn). Auger electron spectroscopy (AES) demonstrated that in the embrittled state these elements are segregated to within the first few monolayers of the high angle boundaries^{5,6}. The segregation is reversible and can be removed by heating at temperatures above the embrittlement range, whilst still within the α field (that is $> 600^\circ\text{C}$). It has been postulated⁷ that the segregation behaviour can be explained using equilibrium thermodynamics. It is proposed that the trace impurities interact more strongly with the major alloying elements than the Fe matrix and hence the chemical potential of the system is reduced by segregation to the high angle boundaries with the formation of bi-dimensional complexes (such as Ni_3Sn_2 , Mn_3P and Cr_3P). No experimental evidence is available for the existence of such complexes. AES gives no information regarding the chemical bonding of the segregants but it has been shown⁸, using X-ray photoelectron spectroscopy (XPS), that the segregated Mn on embrittled fracture surfaces of a 2% Mn steel is in a different chemical state to that on unembrittled surfaces. We report here preliminary results from a specially prepared 3% Ni steel, doped with ^{119}Sn , which were obtained using Mössbauer spectroscopy to monitor changes in the nearest-neighbour atoms of the Sn atoms as a function of heat treatment in the temper embrittlement temperature range.

Argon arc melting was used to prepare the high purity alloy of composition (wt%) Ni (3.4); ^{119}Sn (0.19); C (0.10); Fe (balance). The cast alloy was rolled to a thickness of 100 μm , an optimum thickness for the Mössbauer measurements, and austenitised for 1 h at $1,120^\circ\text{C}$ followed by a water quench. Heat treatments were chosen to produce

Table 1 Heat treatment schedule

Condition	1120 °C	650 °C	500 °C	650 °C
As quenched	1 h, water quench			
Unembrittled	1 h, water quench	1 h		
Embrittled	1 h, water quench	1 h	1,000 h	
De-embrittled	1 h, water quench	1 h	1,000 h	1 h

conditions representative of: the martensitic water quenched material, the unembrittled state, the embrittled state and the de-embrittled state, as detailed in Table 1. Mössbauer spectra were recorded in transmission at 77 K using a CaSnO_3 source and a constant acceleration spectrometer.

The Mössbauer spectra in Fig. 1 were found to consist mainly four components tentatively identified with Sn atoms having 0, 1, 2, or 3 Ni nearest neighbours. As the field in pure Fe is -8.5T and in Ni is $+2\text{T}$, the magnitude of the field is expected to decrease with increasing number of Ni nearest neighbours. At least-squares computer programme adjusted the intensities and magnitude of the fields of the four components to fit the spectra, and the percentages of the components are shown in Table 2. The errors are estimated to be $\pm 5\%$. With increasing number of Ni neighbours a progressive positive isomer shift was observed, indicating an increasing s electron density at the Sn nuclei. The total shift of 3 Ni neighbours was 0.22 mm s^{-1} .

For the as quenched material, the analysis showed a slightly greater percentage of Sn atoms having Ni nearest

Fig. 1 Mössbauer spectra obtained for *a*, the as quenched martensitic material; *b*, the unembrittled state and *c*, the embrittled state. On de-embrittling, the spectra reverted to that of the unembrittled state.

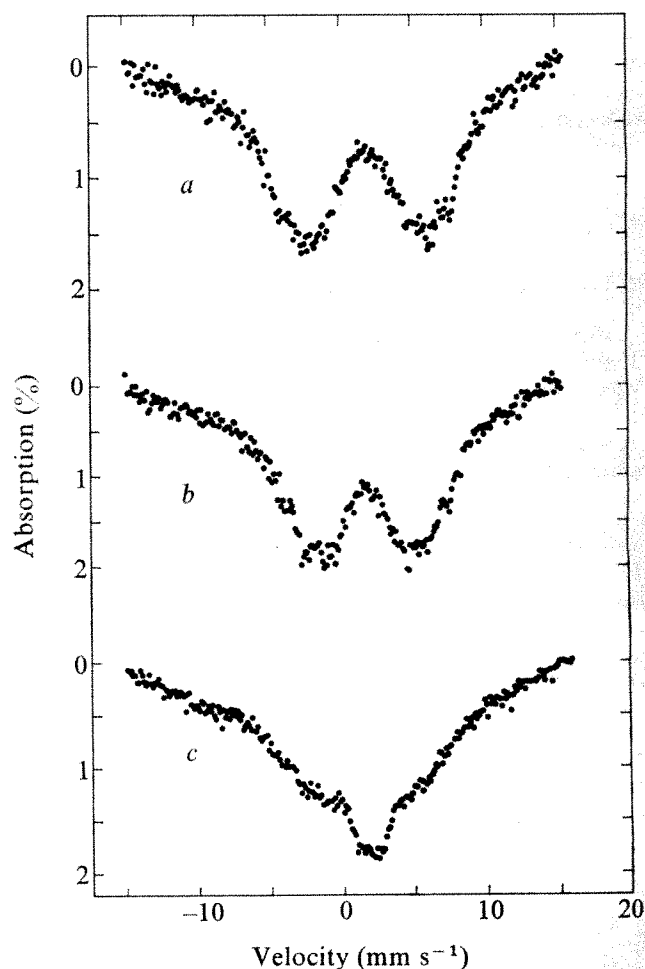


Table 2 Percentage of Sn atoms

No. of Ni nearest neighbours	0	1	2	3
Calculated value for random distribution (bcc)	76	21	3	0
As quenched	71	24	5	0
Unembrittled	62	32	10	6
Embripped	33	23	22	22
De-embripped	53	26	13	8
Field (T)	-8.5	-4.9	-2.1	-0.7

neighbours than that predicted from a random solid solution, indicating the presence of a Ni-Sn interaction within the γ field. Tempering at 650 °C (the unembrittled state) produced a further increase in the percentage of Sn atoms having Ni neighbours. On tempering at 500 °C, a dramatic change in the spectrum was noted and the analysis showed this is due to an increased number of Sn atoms having two or three Ni nearest neighbours. On re-heating at 650 °C (the de-embrittled state) these components were removed from the spectrum which reverted to that of the unembrittled state. The possibility of compound formation during tempering at 500 °C was examined by a comparison with the Mössbauer spectra produced by Ni_3Sn_2 or Ni_3Sn_4 . Within the accuracy of the experiments, the distinction between compound formation and Sn having three Ni nearest neighbours could not be resolved but is the subject of further investigation.

Examination of thin foils of the specimens in the embrittled condition using transmission electron microscopy failed to identify any compound formation either within the grains or at the grain boundaries. However, since AES⁹ has demonstrated that Ni and Sn are segregated at the boundaries of temper embrittled steels, it is reasonable to conclude that the modification of the spectra on ageing at 500 °C is due primarily to a grain boundary centred phenomenon associated with either the formation of a bi-dimensional phase of the type Ni_3Sn_2 (or Ni_3Sn_4) or due to an increased Ni-Sn coupling. The removal of this 'phase' on re-heating at 650 °C, above the embrittling temperature range, indicates that it has a critical role in the mechanism of temper embrittlement.

This work has demonstrated the existence of a reversible Ni-Sn interaction when subjected to a classical embrittling-de-embrittling treatment. This interaction is considered to be primarily centred at the grain boundaries and is related to either the increased number of Ni nearest neighbours of the Sn atoms or due to a bi-dimensional compound formation of Ni_3Sn_2 or Ni_3Sn_4 . Our work has shown that Mössbauer spectroscopy is a powerful tool for monitoring the environment of the Sn impurity atoms in temper embrittled steels as a function of heat treatment, and a similar evaluation may also be possible for Sb.

B. C. EDWARDS

B. L. EYRE

T. E. CRANSHAW

Metallurgy Division,

Nuclear Physics Division

Atomic Energy Research Establishment,

Harwell,

Didcot, Oxfordshire, UK

Received 13 June; accepted 14 July 1977.

- McMahon, C. J., Jr *Am. Soc. Test. Mater. STP* 407, 127-167 (1968).
- Steven, W. & Balajiva, K. *J. Iron Steel Inst.* 193, 141-147 (1959).
- Low, J. R., Jr, Stein, D. F., Turkalo, A. M. & Laforce, R. P. *Trans. Am. Inst. Min. Metall. Petrol. Engrs* 242, 14-24 (1968).
- Krahe, P. R. & Guttman, M. *Scripta Metall.* 7, 387-394 (1973).
- Marcus, H. L. & Palmberg, P. W. *Trans. Am. Inst. Min. Metall. Petrol. Engrs* 245, 1664-66 (1969).
- Stein, D. F., Joshi, A. & Laforce, R. F. *Trans. Am. Soc. Metals* 62, 776-783 (1969).
- Guttman, M., *Surface Science* 53, 213-227 (1975).
- Coad, J. P., Rivière, J. C., Guttman, M. & Krahe, P. R. *Acta Metall.* 25, 161-171 (1977).
- Edwards, B. C., Bishop, H. E., Rivière, J. C. & Eyre, B. L. *Acta Metall.* 24, 957-967 (1976).

Water content of Russian tektites

THE first occurrence of tektites in Russia was reported recently by Florensky¹⁻³, who named them irgizites, and concluded that they were produced by fusion of terrestrial rocks at the Zhamanshin impact structure where they are found. The Zhamanshin impact structure is approximately 10 km in diameter and is 200 km north of the Aral Sea (~49°N, ~59°E). The irgizites have the splash forms that are commonly found among other tektites and impactites, for example, indochinites and Wabar glass. Although Florensky clearly describes the field occurrence, shapes, general petrography, major element chemistry and origin of the irgizites, the reported bulk chemical analysis of the major elements includes neither ferrous/ferric iron nor water³. Thus, data for two of the most widely accepted chemical criteria for the identification of tektites have not previously been available for irgizites. This note reports investigations into their water content.

Seven irgizites were obtained, one of which was available for destructive analysis. A thin plate was cut from this specimen, polished, and the water content of the glass was determined using the technique of Gilchrist *et al.*⁴ from the infrared absorption spectrum (Fig. 1). A moldavite and a terrestrial obsidian sample were run under the same conditions for comparison. The actual water determination for the irgizite was made on a slice 2.1 mm thick, and the value obtained was 0.051 weight per cent water. This figure is greater than the water content of the presently recognised tektites, which average approximately 0.01 wt % (ref. 4).

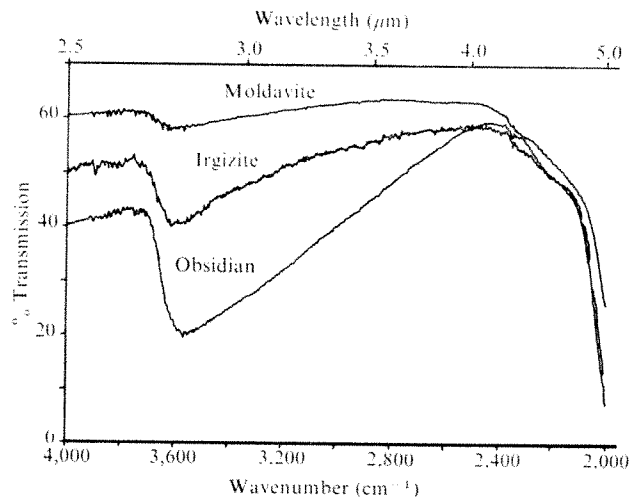


Fig. 1 Infrared absorption spectra of a moldavite, irgizite and an obsidian. Spectra were obtained from doubly polished sections 0.290 mm thick (moldavite), 0.275 mm (irgizite) and 0.180 mm (obsidian). The respective water contents are: moldavite, 0.0092; irgizite, 0.051; obsidian, 0.156 wt %. The water content of the irgizite is greater than that of true tektites.

In thin section, the body glass of the irgizite is a light olive brown colour, similar to many bediasites, and probably indicates a high ferrous/ferric iron ratio, but sufficient material was not available for a wet chemical determination. Partial electron microprobe analyses of the irgizite glass show a range of composition that is commonly found within a single tektite, and confirm the presence of lechatelierite inclusions³. The body glass of the irgizite is very clean with schlieren and slightly different colour and refractive indices; it also contains a number of spherical to elliptical bubbles, congruent in number and size with the bubbles in many tektites. There are no microlites nor any other traces of devitrification or

hydration. A few tiny and rare mineral grain inclusions are present, but they are too small to identify.

The piece of irgizite glass we examined seems to be a typical tektite glass in all respects except for the greater water content and possibly the, as yet undetermined, ferrous/ferric iron ratio. Whether or not the irgizites should be classified as tektites is a moot question. Perhaps they should be termed wet tektites, but regardless of their name, they are extremely similar to, and have originated by, the same basic process as true tektites.

Although the fundamental question of the place and mode of origin of tektites has been resolved for some years⁵⁻⁹, the irgizites are an important link in the chemistry and petrography of terrestrial impactites from craters of small to intermediate size.

The authors are indebted to Walter Zeitschel of Hanau for providing the specimens of irgizites used in this work.

ELBERT A. KING

Department of Geology,
University of Houston
and Mineralogical Institute,
University of Tübingen,

JORG ARNDT

Mineralogical Institute,
University of Tübingen,
74 Tübingen, Wilhelmstrasse 56, FRG

Received 17 June; accepted 30 June 1977.

¹ Florensky, P. V. *Astron. Vestnik*, 9, 237-244 (1975).

² Florensky, P. V. *Priroda* 1, 85-87 (1976).

³ Florensky, P. V. *NASA Tech. Trans. NASA-TT-F-16765* (1976).

⁴ Gilchrist, J., Thorpe, A. N. & Senthle, F. E. *J. geophys. Res.* 74, 1475-1483 (1969).

⁵ Spencer, L. J. *Nature* 131, 117-118, 876 (1933).

⁶ Schnetzler, C. C., Pinson, W. H. & Hurley, P. M. *Science* 151, 817-819 (1966).

⁷ Faul, H. *Science* 152, 1341-1345 (1966).

⁸ King, E. A. in *Space Geology: An Introduction* (Wiley, New York, 1976).

⁹ King, E. A. *Am. Sci.* 65, 212-218 (1977).

Source models to account for Lake Mungo palaeomagnetic excursion and their implications

INDICATIONS of large and rapid excursions in the direction of the ancient geomagnetic field continue to create considerable excitement and debate in palaeomagnetic circles. Barbetti and McElhinny¹ have published detailed evidence of a remarkable excursion at Lake Mungo, Australia dated at about 30,000 yr ago, on which they had briefly reported previously². I consider here various simple sources, all in the Earth's outer core, that could account for that excursion—an eccentric radial dipole or current loop, an eccentric horizontal dipole, and a pair of eccentric radial current loops of opposite sign—and discuss the merits of each in light of the modern geomagnetic field and with regard to the magnitude and spatial extent of the anomalous field produced.

Because most of the excursions proposed so far have been found in sediments and have not been convincingly documented over a sizeable portion of the globe, several workers have suggested that

some or most of them may reflect sedimentological rather than geomagnetic phenomena³⁻⁵. The record of the Lake Mungo excursion, however, is contained in sedimentary material that was baked in prehistoric aboriginal fireplaces. Thus, sedimentological phenomena cannot be invoked to explain the anomalous directions because the natural remanent magnetisation is not detrital or diagenetic in origin but rather is thermoremanent. This type of magnetisation is not only the most reliable recorder of ancient field direction but is also the best suited for making estimates of ancient field intensity.

The Lake Mungo excursion is especially intriguing because of the very high field intensity that Barbetti and McElhinny¹ have found accompanying the large swing in field direction. The intensity estimates are as high as 1.83 Oe, about twice as strong as anywhere on the surface of the Earth today and three times what would be produced at that latitude by the maximum geomagnetic dipole moment previously reported for the past 8,500 yr (ref. 6). While the field was abnormally intense its direction was shallowly inclined first roughly west and then roughly east, very far from the normal field direction of an approximately axial and centred dipole.

The explanation of this phenomenon with the least astonishing consequences is that some of the palaeointensity measurements are erroneously high. It is well known that ancient field intensities are far more difficult to determine than directions because of the many possibilities for systematic error⁷. Nonetheless, we cannot easily dismiss these extraordinarily high palaeointensities. Barbetti and McElhinny¹ carefully discuss the probable effects of a great number of possible systematic errors and conclude that their estimates of palaeointensity should be regarded as minimum values. Moreover, the high values were found at three sites in samples of two different sorts of baked sediment, whereas at several other sites these same materials yield both normal and unusually low estimates of palaeointensity. Thus, it is most useful at this point to accept the measurements of direction and intensity as at least approximately correct and to examine other explanations and their consequences.

The failure so far to find convincing evidence for any single excursion at widely separated points on the globe has led to the suggestion that excursions are manifestations of the non-dipole field^{8,9}. Likewise, Barbetti and McElhinny¹ propose that the most likely cause of the Lake Mungo excursion is the occurrence of a very large intensity non-dipole feature which may be modelled approximately by an eccentric, radially-oriented dipole in the core near the core-mantle boundary beneath Lake Mungo. They point out that such a radial dipole at 0.5 R (offset from the centre of the Earth by 0.5 of the Earth's radius) would need a moment of only one-eighth that of the main geocentric dipole to produce at the point on the surface nearest to it a field greater than or equal to that of the main dipole, whereas on the far side of the Earth it would produce a field only 1/27 as large.

Even higher field intensities, however, are required elsewhere on the surface of the Earth when one attempts to apply the radial dipole model in a quantitative manner to the Lake Mungo excursion (Fig. 1a). To do this, I have assumed that the dipole field at the time was axial and had an intensity 0.3 Oe at the site. This is the most reasonable assumption in accordance with the data¹, but

Table 1 Solutions for radial dipoles corresponding to Lake Mungo excursion

Non-dipole field				Nearer solution				Farther solution			
Site	F_N	F_E	F_D	λ	ϕ	M/M_0	θ	λ	ϕ	M/M_0	θ
F7*	-0.68	-0.76	0.27	-52	81	-1.4	48	13	186	2.3	62
F12	-0.44	-1.81	0.14	-29	80	-3.1	53	-8	199	3.6	57
F8	-0.06	0.57	0.14	-26	198	-0.8	48	-11	80	1.3	62
F9*	-0.37	1.18	0.25	-34	203	-1.8	49	-3	87	2.7	60

Sites are those of Barbetti and McElhinny². F_N , F_E , F_D are north, east, downward non-dipole field components in Oe (10^{-4} T). λ , ϕ , and M/M_0 are north latitude, east longitude, moment divided by present geocentric dipole moment of radial dipole. θ is great-circle angle in degrees between radial dipole and site.

*Palaeointensity is average of values derived from ovenstone and baked sediment².

other plausible choices such as the present inclined dipole field of approximately 0.6 Oe at Lake Mungo do not seriously change the results. The differences between the palaeomagnetically determined total field vectors and the presumed dipole field vector are the ancient non-dipole field vectors (Table 1), for which one seeks to find the appropriate radial dipole sources.

Two possible solutions always exist for a radial dipole at any particular depth that can produce a given field at a site on the surface. These can be found using standard formulas¹⁰ and an iterative technique. The dipole nearer to the site naturally has a smaller moment than the one further away that produces the same field. Thus, it is the nearer dipole that is the more likely to correspond to the real physical situation if the non-dipole field at the site is in fact produced by only one such source. The depth of the radial dipole that is best used depends on the width of the anomaly to be modelled at the surface. Assuming non-dipole features in the past to have been similar to those of today, offsets of 0.35–0.45 R are most appropriate. These correspond to half-widths of 44–56° great circle arc for the vertical component of the anomaly.

The solutions for the positions and strengths of radial dipoles with offset 0.40 R that would produce the non-dipole field vectors for the Lake Mungo excursion are given in Table 1. The nearer solutions are inward-pointing dipoles at southerly latitudes of 26° or higher and at longitudes around 80°E or 200°E for the sites with westerly or easterly palaeomagnetic declinations, respectively. The farther solutions are only about 11° more distant on the average than the nearer solutions, and are outward-pointing dipoles lying within 13° of the equator at longitudes around 190°E or 85°E. The solutions are listed in order of presumed decreasing age and span a period of 2,000–3,000 yr (ref. 2).

The moments of even the nearer radial dipoles are enormous, ranging from 0.8–3.1 times the present geocentric dipole moment, and the alternate farther dipoles are up to 60% stronger than the nearer ones. These hypothetical dipoles would cause extremely large intensities at the points on the surface nearest to them of 2.3–8.8 Oe. Even at the antipodal points the intensities are about 0.2–0.7 Oe, certainly not negligible compared with usual field intensities.

Shifting the radial dipole toward or away from the centre of the Earth does little to diminish the excessively high field intensities. A radial dipole just below the core–mantle boundary (0.54 R) or just above the inner core (0.20 R) that produces the largest non-dipole field vector inferred for Lake Mungo (that at site F12, Table 1) would produce field intensities over the whole Earth ranging from 10.6–0.3 Oe or 6.1–1.8 Oe, respectively. Likewise, replacing the simple point dipole by a distributed source such as a circular loop

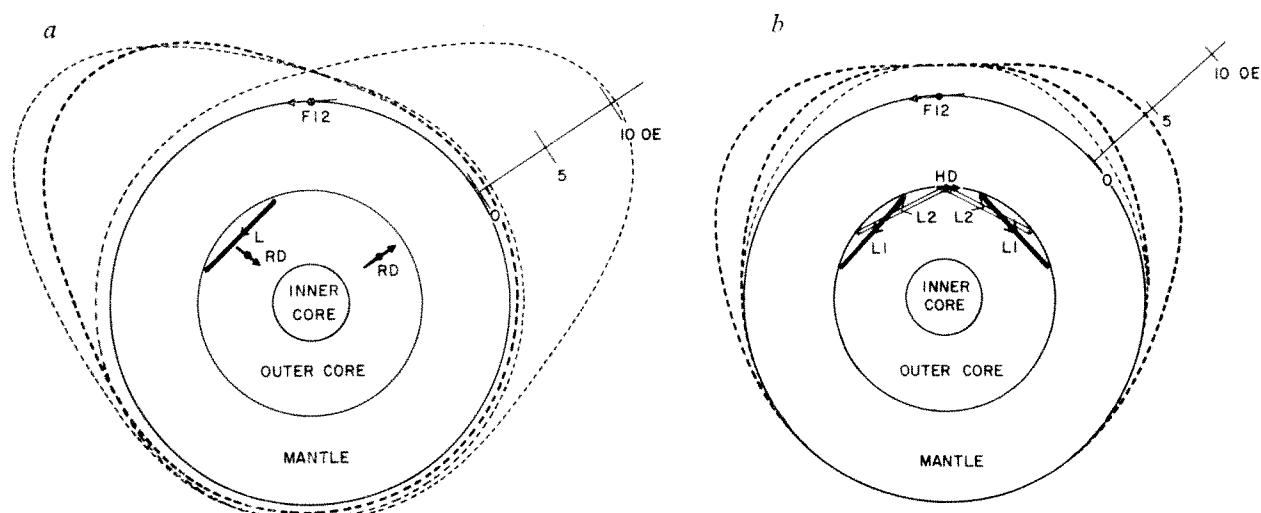
of current at the core–mantle boundary is only a minor improvement. Such a current loop with radius 44% of the radius of the core, which would cause an anomaly at the surface of about the same width as a radial dipole at 0.40 R, would bring about intensities over the world ranging from 7.2–0.4 Oe, while producing the largest non-dipole field vector inferred for Lake Mungo. Variations of the total field intensity for various of these models are illustrated in Fig. 1a.

The only way for such a large, almost horizontal non-dipole field as apparently occurred at Lake Mungo to exist without much larger fields existing simultaneously elsewhere on the Earth is to relax the assumption that the cause was a simple, radial source. When two adjacent radial sources have opposite signs, their horizontal components add between them and the area dominated by their combined fields is much less than for a single source. For example, consider the current loop described above and a slightly stronger current loop of opposite sign, 96° away from the former on the other side of Lake Mungo, that would also produce the same field at site F12. If reduced to half their strengths, the two loops together would likewise cause the required non-dipole field at site F12, but over the rest of the Earth they would produce fields that varied from only 4.5 to almost 0 Oe (Fig. 1b). If the current loops were allowed to overlap slightly, the most efficient configuration would be two current loops of equal strength and opposite sign spaced 54° apart. Such a pair would cause fields that varied from only 2.3 Oe to exactly 0 Oe over the globe (Fig. 1b), while producing the required 1.87 Oe at site F12. The spacing of 54° lies at the short end of the observed range of about 50–100° for the spacing of adjacent features of opposite sign of the modern non-dipole field¹¹.

The other assumption that might be relaxed is that the source is radial. A horizontal dipole at the core–mantle boundary would only require a strength of about 20% or less of the present main geocentric moment to account for the non-dipole fields inferred at Lake Mungo. This dipole would cause field intensities over the globe varying from about 2.0 Oe to less than 0.05 Oe (Fig. 1b). Combinations of two horizontal dipoles could reduce still further the maximum field intensities and the area of the globe significantly affected.

What, then, is the most likely source for the very high intensity Lake Mungo excursion? A horizontal eccentric source or current loop is the most efficient in terms of causing the least disturbance of the field elsewhere around the world, but there is little basis for expecting one to exist since the modern non-dipole field is dominated by a few large features that are most simply modelled by radial sources^{10,11}. Single radial sources, however, would produce

Fig. 1 Magnetic field intensity at Earth's surface due to various sources in outer core. Dashed lines indicate total field, zero if coincident with surface of Earth. Each source produces the palaeomagnetic non-dipole field at Lake Mungo site F12 (Table 1). *a*, Single radial sources: radial dipole at 0.40 R (RD), light dashed lines; current loop (L), heavy dashed line. *b*, Paired radial sources or horizontal source: current loops of opposite sign (L1 or L2), heavy dashed lines; horizontal dipole (HD), light dashed line.



extremely high fields over very large areas and significantly high anomalous fields over the entire globe. Thus, they are improbable unless simultaneous worldwide effects are discovered. The most appealing explanation seems to be a combination of two radial eccentric sources of opposite sign. In their most efficient configuration such a pair would cause a maximum field of about 2.3 Oe, while producing the required non-dipole field at Lake Mungo (site F12), and significant effects would occur as far away as south-eastern Asia and India, southern Africa, and much of Antarctica and the South Pacific Basin.

To produce all four non-dipole fields listed in Table 1, such a pair of sources would have to change signs and move about 15° N and S, in a similar fashion to the oscillating radial dipoles of fixed average position recently postulated by Creer¹² in explanation of geomagnetic secular variation. Although it could well be coincidental, it is interesting that two of the oscillating dipoles proposed by Creer lie within 10° and 25° of the average positions of the optimally-spaced pairs of current loops calculated to produce the field directions of the Lake Mungo excursion.

Obviously, there is a critical need for additional records of the Lake Mungo excursion in other places around the world. Only these will provide firm answers to fundamental questions such as whether the excursion is an expression of the dipole or non-dipole field and whether the very high field intensities inferred from the experimental data are in fact real. If the non-dipole field is responsible, such records are also necessary to determine the shape, number and configuration of the sources. At present, one can only conclude that if the very high field intensities are approximately correct and if the excursion was caused by non-dipole features similar in configuration to the modern ones, then unusually strong non-dipole fields must have occurred simultaneously over a significant portion of the rest of the world.

I thank Dr M. F. Barbetti, Dr M. W. McElhinny, Dr C. S. Grommé and Dr A. Cox for discussions, and the Australian National University for partial support provided by a visiting research fellowship.

R. S. COE

Earth Sciences Board,
University of California,
Santa Cruz, California 95064

Received 2 May; accepted 24 June 1977.

- ¹ Barbetti, M. F. & McElhinny, M. W. *Phil. Trans. R. Soc. Lond.* **A281**, 515–542 (1976).
- ² Barbetti, M. F. & McElhinny, M. W. *Nature* **239**, 327–330, 1972.
- ³ Verosub, K. L. *Science* **190**, 48–50, 1975.
- ⁴ Verosub, K. L. & Banerjee, S. K. *Rev. Geophys. Space Phys.* **15**, 145–155, 1977.
- ⁵ Thompson, R. & Berglund, B. *Nature* **263**, 490–491, 1976.
- ⁶ Cox, A. *J. geophys. Res.* **73**, 3247–3260, 1968.
- ⁷ Coe, R. S. *J. Geomag. Geoelect.* **19**, 157–179, 1967.
- ⁸ Denham, C. R. *J. Geomag. Geoelect.* **26**, 487–498, 1974; *Earthplanet. Sci. Lett.* **29**, 422–434, 1976.
- ⁹ Harrison, C. G. A. & Ramirez, E. *J. Geomag. Geoelect.* **27**, 139–151, 1975.
- ¹⁰ Allredge, L. D. & Hurlwitz, L. *J. geophys. Res.* **69**, 2631–2640, 1964.
- ¹¹ Bullard, E. C., Freedman, C., Gellman, H. & Nixon, J. *Phil. Trans. R. Soc. Lond.* **A243** 67–92, 1950.
- ¹² Creer, K. M. *Geophys. J. R. astr. Soc.* **48**, 91–109, 1977.

Trace elements in zooplankton particulate products

It has become increasingly evident that biogenous processes in oceanic surface layers play an important role in removing trace elements from sea water and transporting them to the sediments^{1,2}. Plankton are strongly implicated in these processes and investigations of the chemistry of these organisms have demonstrated their ability to accumulate trace elements to relatively high levels^{3,4}. Zooplankton metabolic activity can be expected to enhance the biogeochemical cycling of trace elements through the release of particulate matter⁵ such as faecal pellets, moulted exoskeletons and eggs. Furthermore, theoretical models^{5,6} on the vertical flux of particulates have emphasised the sinking of large particles, similar to those produced by zooplankton, as a mechanism for rapid transport of particulate matter

and associated trace elements out of the mixed layer. Nevertheless, little, if any, data exist on the trace element composition of these biogenic particles, and Boyle *et al.*⁷, interpreting their Pacific Ocean Cd profiles in terms of probable Cd regeneration from sinking biogenic debris, have stressed the need for information on the trace element composition of planktonic particulate matter. This note reports the concentrations of 18 trace elements in freshly released faecal pellets, moults and eggs from a representative planktonic crustacean. The high levels of many trace elements found in these biogenic products, relative to concentrations in the plankton which produce them, clearly indicate the importance of these particulates in oceanic trace element biogeochemical cycles.

Many euphausiids (*Meganyctiphanes norvegica*) were collected during one night in the surface waters off Monaco by making several, short oblique tows with a midwater trawl. Approximately 1,000 individuals were placed in a faecal pellet collection system⁸ and several hundred milligrams (wet) of pure faecal pellets were retrieved after 15 h. The remaining euphausiids were split into two groups; one was maintained in the laboratory⁹ until a sufficient number of moults had been collected and the other was killed for whole body analysis. Eggs released by females were concentrated from the water on fine mesh nylon netting. Microplankton, on which *M. norvegica* were feeding, were collected at the same time for purposes of comparing their trace element contents with levels in the euphausiid faecal pellets. All samples were briefly rinsed with doubly distilled water to remove adhering sea salts and oven-dried at 60 °C. Separate aliquots of material intended for Hg analysis were freeze-dried in order to avoid Hg loss through volatilisation. Care was exercised to avoid metal contamination at all stages of collection and sample preparation. Microplankton samples were carefully screened for possible metal-particle contamination using the Martin and Knauer⁴ method. Samples along with appropriate blanks and standards were analysed by flameless or flame atomic absorption spectrophotometry¹⁰ and instrumental neutron activation¹¹.

Trace element concentrations in euphausiids, their particulate products and the food they eat are given in Table 1. Since all samples were derived from the same water mass, realistic comparisons can be made between trace element levels in the particulates and those in the organisms which produced them. For most elements, faecal pellets contained the highest trace element levels with lesser amounts in moults and eggs. Trace element concentrations in faecal pellets were significantly higher than levels in whole euphausiids (as much as 1,000 times) except for Sr and possibly Hg and Se. The natural diet of *M. norvegica*, an omnivore, is extremely varied¹² but consists primarily of phytoplankton, small zooplankton and detritus. Although it is difficult to define precisely the exact composition of their diet at any given moment, examination of euphausiid stomach contents and faecal pellets indicated that the samples were feeding on many of the species noted in the microplankton. Comparison of the trace element content in microplankton and euphausiid faecal pellets in Table 1 shows that, except in the case of Sr, euphausiids readily concentrate ingested trace elements in their faeces. Faecal pellet-microplankton concentration ratios ranged as high as 670 with the greatest degree of concentration noted for trace elements not biologically essential such as Ce, Sb, Cs, Eu and Sc. The high concentration ratio for Fe, a biologically essential element, is possibly a result of ingesting a particulate form of this metal which may not be easily assimilated. Boothe and Knauer¹³ have also reported a relatively high concentration of Fe in crab faeces relative to that in their food.

With the exception of Cu, Cs, Hg and Se, trace element concentrations in moults were also significantly higher than

Table 1 Trace element concentration ($\mu\text{g g}^{-1}$ dry) in a planktonic euphausiid, its particulate products and the microplankton it ingests

Sample	Wet-dry weight ratio	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce*	Cs*	Eu*	Hg*	Sb*	Sc*	Se*	Sr*
Faecal pellets	4.4	2.1	9.6	3.5	38	226	24,000	243	20	34	950	200	6.0	0.66	0.34	71	2.8	6.6	78
Moult	4.6	2.9	2.1	0.80	5.3	35	232	11.7	6.7	22	146	1.2	0.019	0.0077	0.17	0.80	0.030	1.9	350
Eggs†	10.0	0.96	0.58	0.80	7.9	17	330	11.5	4.3	8.9	318								
Whole euphausiid	4.7	0.71	0.74	0.18	0.85	48	64	4.2	0.66	1.1	62	0.21	0.062	0.0023	0.35	0.071	0.0090	4.4	117
Microplankton‡	10.7	0.67	2.1	0.87	4.9	39	570	17.9	8.1	11	483	0.30	0.080	0.013	0.05	0.22	0.13	2.7	520

*Determined by instrumental neutron activation analysis.

†Insufficient sample for neutron activation analysis.

‡Principally copepods, phytoplankton, chaetognaths and detritus retained on nylon netting of 76 μm aperture.

corresponding levels in whole individuals (Table 1). Bertine and Goldberg¹⁴ analysed several trace elements in shrimp and found that Zn and Ag were higher in dissected exoskeleton than in internal tissues. When small pelagic crustaceans moult, only the thin outer layer of the exoskeleton is shed. If surface adsorption predominates in the accumulation of many of the trace elements on to chitinous moults, as is the case for several radionuclides¹⁵, it is conceivable that moults will contain higher concentrations of these elements than the entire exoskeleton.

M. norvegica moults average 7.7% of the organism's dry weight; from this, rough estimates of the fraction of the animal's trace element content contained in the moult can

than moults in effecting element transport, since egg deposition is seasonal. Zooplankton faecal pellets, which are produced at a higher rate¹⁹ and decompose more slowly²⁰ than moults, clearly have the greatest potential for transporting trace elements to depth. Recent finding of large numbers of intact faecal pellets in deep waters^{21, 22} supports this contention. Trace element flux rates resulting from the release of moults and faeces by euphausiids can be calculated and are given in Table 3. From a comparison of the rates it is clear that, with the exception of Sr, faecal pellet flux is a major contributor to the biogenic vertical transport of the elements investigated. Thus, vertical transport through faecal pellets may be an important factor

Table 2 Fraction of euphausiid trace element body burden contained in the moult

	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce	Cs	Eu	Hg	Sb	Sc	Se	Sr
% in moult*	31	22	34	48	6	28	21	78	≈150	18	44	2	26	4	87	26	3	23

*Calculations based on p.p.m. dry weight values listed in Table 1.

be calculated (Table 2). Clearly, for many trace elements, a large fraction of the euphausiid's total body burden is associated with its outer surface. The reason for the anomalously high Pb percentage is not readily explainable and measurements made on another set of samples led to a similarly high percentage. The possibility of contamination can not be entirely ruled out; however, Pb is known to be highly concentrated on the epidermis of marine fish¹⁶, and

influencing the oceanic residence times of these elements. The degree to which faecal pellets and other biogenic debris transport their trace element load to depth will be a function of trace element regeneration time for these sinking materials. Considering the high trace element content of freshly released zooplankton particulate matter, some attempt to understand *in situ* trace element regeneration kinetics in these products is needed.

Table 3 Trace element flux rate contribution (μg per kg euphausiid per d) by euphausiids*

	Ag	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Ce	Cs	Eu	Hg	Sb	Sc	Se	Sr
Faecal pellets	80	360	130	1,400	8,600	910,000	9,200	760	1,300	36,000	7,600	230	25	13	2,700	110	250	3,000
Moult	26	19	7	48	320	2,100	110	60	200	1,300	11	0.2	0.07	1.5	7	0.3	17	3,200

*Based on typical *M. norvegica* faecal pellet and moult production rates¹⁹ of 0.038 and 0.009 g dry matter per g dry euphausiid per day, respectively. Flux due to egg deposition is not considered since egg production takes place for only a brief period during the year¹⁹.

it may be that the cast moult adsorbed additional Pb on its inner surface before it was removed from the water. Nevertheless, the implication is that most or all of the Pb associated with euphausiids is located on the surface of the exoskeleton.

The relatively high concentrations of so many trace elements in zooplankton particulates hold important implications for marine biogeochemical cycles. Pelagic crustaceans moult as often as every few days^{9,17,18}, in some cases throughout their entire lifespan. Consequently, sinking moults may either release trace elements to the deeper waters or sediments during decomposition or, if eaten, provide a rich source of trace elements to organisms that consume them. Released eggs, often also displaying a relatively high trace element content, will be much less important

The International Laboratory of Marine Radioactivity operates under a tripartite agreement between the International Atomic Energy Agency, the Government of the Principality of Monaco and the Oceanographic Institute at Monaco. Support for this work is gratefully acknowledged. I thank P. Strohal and B. Oregioni for analytical assistance.

SCOTT W. FOWLER

International Laboratory of Marine Radioactivity,
Musée Océanographique, Monaco

Received 8 June; accepted 30 June 1977.

¹ Chester, R. & Aston, S. R. in *Chemical Oceanography* 2nd edn 6, 281–390 (eds Riley, J. P. & Chester, R.) (Academic, New York, 1976).

- ² Wallace, G. T., Hoffman, G. L. & Duce, R. A. *Mar. Chem.* **5**, 143-170 (1977).
- ³ Lowman, F. G., Rice, T. R. & Richards, F. A. in *Radioactivity in the Marine Environment* 161-199 (National Academy of Sciences, Washington DC, 1971).
- ⁴ Martin, J. H. & Knauer, G. A. *Geochim. cosmochim. Acta* **37**, 1639-1653 (1973).
- ⁵ Tsunogai, S., Nozaki, Y. & Minagawa, M. *J. oceanog. Soc. Japan* **30**, 251-259 (1974).
- ⁶ McCave, I. N. *Deep Sea Res.* **22**, 491-502 (1975).
- ⁷ Boyle, E. A., Sclater, F. & Edmond, J. M. *Nature* **263**, 42-44 (1976).
- ⁸ La Rosa, J. *Deep Sea Res.* **23**, 995-997 (1976).
- ⁹ Fowler, S. W., Small, L. F. & Keckes, S. *Mar. Biol.* **11**, 45-51 (1971).
- ¹⁰ Fowler, S. W. & Oregioni, B. *Mar. pollut. Bull.* **7**, 26-29 (1976).
- ¹¹ Stroh, P., Tuta, J. & Kolar, Z. *Limnol. Oceanogr.* **14**, 265-268. (1969).
- ¹² Mauchline, J. & Fisher, L. R. *Adv. mar. Biol.* **7**, 174-199 (1969).
- ¹³ Boothe, P. N. & Knauer, G. A. *Limnol. Oceanogr.* **17**, 270-274 (1972).
- ¹⁴ Bertine, K. K. & Goldberg, E. D. *Limnol. Oceanogr.* **17**, 877-884 (1972).
- ¹⁵ Fowler, S. W. & Small, L. F. in *Design of Radiotracer Experiments in Marine Biological Systems*, Tech. Rep. Ser. No. 167, 63-84 (IAEA, Vienna, 1975).
- ¹⁶ Chow, T. J., Patterson, C. C. & Settle, D. *Nature* **251**, 159-161 (1974).
- ¹⁷ Lasker, R. *Nature* **203**, 96 (1964).
- ¹⁸ Martin, J. H. *Limnol. Oceanogr.* **15**, 756-761 (1970).
- ¹⁹ Small, L. F., Fowler, S. W. & Keckes, S. in *Radioactive Contamination of the Marine Environment* 437-452 (IAEA, Vienna, 1973).
- ²⁰ Small, L. F. & Fowler, S. W. *Mar. Biol.* **18**, 284-290 (1973).
- ²¹ Wiebe, P. H., Boyd, S. H. & Winget, C. J. *mar. Res.* **34**, 341-354 (1976).
- ²² Soutar, A., Kling, S. A., Crill, P. A., Duffrin, E. & Bruland, K. W. *Nature* **266**, 136-139 (1977).

Weis-Fogh clap and fling mechanism in *Locusta*

WEIS-FOGH discovered a mechanism of lift generation¹ when he observed, from slow motion films, that the tiny parasitic wasp *Encarsia* clapped its wings together at the top of the stroke during hovering flight; the clap was followed by a 'flinging open' of the wings, as they rotated about a common axis where the hindwing trailing edges were in contact. He calculated that the insect generated greater lift than could be explained by steady-state aerodynamics. The importance of the clap and fling in generating this extra lift is now well established²⁻⁴. We report here that slow motion film of natural flight has revealed this mechanism in locusts and that it occurs during forward flight to produce extra lift.

Swarms of *Locusta migratoria* were filmed with a high-speed camera in Australia and New Guinea. Film taken pointing directly towards an approaching and climbing swarm revealed that the hindwings of the locusts met at the top of the stroke in a similar fashion to that observed by Weis-Fogh in *Encarsia*. During the clap the tips of the hindwings meet, leaving a characteristic gap lower down towards

the wing bases. At the beginning of the downstroke, the leading edges of the hindwings part, but the more posterior vannal portions remain touching; as the hindwings move further down, the two wings eventually become completely separated from one another and they then progress as in a normal stroke. Significantly this clap and fling stroke is seen only in climbing locusts, which need greater lift than locusts in horizontal flight. Aspects of the stroke can be seen in Fig. 1.

The hindwing movement on the upstroke, before the clap, is noticeably faster than in a non-clapping stroke, and Fig. 2a shows this remarkably rapid upstroke which can be twice as fast as its forewing counterpart; hindwing tip speeds up to 10 m s^{-1} have been calculated from the film. Differences in phase between the fore and hindwings during the clap stroke seem to be essentially similar to those in the normal non-clap stroke (see Fig. 2b), and thus the hindwings move through a greater amplitude before the clap simply by moving faster than usual, reaching the top of their stroke with about the same phase lead over the forewings as in a non-clap stroke. Only the amplitude difference between fore- and hindwings changes markedly, and this results in a characteristic configuration with the hindwings pointing vertically and the forewings pointing horizontally (see Fig. 1).

On some of the high-speed film an occasional butterfly (unknown species) was seen flying across the field of view. Like the locusts, they also performed a wing clap at the top of the stroke⁴; in some cases the clap together was not immediately followed by separation as in the locusts, and instead the wings were held closed for a fraction of a second. The clap was observed both in approximately horizontal and vertical flight.

The clapping of the locust hindwings might be expected to make a noise, as is the case with pigeons taking off⁵. There are several reports of locust flight noises, especially during take-off⁶. Experiments performed in the field^{7,8} showed that the hindwings were responsible, and variously attributed the noise to the interaction of hindwings with back legs⁹, or to stretching of the vannal area of each

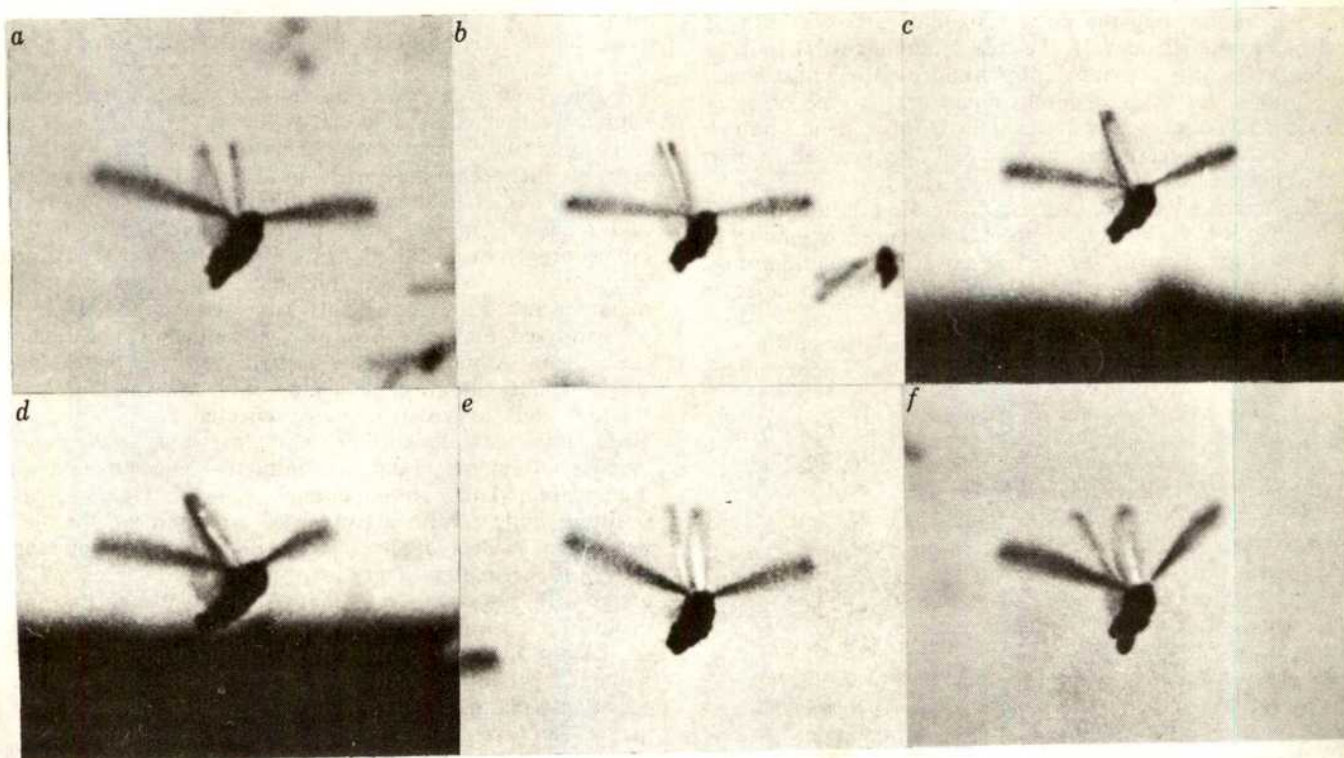


Fig. 1 Sequence of hindwing clap and fling in *Locusta*. Wings meet directly above the body and then at the start of the downstroke, the leading edges open, leaving more posterior portions of the wing still in contact. (Each frame is taken from a different locust.)

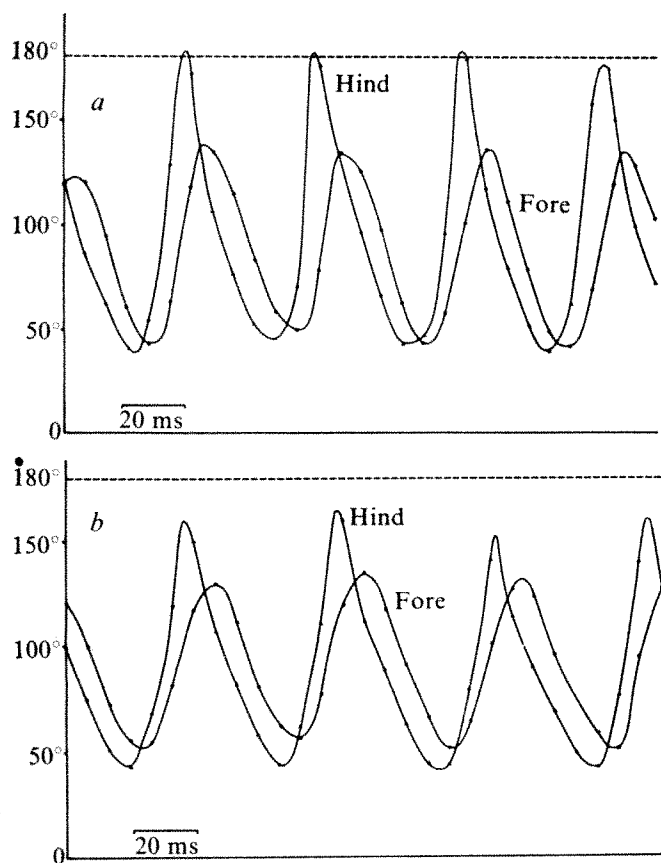


Fig. 2 *a*, Wingbeat cycles of right fore and hindwings of a locust performing clap. *b*, Wingbeat cycles of a different locust during non-clap strokes. 180° refers to wings in vertical clap position, and thus 90° is wings in horizontal position.

hindwing¹⁰. We suggest, however, that the noise is caused by the hindwings clapping together, this being likely to make a louder noise than the more proximal parts of the hindwings striking the legs. In the case of the locust *Dissosteira carolina*¹¹, which can apparently produce noise whilst hovering over a nearly fixed point, it performs a type of aerial dance and noise display thought to be of a sexual nature; we therefore suggest that the clap and fling mechanism may have a secondary sexual function in this case.

The significant aspect of these findings, both in locust and butterfly, is that the clap and fling mechanism seems to be used in forward flight as well as hovering, and thus must now be recognised as an aspect of primary importance in insect flight.

We thank Professor Sir James Lighthill for encouragement and advice, and CSIRO, Canberra for support in the field.

R. J. COOTER
P. S. BAKER

Centre for Overseas Pest Research,
London W8, UK

Post-metamorphic eye migration in *Rana* and *Xenopus*

CONTRADICTORY results have been obtained with *Xenopus laevis* and *Rana pipiens* in experiments directed at evaluating the possible role of visual experience in assuring proper binocular relations in the optic tectum. Gaze, Keating, and colleagues, working with *Xenopus*, reported that, in certain circumstances, experimental alteration of the contralateral visuotectal projection by eye rotation results in a modification of the ipsilateral projection so as to re-establish appropriate binocular relations^{1,2}. Jacobson and co-workers, however, working with *Rana*, reported no change in the ipsilateral projection after experimental rotation of the eye³⁻⁶. Based on the hypothesis that the ability to respond to experimental perturbation of interocular relationships would correlate with the amount of variation in interocular relationships occurring normally, we predicted that *Rana* might exhibit substantially less post-metamorphic eye migration than *Xenopus*. We report here results which confirm this prediction.

In both *Xenopus*⁷ and *Rana*³, the ipsilateral visuotectal projection develops during metamorphosis, when the eyes migrate from lateral positions, with little or no overlap of their visual fields, to positions with significant binocular overlap. Because we were concerned with possible changes in interocular relationships occurring after the ipsilateral projection has developed, we concentrated our attention on relative eye position from the completion of metamorphosis onwards. Keating⁸ drew attention to the fact that eye migration continues for some period post-metamorphically in *Xenopus*. We here provide quantitative confirmation of a continual change in relative eye position in post-metamorphic *Xenopus*. In contrast, we have found that relative eye position is stable post-metamorphically in *Rana*. The photographs in Fig. 1 illustrate these basic conclusions. Figure 1*a* shows the relative eye position in a *Xenopus* froglet in the final stage of metamorphosis, as well as in an older juvenile and in an adult. The eyes continue to migrate dorsally, towards each other, after the end of metamorphosis. Similar photographs, however, for the *Rana* froglet and adult (Fig. 1*b*) show strikingly similar relative eye positions.

To quantitate these observations we devised a modification of the techniques used by Fite⁹ to determine the extent of visual fields with relation to body axes in frogs and toads. *R. pipiens* and *X. laevis* were reared in the laboratory from fertilised eggs and adults were obtained commercially. At known metamorphic stages or post-metamorphic ages individuals were either anaesthetised or paralysed and mounted at the centre of and facing towards an Aimark projection perimeter. An ophthalmoscope held in a moveable clamp was used to illuminate the eye and to visualise light reflected from within it. Once this was done the ophthalmoscope was moved peripherally in the visual field until the reflected light just disappeared. The ophthalmoscope was then kept in this position while a mirror was used to intercept the light beam and reflect it back through the ophthalmoscope onto the perimeter where its position was noted as an angle with respect to body axes. Repetitions of this procedure for both eyes with different directions of movement of the ophthalmoscope outlined the extent of the anterior half of the two visual fields. The whole process was then repeated with the animal facing away from the perimeter, giving the posterior half of the visual fields.

Results are shown in Fig. 2. In all cases, data from four or more animals have been included to indicate the range of variations which along any meridian or

Received 23 May; accepted 7 July 1977.

- ¹ Weis-Fogh, T. *J. exp. Biol.* **59**, 169-230 (1973).
- ² Lighthill, J. *J. Fluid Mech.* **60**, 1-17 (1973).
- ³ Lighthill, J. in *Swimming and Flying in Nature 2* (eds Wu, T. Y., Brokaw, C. J. & Brennen, C.) (Plenum, New York, 1975).
- ⁴ Ellington, C. in *Swimming and Flying in Nature 2* (eds Wu, T. Y., Brokaw, C. J. & Brennen, C.) (Plenum, New York, 1975).
- ⁵ Weis-Fogh, T. in *Insect Flight* (ed. Rainey, R.) (Blackwell, London, 1976).
- ⁶ Haskell, P. T. *Insect Sounds* (Witherby, London, 1961).
- ⁷ Isely, F. *Ent. News* **47**, 199-205 (1936).
- ⁸ Karny, H. *Stettin. ent. Ztg* **49**, 112-129 (1908).
- ⁹ Haskell, P. T. *J. Insect Physiol.* **1**, 52-75 (1957).
- ¹⁰ Uvarov, B. *Locusts and Grasshoppers* (Imperial Bureau of Entomology, London, 1928).
- ¹¹ Pierce, G. *The Songs of Insects* (Harvard, Cambridge, Massachusetts, 1948).

parallel rarely exceeds 10° . A fair amount of this variation is attributable to uncertainties in the procedure itself, since repeated determinations in a single animal give comparable variations. It is clear from Fig. 2a and b that there is little or no difference in relative eye position between an immediately post-metamorphic and an adult *Rana*. For *Xenopus*, however, there is a significant amount of dorsal movement of the eyes subsequent to metamorphosis (Fig. 2c, d). The migration involves a shift of the visual axis of about 30° , is half completed at 7 wk, and still in progress at six months (Fig. 2e). There is apparently no substantial change in monocular visual field size or shape over this period.

The significance of the continuing eye migration in *Xenopus*, as pointed out by Keating⁸, is that, unless there is complicated reorganisation of the retinae, the region of one retina which is directed at the same part of visual space as a given region on the other is constantly changing. In these circumstances constant readjustment of the binocular interactions by the use of visual experience may be an essential developmental mechanism for maintaining the appropriate interactions between the two eyes. The results of experimental rotation of one eye may be interpreted as indicating the presence of such a mechanism operating during normal development. In *Rana*, however, as we have shown, the developmental problem of constant readjustment of binocular interactions is not present or, at least, is very much less. A mechanism for matching

Fig. 1 Photographs illustrating eye position at metamorphic climax and subsequently in a, *X. laevis* and b, *R. pipiens*. In each case, the smallest animal is one just at the completion of metamorphosis and the largest is 1 yr or more post-metamorphic. For *X. laevis*, an additional animal aged 4 wk post-metamorphosis is also shown.

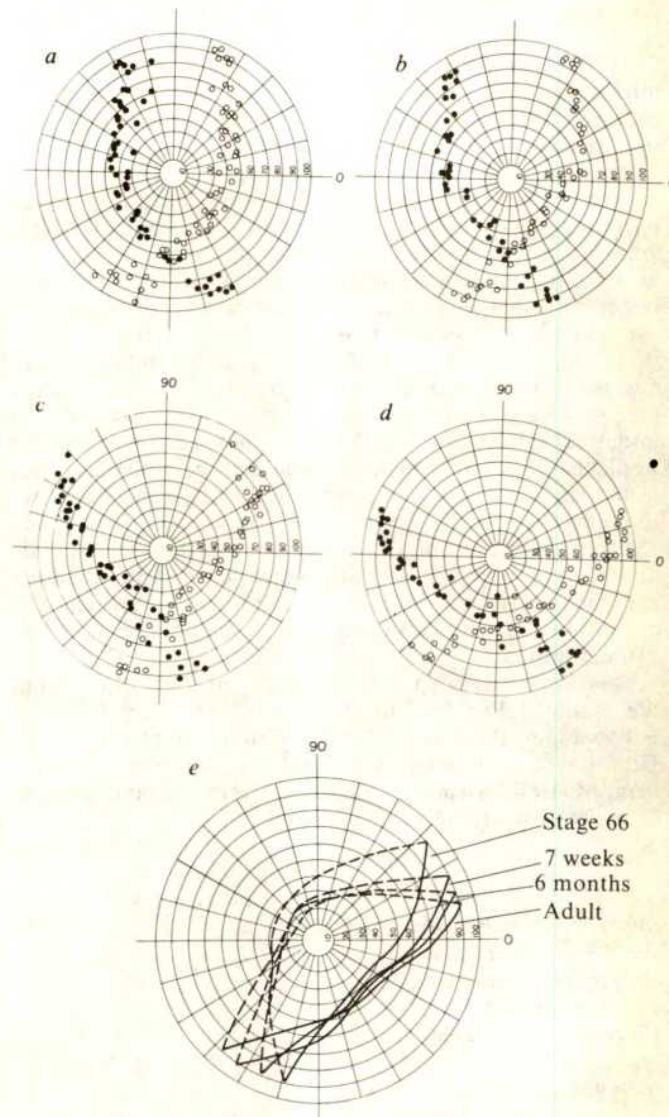
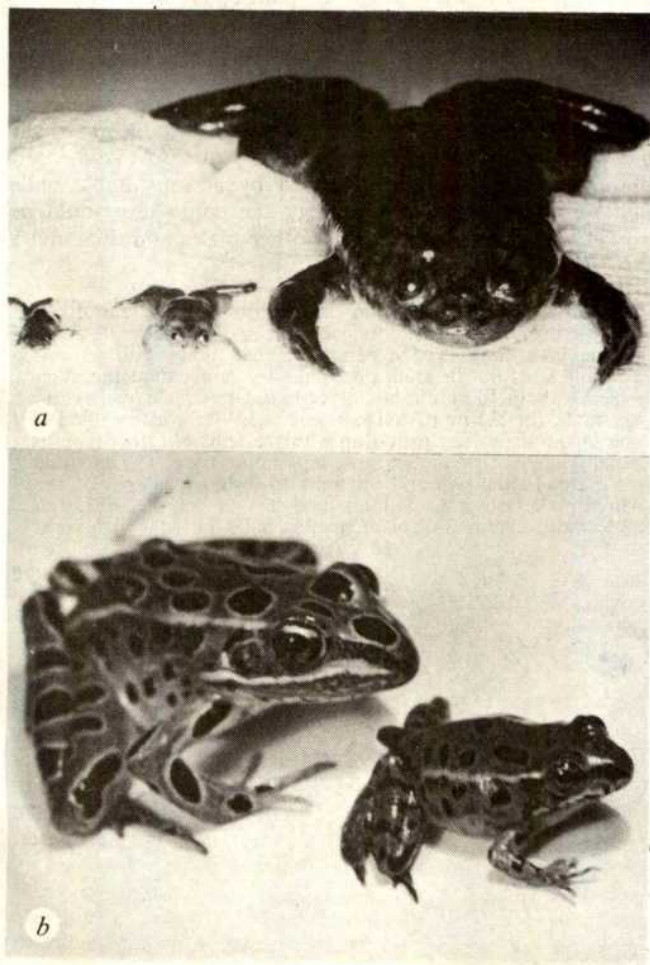


Fig. 2 Visual field boundaries for *R. pipiens* and *X. laevis*. Anterior visual field boundaries for the left (●) and right (○) eyes are shown for metamorphic climax *Rana* (a) and adult *Rana* (b) and for *Xenopus* (c, metamorphic climax; d, adult). Each map represents a hemisphere with the centre raised from the page. The animal should be pictured as sitting inside the hemisphere and facing the reader. The animal's eyes are in the plane of the page and centred on the intersection of planes corresponding to the vertical and horizontal meridia of the map. The symbols are actual border points for several different animals obtained as described in the text. Similar maps obtained for the posterior visual field boundaries are not shown. Data are from five animals in a, c and d and four in b. From maps like these, average visual field boundaries were estimated to illustrate the time course of eye migration in *Xenopus* (e). In (e), the visual field boundary of only the right eye of animals at the indicated ages is shown. The map, however, should this time be interpreted as a complete sphere with the posterior borders of the visual field (broken lines) being seen through the transparent anterior hemisphere.

the ipsilateral to the contralateral visuotectal projections may be correspondingly absent or significantly less effective and hence not lead to the same sequelae to experimental eye rotation as in *Xenopus*. Following this line of argument, our findings not only provide a possible explanation for the discrepant experimental results in *Rana* and *Xenopus* but also support the suggestion of Keating that visual experience is important in assuring proper binocular interaction in conditions of continuing eye migration⁸.

On the other hand, the differences between *Rana* and

Xenopus may be more quantitative than qualitative. In reviewing the mammalian literature, Grobstein and Chow^{10,11} put forward the proposition that binocular interaction may necessarily depend on visual experience because developmental variability makes genetic information inadequate to assure proper pairing of retinal regions. They also suggested that the influence of visual experience is probably constrained to some restricted range of operation by the genetically based developmental program (a suggestion earlier made by Shlaer¹²). It may well be that the range of operation in *Xenopus* is quite large, corresponding to the range of relative eye positions over which the system must operate, whereas in *Rana* it is quite small. Thus, the fairly large eye rotations which are necessary experimentally to be sure that a rotation has been accomplished lead to a compensation of the ipsilateral projections in *Xenopus* but not in *Rana*. It would be of interest to know whether the ipsilateral projection in *Rana* will compensate following very small eye rotations.

After submission of this paper it came to our attention that Keating¹³ has recently also suggested that the differing experimental results in *Rana* and *Xenopus* may relate to quantitative rather than qualitative differences between the two organisms.

This work was supported by PHS grant EY-01658 from the National Eye Institute, an Alfred P. Sloan Research Fellowship to P.G., and a Prather Fellowship to C.C. of the Department of Biology. We thank M. Jacobson, J. Goldberg, M. Hollyday and P. Ulinski for critical comments and C. Bailey for typing of manuscript.

PAUL GROBSTEIN
CHRISTOPHER COMER

Department of Pharmacological
and Physiological Sciences,
University of Chicago
947 East 58th Street
Chicago, Illinois 60637

Received 15 April; accepted 5 July 1977.

- 1 Gaze, R. M., Keating, M. J., Szekely, G. & Beazley, L. *Proc. R. Soc. B* 175, 107-149 (1970).
- 2 Keating, M. J. & Feldman, J. D. *Proc. R. Soc. B* 191, 467-474 (1975).
- 3 Jacobson, M. *Proc. natn. Acad. Sci. U.S.A.* 68, 528-532 (1971).
- 4 Jacobson, M. & Hirsch, H. V. B. *Brain Res.* 49, 47-65 (1973).
- 5 Skarf, B. *Brain Res.* 51, 352-357 (1973).
- 6 Skarf, B. & Jacobson, M. *Expl Neurol.* 42, 669-686 (1974).
- 7 Beazley, L., Keating, M. J. & Gaze, R. M. *Vision Res.* 12, 407-410 (1972).
- 8 Keating, M. J. *Br. med. Bull.* 30, 145-151 (1974).
- 9 Fite, K. V. *Behav. Biol.* 9, 707-718 (1973).
- 10 Grobstein, P. & Chow, K. L. in *Neural and Behavioral Specificity* (ed. Gottlieb, G.) 155-193 (Academic, New York, 1976).
- 11 Grobstein, P. & Chow, K. L. *Science* 190, 352-358 (1975).
- 12 Shlaer, R. *Science* 173, 638-641 (1971).
- 13 Keating, M. J. *Phil. Trans. R. Soc. B* 278, 277-294 (1977).

Optimal mate selection in the toad *Bufo bufo*

SEXUAL reproduction can no longer be regarded as a cooperative venture in which males and females are selected to achieve the same goals. The optimal reproductive strategy for a male is often very different from that for a female^{1,2}. We report here that, in the wild, toads (*Bufo bufo*) do not pair up at random, that this comes about through male-male competition probably influenced by the behaviour of the female and that mating involves a compromise between different male and female optima.

Toads visit ponds to breed in early spring. All the year's reproductive activity takes place within a few weeks, after which the toads leave the pond and remain on land until the next year³. Our observations were made at a pond near Oxford in March and April 1977. Every female we found in the pond was already in amplexus; that is a male was clasped onto her back, gripping her pectoral girdle with his forearms. Males greatly outnumbered females, however,

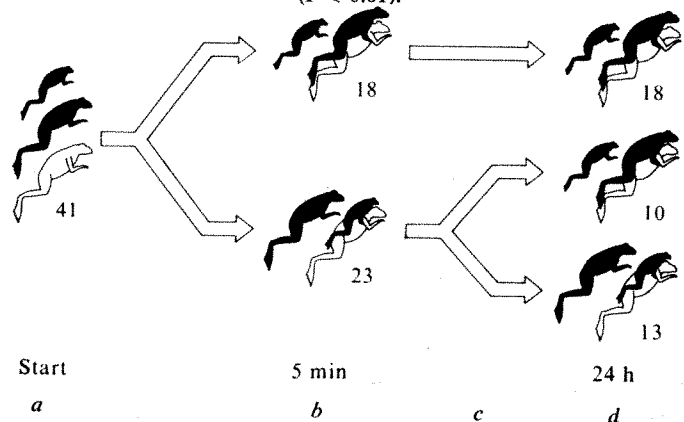
and during 11 d we found that $84.9 \pm 9.3\%$ (mean ± 1 s.d.) of the males were unpaired. The excess of males on any one day probably occurred because males can mate more than once and individuals spend most of the breeding season in the pond, whereas females, who can only spawn once, visit the pond for a shorter time. Thus at any particular time most of the male population is in the pond but only a small fraction of the female population^{3,4}.

We measured the snout-vent lengths of males and females we found in amplexus. Before the start of spawning there was no significant correlation between the length of males and females in amplexus (on one day, $r = 0.094$, $n = 32$; on another day, $r = 0.299$, $n = 26$). Small males were just as likely to be paired with large females as were large males. But when we measured the length of pairs actually engaged in spawning, we found a significant relationship ($r = 0.567$, $n = 16$, $P < 0.05$). By the time spawning had started the large females were paired with the large males. This presents two problems; first, what is the mechanism by which this non-random mating comes about and second, what is its functional significance?

What is the mechanism by which non-random mating comes about? In the laboratory we found that even males that were 30 mm smaller than their mates were easily able to maintain amplexus for a week or more. Thus the female herself is apparently unable to displace a male, even if he is very small. In the wild, however, we often found two males tussling over the possession of a female. To investigate the effect of male-male competition we performed a series of experiments in the laboratory, summarised in Fig. 1. The results showed that, just as in the wild, pairing was random at first but after some time large males were more likely to be paired with large females because they could achieve successful takeovers.

We conclude that male-male competition is the mechanism by which the change in pairing from pre-spawning to spawning comes about in the wild. Because it is the female who is in control of locomotion of the pair (her legs are in the most free position for swimming) it seems likely that she will be able to influence whether male-male competition takes place or not. If she has a suitable mate she may swim away from other males in order to avoid interference⁵. If she has been grabbed by an unsuitable male then we suggest that one strategy she could use would be to swim over to where there are other males and thus invite

Fig. 1 Male-male competition experiments in toads. Results of 41 experiments, each with one large male, one small male and a female in a tank. The experiments were done with various absolute sizes but in each, one male was larger than the other male by about 10 mm in body length. *a*, One female (white) and two males (black) are placed in a tank. *b*, Within a few minutes one of the males has paired up with the female. Either male is equally likely to be the first to adopt amplexus. *c*, The unpaired male attacks the pair and attempts to dislodge his rival. The paired male lashes out with his hind legs in defence. *d*, Large males can sometimes displace smaller males but not vice versa ($P < 0.01$).



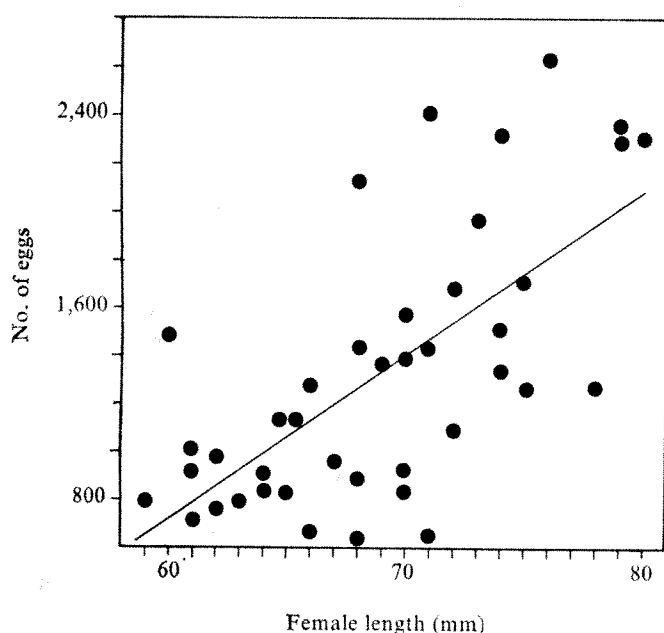


Fig. 2 Relationship between female body length, measured from snout to vent, and number of eggs laid. Results obtained from pairs spawning in individual tanks in captivity. A sample of 10 of the females was dissected and all had laid their full clutch of eggs. Fitted regression, $y = 67.47x - 3316.68$. A polynomial does not give a significantly better fit than the straight line. Correlation coefficient, $r = 0.669$, $P < 0.001$.

male-male competition. Obviously all this waste of time and energy could be avoided if the female found a suitable mate in the first place. In our laboratory experiments successful takeovers took on average 7 h to complete (mean \pm 1 s.d. = 420.3 ± 321.8 min, $n = 10$). Although we have no evidence for our species, in many anurans the female uses the call of the male as a basis for mate choice⁶. In this context it is interesting that different sized males have different calls so it is possible that a female might select a male of suitable size on the basis of his call⁷.

What is the functional significance of the non-random mating found in the wild? To investigate this question we paired up toads in the laboratory with partners of a size that they would not have mated with in the wild, to see if reproductive success was thereby decreased. We measured reproductive success in terms of the number of fertilised eggs produced. For any given pair this will depend on the number of eggs laid by the female and the percentage of them that are successfully fertilised by the male. Figure 2 shows that, as in other amphibians⁸, the number of eggs laid by a female is related to her size; large females lay more eggs.

As with all cases of external fertilisation, the male has the problem of timing his release of sperm so that it coincides with the female's release of eggs. Precise synchrony of gamete release is presumably important for efficient fertilisation. In many anurans it seems that the stimulus for sperm emission is the passage of the extruding eggs over the venter, cloaca or feet of the male⁹. In some species, just before egg emission, the female arches her back to bring her cloaca in close contact with that of the male⁹.

Figure 3 shows that the degree of matching in size between male and female affects fertilisation success. Where the male is relatively small compared with the female the fertilisation success is lower than where the partners are matched for size. We suggest that the most likely explanation for this relationship is that relatively small males are inefficient at detecting egg release and thus synchronising their timing of emission of sperm. Alternatively where the cloaca of the male is not close to that

of the female, the sperm must have further to swim to reach the eggs and this may decrease fertilisation success.

From the relationship between female length and egg number (Fig. 2) and percentage fertilisation and relative size of male and female (Fig. 3), it is possible to calculate the optimum mate size for a male or female of any given size. To do this we assumed that what will be maximised is the number of fertilised eggs (tadpoles) produced per breeding season. For any given pairing this is simply the number of eggs (calculated from female body length) multiplied by the percentage of these eggs that are successfully fertilised (determined by the relative size of the male and female). To achieve maximum fertilisation of their eggs, females should prefer the largest males. Males, on the other hand, should prefer a different optimum. For any given sized male the number of fertilised eggs increases with female size up to a maximum and then declines. This is because with relatively small females he can achieve efficient fertilisation but small females produce only a few eggs, while at the other extreme, relatively large females lay more eggs but the male can only fertilise a small proportion of them. To maximise the number of fertilised eggs, males should prefer females about 10–20 mm larger than themselves.

Figure 4 shows that there is a conflict between the sexes in the optimal mate size for achieving the maximum number of fertilised eggs. The pairings we observed in the wild mostly fall in between the male optimum and the female optimum. Because females are on average larger than males, the points tend to fall closer to the male optimum. This, however, does not necessarily mean that the males are winning the battle of the sexes. The theoretical optima curves were calculated with the assumption that individuals will be selected to maximise the number of tadpoles produced per year. In long-lived species like toads, however, individuals may be prepared to sacrifice maximal yearly reproduction if by so doing they increase their chance of survival to breed again in future years^{9,10}. Indeed, offspring quantity may be an inappropriate measure of fitness; we have ignored the possible importance of offspring quality (as determined, for

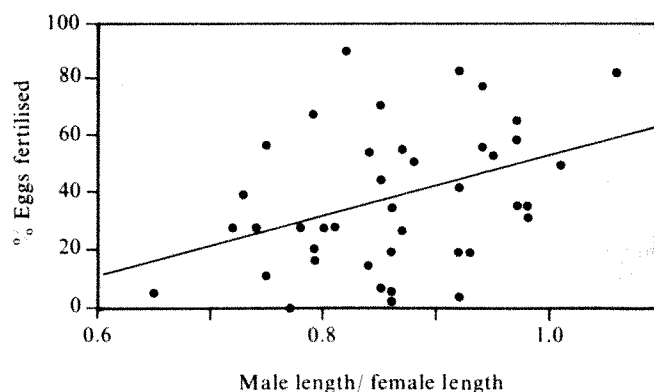


Fig. 3 The effect of relative male and female size (body length, snout to vent) in amplexing pairs of toads on the percentage of the eggs that are successfully fertilised. The smaller the male is relative to the female, the smaller the proportion of fertilised eggs. Pairs of toads were collected from the wild, parted and then formed into new pairs with various sizes of male and female in amplexus. Each pair was kept in a separate tank. The number of fertilised and unfertilised eggs were scored 15 d after egg laying. At this stage the fertilised eggs had developed into small tadpoles, while unfertilised eggs remained as unchanged spheres. None of 100 unchanged eggs examined microscopically from each pair had undergone any cell division. Fitted regression, $y = 104.73x - 52.36$. Correlation coefficient, $r = 0.379$, $P < 0.02$. When the effect of egg number on percentage fertilisation is partialled out, the effect of relative size is still significant ($P < 0.05$). Likewise, it is still significant ($P < 0.02$) when the effect of absolute size of male is partialled out.

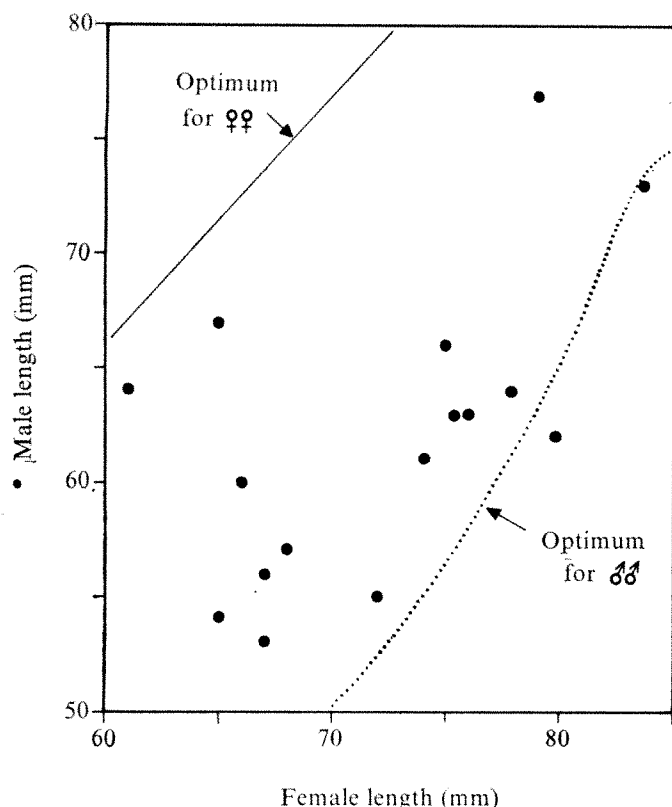


Fig. 4 The conflict between males and females in the optimum size of mate assuming that what is maximised is the number of fertilised eggs produced per year. The female line is a minimum because no experimental pairings were done with values greater than 1.1 (for male length divided by female length). If the line in Fig. 3 continued to rise with relatively larger males, then the female optimum curve should be shifted to the left so that larger males are preferred than is indicated. Observed pairings in the wild in the process of spawning (solid circles), fall in between the male and female optima. (Correlation coefficient, $r = 0.567$, $P < 0.05$.)

example, by egg size⁸). Finally we have examined mate selection solely with respect to body size whereas it seems certain that other characters will also influence fitness. In spite of these reservations, as far as we know these data provide the only clear evidence, apart from that from one laboratory study¹¹, that individuals can increase their fertility by non-random choice of mates.

We thank Christopher Perrins and David McFarland for facilities in the Zoology Department at Oxford, J. A. Pye for permission to study at his pond, Ken Marsland and Michael Rayner for help with the experiments, Richard Dawkins and John Krebs for comments on the manuscript and Maggie Norris for typing it.

N. B. DAVIES
T. R. HALLIDAY

Edward Grey Institute and
Animal Behaviour Research Group,
Department of Zoology,
University of Oxford, UK

Received 22 June; accepted 19 July 1977.

- ¹ Trivers, R. L. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) 136–179 (Heinemann, London, 1972).
- ² Williams, G. C. *Sex and Evolution* (Princeton University Press, Princeton, 1975).
- ³ Smith, M. *The British Amphibians and Reptiles* (Collins, London, 1973).
- ⁴ Savage, R. M. *The Ecology and Life History of the Common Frog* (Pitman, London, 1961).
- ⁵ Martof, B. S. & Thompson, E. F. *Behaviour* 13, 243–258 (1958).
- ⁶ Salthe, S. N. & Mecham, J. S. in *Physiology of the Amphibia*, 2 (ed. Loftis, B.) 309–521 (Academic, London, 1974).
- ⁷ Licht, L. E. *Can. J. Zool.* 54, 1277–1284 (1976).
- ⁸ Salthe, S. N. *Am. Midl. Nat.* 81, 467–490 (1969).
- ⁹ Tinkle, D. W. *Am. Nat.* 103, 501–516 (1969).
- ¹⁰ Elliott, P. F. *Am. Nat.* 109, 281–287 (1975).
- ¹¹ Maynard Smith, J. *J. Genet.* 54, 261–279 (1956).

Use of Ficoll–sodium metrizoate density gradient to separate human X- and Y-bearing spermatozoa

SEVERAL claims have been made about the successful separation of Y-bearing spermatozoa in the past¹. The most recent claim by Ericsson *et al.*² has been disputed^{3,4}. We have attempted to develop a method for separating human X- and Y-bearing spermatozoa, without affecting the motility or the variability of these vital cells. We report here a successful separation of human X- and Y-bearing spermatozoa using a two-step procedure with Ficoll–Sodium metrizoate density gradient. The motility and viability of enriched fractions of spermatozoa was found to be slightly reduced. The reduction was mainly due to centrifugation and not the density gradient. The ability to predetermine the sex of the offspring before conception would have great clinical and sociological significance⁵.

Semen was collected from normal human volunteers. Only samples with a count of over $6 \times 10^7 \text{ ml}^{-1}$ and a motility of at least 50% were used for the experiments. Experiments were performed within 1 h of sample collection.

Ficoll-400 (Pharmacia) and sodium metrizoate (Nyeguard) density gradients were used for the separation. Each semen sample was diluted 20-fold with phosphate buffered saline (PBS), pH 7.2 and centrifuged at 100g for 20 min. The spermatozoa were washed twice with the same buffer, in identical conditions.

In a one-step separation technique the washed spermatozoa were suspended in PBS and made up to the original volume of the semen. The density gradient was prepared by mixing 2.4 ml of 8% Ficoll with 1.0 ml of 32.8% sodium metrizoate. The density of this gradient was 1.08. The spermatozoa suspension was layered on Ficoll–sodium metrizoate density gradient and centrifuged for 20 min at 100g. After centrifugation different layers were separated and suspended in PBS, and the count adjusted to 10^6 ml^{-1} . Smears of these suspensions were stained for the detection of the fluorescent body.

In a two-step separation technique the first step separation was carried out as above. The enriched fractions obtained after the first step were again layered on 15% Ficoll of density 1.04. This was centrifuged for 10 min at 100g. The spermatozoa from the different layers were separated and smears were made for staining.

A modification of the method of Barlow and Vosa⁶ was used for staining the fluorescent body. The smears were air dried, fixed in methanol for 15 min stained by immersing the slides in 0.5% quinacrine hydrochloride (Sigma) for 15 min and washed in running tap water. Excess dye was removed by immersing the slide in PBS at pH 5.6, for 15 min. The slides were mounted in glycerolphosphate buffer (1:9), and examined under oil immersion with dark field condenser of Olympus fluorescent microscope with barrier filter Y-50 and exciter filter B₁. About 10 fields with a minimum of 100 spermatozoa were counted, from each slide, for the presence of the fluorescent body.

After centrifugation with Ficoll–sodium metrizoate it was seen that some of the spermatozoa had settled down and there was a very clear interface which was also rich in spermatozoa. The sediment (S) was rich in Y-bearing spermatozoa, whereas the interface consisted mostly of X spermatozoa (I). The mean percentage of Y-bearing spermatozoa was $43 \pm 2.7\%$ in unseparation (Table 1). The interface contained $74.7 \pm 2.9\%$ X spermatozoa, whereas the percentage in unseparated suspension spermatozoa, whereas the percentage in unseparated suspension was $56.3 \pm 2.7\%$.

In the two-step separation process the percentage of Y-bearing spermatozoa following the initial one-step separation (S₁) was $73.2 \pm 1.0\%$ and this increased only to $76.9 \pm 2.1\%$ after the second separation step (S₂) (Table 2). The results of the one and two step separation procedures with different samples were very consistent. Table 3 shows the percentages of X-spermatozoa before and after two-step separation. The mean percentage of X spermatozoa in the unseparated sample was 54.2 ± 3.2 . The first step procedure

Table 1 Percentages of X- and Y-bearing spermatozoa separated on Ficoll-sodium metrizoate density gradient (1.08) and centrifuged for 20 min

Experiment no.	% Y spermatozoa in washed samples before separation	% Y spermatozoa following separation (S)	% X spermatozoa before separation (I)	% X spermatozoa after separation
1	41.9	70.4	58.1	68.9
2	46.6	70.8	53.4	73.3
3	42.6	72.4	57.4	73.8
4	43.3	72.7	56.7	78.4
5	39.8	77.8	60.2	77.8
6	41.9	72.4	58.1	77.6
7	45.8	72.7	54.2	74.6
8	48.4	73.2	51.6	74.2
9	43.0	74.5	57.0	74.1
Mean \pm s.d.	43.7 \pm 2.7	72.8 \pm 2.2	56.3 \pm 2.7	74.7 \pm 2.9

Spermatozoa were washed twice with PBS pH 7.2 before layering on density gradient.

yielded a fraction containing 74.7 ± 2.9 and the second step 80.6 ± 6.3 of X spermatozoa.

Our results demonstrate that the percentage of Y-bearing spermatozoa in fractions S_1 and S_2 and that of X spermatozoa in fractions I_1 and I_2 are considerably higher than in unseparated samples. It is known that spermatozoa in the semen of normal fertile human males, show considerable heterogeneity⁷. The X- and Y-bearing spermatozoa so separated seemed to be uniform in size and shape in each of the concerned fractions.

it may be assumed that the calculated percentage of the Y-spermatozoa in the one step procedure might be about 82% and in two-step separation it might be as high as 86%.

The Ficoll-sodium metrizoate density gradient has been used extensively for the separation of lymphocytes without any functional damage to cells⁹. Ficoll has also been used for washing spermatozoa¹⁰, and is known not to damage the cells. We have carried out a few experiments to check the motility and viability of spermatozoa following separation. We found that the original

Table 2 Percentage of Y-bearing spermatozoa following two-step separation

Experiment no.	% Y spermatozoa before separation	% Y spermatozoa following one-step separation (S_1)	% Y spermatozoa following two-step separation (S_2)
1	41.9	72.4	80.9
2	45.8	72.7	75.9
3	48.4	73.2	77.9
4	43.0	74.5	77.8
5	41.3	74.2	75.0
6	46.3	71.6	75.0
7	44.2	73.5	76.0
Mean \pm s.d.	44.4 \pm 2.6	73.2 \pm 1.0	76.9 \pm 2.1

The first separation (S_1) was carried out by layering washed spermatozoa on Ficoll-sodium metrizoate gradient of density 1.08. The spermatozoa thus separated were again layered on 15% Ficoll of density 1.04 (S_2).

It is of interest to note that the mean percentage of Y-bearing spermatozoa in semen samples used in the first set of experiments was $43.7 \pm 2.7\%$ and in the second set of experiments $44.4 \pm 2.6\%$. It is generally accepted that 50% of spermatozoa possess Y chromosomes. The lower percentage of Y-bearing spermatozoa observed by us could be due to the fading of fluorescence. It could also be that the ratio was low because of the difficulty of resolving fluorescent body which lies within the dense chromatin⁶. Yet, another reason may be the low uptake of the fluorescent dye by some spermatozoa⁸. If we take all these factors into consideration

motility (85%) was slightly reduced (75%) after the first centrifugation, and motility was further reduced to 58% after another centrifugation with PBS. But, when spermatozoa were layered on Ficoll-sodium metrizoate and centrifuged, motility was 65% and 70% in the interface and the sediment fractions respectively. Thus the gradient has a protective action on the cells, preserving sperm motility. Viability was slightly reduced after two centrifugations, however. This reduction in motility and viability is mainly due to centrifugation and not due to the effect of density gradient. It has been reported¹¹ that following artificial insemination (AI) there

Table 3 Percentage of X-bearing spermatozoa following two-step separation

Experiment no.	% X spermatozoa before separation	% X spermatozoa following one step separation (I_1)	% X spermatozoa following two-step separation (I_2)
1	58.1	77.6	94.4
2	54.2	74.6	80.5
3	51.6	74.2	76.5
4	57.0	74.1	76.6
5	48.7	78.8	78.8
6	53.7	69.8	77.2
7	55.8	73.5	80.2
Mean \pm s.d.	54.2 \pm 3.2	74.7 \pm 2.9	80.6 \pm 6.3

The first separation (I_1) was carried out by layering washed spermatozoa on Ficoll-sodium metrizoate gradient of density 1.08. Spermatozoa thus separated were again layered on 15% of Ficoll of density 1.04 (I_2).

was an increased number of female offsprings when insemination was carried out 3–4 days before ovulation and an increased probability of males as the ovulation approached. The probability of producing offspring of a desired sex would be enhanced if AI was carried out at the appropriate time¹¹.

PADMA R. SHASTRY
UMASHASHI C. HEGDE
SHANTA S. RAO

*Institute for Research in Reproduction,
Jehangir Merwanji Street, Parel,
Bombay 400 012, India*

Received 9 May; accepted 13 June 1977.

- ¹ Beatley, R. A. *Biol. Rev.* **45**, 73–119 (1970).
- ² Ericsson, R. J., Langevin, C. N. & Nishino, M. *Nature* **246**, 421–424 (1973).
- ³ Evans, J. M., Douglas, T. A. & Renton, J. P. *Nature* **253**, 352–354 (1975).
- ⁴ Ross, A., Robinson, J. A. & Evans, H. J. *Nature* **253**, 354–355 (1975).
- ⁵ *Lancet* **i**, 203–204 (1974).
- ⁶ Barlow, P. & Vosa, C. G. *Nature* **226**, 961–962 (1970).
- ⁷ Hafex, E. S. E. & Kanagawa, D. U. M. *Fertil. Steril.* **24**, 776–787 (1973).
- ⁸ Roberts, A. M. & Goodhall, H. *Nature* **262**, 493–494 (1976).
- ⁹ Boyum, A. *Scand. J. clin. Lab. Invest.* **21**, suppl. 97 (1968).
- ¹⁰ Harrison, J. J. *Reprod. Fert.* **48**, 347–353 (1976).
- ¹¹ Guerrero, V. R. *Int. J. Fert.* **15**, 221–225 (1970).

Antigen expression on early mouse trophoblast

THE mammalian foetus inherits paternal as well as maternal genes and can be considered to constitute a naturally occurring and highly successful allograft¹. It has been suggested that an important factor in the survival of the foetus in an immunocompetent histoincompatible mother might be the presence of an antigenically deficient barrier of foetal trophoblast cells^{2–4}. Cell surface antigens determined by minor (non-H-2) histocompatibility loci have been demonstrated on cleavage stage mouse embryos⁵, and subsequently major (H-2) as well as non-H-2 determinants have been detected on the trophectoderm of pre-implantation mouse blastocysts^{6,7}. The expression of these antigens, however, is considerably reduced, below detectable levels in the case of H-2, on blastocytes which have been activated for implantation from a state of experimentally induced delay^{6,7}. In addition, immunofluorescence⁸, immunoperoxidase⁷ and mixed haemadsorption (MHA)⁹ studies have failed to demonstrate histocompatibility antigens on the trophoblast component of blastocysts cultured as outgrowths *in vitro*. In contrast to all these findings, however, a recent report has claimed to demonstrate both maternally and paternally inherited antigens on blastocyst outgrowths by means of an MHA technique¹⁰. We have now confirmed this report and reconciled the apparently contradictory results by investigation of the identity of the antigens expressed by the trophoblast at this early stage of its differentiation in four mouse inbred strains.

Blastocysts from spontaneously ovulating females were collected approximately 80 h after mating by flushing the excised uteri with phosphate-buffered saline (PBS). After removal of the zona pellucida by digestion with 0.5% Pronase, embryos were cultured in groups of four or five in the wells of plastic migration plates (Sterilin) under RPMI 1640 medium. MHA tests were performed after 96 h in culture. Blastocyst outgrowths were incubated for 2 h with various dilutions of specific alloantisera either obtained commercially (Searle Diagnostic) or raised between inbred strains of mice by sequential intraperitoneal injections of adult spleen cells. After thorough washing with PBS, a 0.5% suspension of freshly prepared indicator sheep red cells (ISRC)¹¹ was added to the cultures and incubation continued for a further 1 h. Cultures were finally washed in PBS and examined by phase contrast microscopy for adherence of ISRC. Scoring was based on a visual assessment similar to that described by Hausman and Palm¹² and was confirmed by an independent observer. The outgrowths were subsequently fixed in 2.5% glutaraldehyde and stained with

Giemsa as a permanent record. The results are summarised in Table 1.

Although both CBA and C57BL outgrowths showed strongly positive adherence of ISRC after incubations with antisera raised against multiple (H-2+non-H-2) or minor (non-H-2) specificities, no adherence was noted when antisera raised between strains congenic for H-2 were used. The results imply that the reactivity previously reported with these strains¹⁰ is not due to antigens specified by the major histocompatibility complex (MHC). Similar adherence was not observed on A outgrowths when tested with an antiserum against multiple specificities raised in C57BL mice, thus confirming earlier results using this strain combination^{9,13}.

CBA anti-C57BL antiserum detects non-H-2 antigens on B10.BR outgrowths, which is probably a reflection of the common genetic background of the C57BL and B10.BR strains¹⁴, and also detects non-H-2 antigens on A strain trophoblast. Non-H-2 antigens, however, cannot be detected on A outgrowths by either C57BL anti-A or C57BL anti-CBA antisera, even though the latter, adsorbed to remove activity against H-2 antigens, is strongly positive in MHA tests against monolayer cultures of A strain embryonic fibroblasts (unpublished observations). Conversely, the C57BL anti-A antiserum, which is negative for A outgrowths, gives a weakly positive reaction with CBA outgrowths, implying that non-H-2 antigens expressed by adult A strain spleen cells and shared by CBA outgrowths are nevertheless not expressed on A strain trophoblast. This reactivity is unlikely to be a result of shared H-2^k specificities since CBA outgrowths do not react with the congenic anti-H-2^k serum. Since outgrowth from F₁ hybrid blastocysts of reciprocal CBA×A matings continue to express CBA-associated antigens, of both maternal and paternal origin (unpublished observations), and A outgrowths express non-H-2 antigens detected by CBA anti-C57BL antiserum, it is unlikely that any gross masking effect is responsible for the non-expression on A outgrowths of those non-H-2 antigens which are shared by CBA trophoblast and adult A strain tissues. It is therefore clear that H-2 antigens are not expressed on trophoblast at this stage of development and also that there are strain differences in the expression of non-H-2 antigens.

It has been recognised that determinants controlled by the MHC are involved in the interaction of T lymphocytes

Table 1 Detection of cell surface antigens on trophoblast of blastocyst outgrowths by mixed haemadsorption assay (MHA)

Antiserum	Antigens detected	CBA (H-2k)	Target outgrowth C57BL (H-2b)	A (H-2kd)	B10.BR (H-2k)
C57BL anti-CBA	H-2k + non-H-2	+++	—	—	—
CBA anti-C57BL	H-2b + non-H-2	—	+++	++	++
C57BL anti-A	H-2kd + non-H-2	+	—	—	ND
C57BL/10ScSn anti-B10.BR	H-2k	—	—	—	—
B10.BR anti-C57BL/10ScSn	H-2b	—	—	—	—
C57BL anti-CBA (abs)*	non-H-2 only	+++	ND	—	ND

+++ , Heavy adsorption of ISRC on all trophoblast cells. ++ , moderate adsorption of ISRC on > 80% of trophoblast cells. + , light adsorption of ISRC on > 50% of trophoblast cells. — , no adsorption of ISRC. (Occasionally one or two cells in some outgrowths showed strong adsorption. These were very likely of inner cell mass origin.) ND, not determined. Each result is based on observation of not less than 10 outgrowths.

*C57BL anti-CBA antiserum was adsorbed twice against equal volumes of washed B10.BR lymphoid cells. Each adsorption was for 1 h at 37 °C and the resulting serum had no residual activity against H-2k as measured by MHA on B10.BR embryonic fibroblasts.

with non-MHC antigens¹⁵. Lack of reactivity of blastocyst outgrowth trophoblast with antisera which are probably directed against the products of the entire MHC may imply an absence not only of those determinants which are necessary for efferent recognition by cytotoxic T lymphocytes (K and D region determinants)¹⁶ but also of those which are necessary for T-cell sensitisation (the I region associated Ia antigens)¹⁷. Thus the absence of MHC products may enable the trophoblast to fulfil an immunological barrier function because it is unlikely that the expression of non-MHC antigens could render it susceptible to damage by maternal T cells. The possible interplay between these components of the trophoblast cell surface and other types of maternal immune response as well as their potential involvement in developmental interactions with maternal and/or foetal cells is unexplored.

It must be emphasised that these findings relate only to the trophoblast of the blastocyst outgrowth, for the antigenic status of trophoblast is not constant throughout its ontogeny and differentiation^{9,13}. This probably reflects a changing functional role for this tissue in the materno-foetal immunological relationship.

I thank Drs E. J. Jenkinson and W. D. Billington for helpful discussion and advice. The work was initiated during the tenure of a graduate studentship from the MRC and subsequently supported by the Rockefeller Foundation.

M. H. SELLENS

Reproductive Immunology Research Group,
Department of Pathology,
The Medical School, University of Bristol,
Bristol, UK

Received 27 May; accepted 22 July 1977.

- ¹ Beer, A. E. & Billingham, R. E. *Adv. Immun.* 14, 1-84 (1971).
- ² Simmons, R. L. & Russell, P. S. *Nature* 208, 698-699 (1965).
- ³ Kirby, D. R. S., Billington, W. D. & James, D. A. *Transplantation* 4, 713-718 (1966).
- ⁴ Edwards, R. G., Howe, C. W. S. & Johnson, M. H. (eds) *Immunobiology of Trophoblast* (Cambridge University Press, London, 1975).
- ⁵ Muggleton-Harris, A. & Johnson, M. H. *J. Embryol. exp. Morph.* 35, 59-72 (1976).
- ⁶ Håkansson, S., Heyner, S., Sundqvist, K. G. & Bergstrom, S. *Int. J. Fert.* 20, 137-140 (1975).
- ⁷ Searle, R. F., Sellens, M. H., Elson, J., Jenkinson, E. J. & Billington, W. D. *J. exp. Med.* 143, 348-359 (1976).
- ⁸ Heyner, S. *Transplantation* 16, 675-678 (1973).
- ⁹ Billington, W. D., Jenkinson, E. J., Searle, R. F. & Sellens, M. H. *Transplant. Proc.* 9, 1371-1377 (1977).
- ¹⁰ Carter, J. *Nature* 262, 292-293 (1976).
- ¹¹ Fagraeus, A., Espmark, J. A. & Jonsson, J. *Immunology* 9, 161-175 (1965).
- ¹² Hausman, S. J. & Palm, J. *Transplantation* 16, 313-324 (1973).
- ¹³ Sellens, M. H. thesis, Univ. Bristol (1976).
- ¹⁴ Klein, J. *Transplantation* 15, 137-153 (1973).
- ¹⁵ Raff, M. C. *Nature* 263, 10-11 (1976).
- ¹⁶ Bevan, M. J. *Nature* 256, 419-421 (1975).
- ¹⁷ Peck, A. B., Janeway, C. A. Jr. & Wigzell, H. *Nature* 266, 841-842 (1977).

Ly-6 is a T-cell differentiation antigen

THERE is ample evidence that membrane antigenic markers change as the cells differentiate along a given pathway. Thus a B cell acquires its receptors, immunoglobulin, as it matures to a functional state. Yet other membrane markers are acquired as cells differentiate from precursor to the activated effector cell stage. For example, the membrane alloantigen Pca-1 is acquired as B cells become antibody-forming cells¹. Another example is the alloantigen Ala-1, presented on activated lymphocytes of both T- and B-cell series, but not on resting cells². Suppressor cells are lysed by anti-Ia antisera³ while their precursors are not⁴, suggesting either the acquisition of Ia antigens on activation, or a change in susceptibility to lysis. This is of interest as the active molecules of suppression, 'suppressor factors', themselves bear Ia antigens⁵. Thus, in this instance there is the acquisition of a membrane marker which is also present in the functional effector molecule. McKenzie and his colleagues recently described a new T-cell alloantigen, Ly-6, which is expressed on the majority of peripheral T cells but only on a small proportion of thymocytes⁶. Using antiserum directed against Ly-6.2 we have published evidence suggesting that this antigen is present on cytotoxic effector cells⁷.

In the work presented here we extend those investigations, showing that while Ly-6 is present on cytotoxic effector cells it is absent from precursor cells, unlike the Ly-1, 2, 3 antigens where precursor cells usually carry the same antigens as effectors⁸. These observations suggest that Ly-6 can be considered as a unique T cell-restricted differentiation antigen that evolves fairly late in the maturation scheme, at least for killer cells.

When various antisera, directed against cell surface allo-antigens were used to phenotype the cytotoxic effector cells (Fig. 1), we found that AKR (Ly-1.2) killer cells express the phenotype Thy-1⁺ Ly-1⁺2⁺3⁺5⁺6⁺, although in other studies using Ly-1.1 positive strains, such as CBA, killer cells bear the phenotype Thy-1⁺ Ly-1⁺2⁺3⁺ (refs 2, 9). To study the phenotype of the precursor cell it was necessary to treat normal spleen cells with the various antisera and complement and then examine the ability of the remaining cells to generate cytotoxic effector cells. The results of three such experiments are shown in Table 1. The data on total activity indicate that the reduction in killing capacity seen in the populations treated with anti-Ly-2.1 or anti-Thy-1.1 is not the result of poor culture viability. It is clear that the phenotype of the killer cell precursor is Thy-1⁺ Ly-1⁺2⁺6⁻ indicating that the alloantigen Ly-6 is either not carried on the precursor cells, or that the amount of antigen present is not sufficient to mediate complement dependent lysis. A third possibility would be that these particular cells are resistant to complement lysis, however, this is unlikely as the cells are susceptible to lysis by other alloantisera of the same titre such as anti-Thy-1 and anti-Ly-2. In two experiments (data not shown) the anti-Ly-6 was used two- and fourfold more concentrated than necessary to have plateau level killing. Results of these assays were similar to those shown. Treatment, however, of normal cell populations using alloantisera at plateau levels of cytotoxicity fails to eliminate

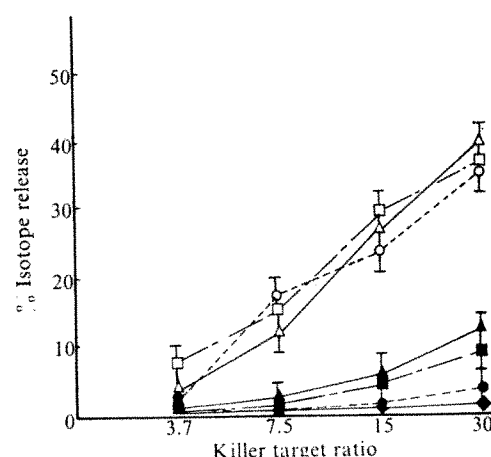


Fig. 1 Treatment of cytotoxic effector cells with various allo-antisera and complement. AKR spleen cells (H-2^k, Thy-1.1, Ly-1.2, Ly-2.1, Ly-5.1, Ly-6.2) at 10×10^6 per ml were incubated with 3×10^6 irradiated (2,000 rad) BALB/c (H-2^d) spleen cells for 6 d in Marbrook tissue culture flasks. The cells were collected and aliquots placed in tubes containing 3×10^6 viable cells. The cells were treated with 0.3 ml of alloantiserum, used at plateau levels of cytotoxicity, for 30 min at 37 °C, washed, and then treated with 0.3 ml complement diluted to 10%, controls included cells treated with media or complement only. The treated cells were then resuspended to 0.5 ml and serially diluted in microtitre plates. Chromium labelled P815 (H-2^d) mastocytoma cells were used as targets, and were added such that the highest killer target ratio was between 20 : 1 and 30 : 1. After initial centrifugation at 200g the assay was incubated for 4 h at 37 °C, centrifuged again, and 100 µl of supernatant aspirated for counting. The % isotope release is calculated as for Table 1. The data are expressed as the mean % isotope release of duplicate values \pm s.e.m. Antisera— α Ly-1.2, Δ ; no addition, \circ ; C', \square ; α Ly-2.1, Δ ; α Ly-6.2, \blacksquare ; α Ly-5.1, \bullet ; α Thy-1.1, \blacklozenge .

Table 1 Effect of pretreatment of normal spleen cells with alloantiserum and complement on ability to generate cytotoxic effector cells

Treatment of normal spleen cells	% Isotope release (total activity)		
	Experiment 1	Experiment 2	Experiment 3
Nil	90±0.5 (1,260)	60±3 (1,020)	70±5 (1,008)
C' alone	83±3.0 (1,162)	58±2 (986)	60±1 (678)
Anti-Ly-1.2+C'	81±2.0 (1,134)	70±4 (1,470)	ND
Anti-Ly-2.1+C'	52±1.0 (899)	20±2 (300)	ND
Anti-Ly-6.2+C'	84±1.0 (1,130)	54±3 (1,080)	57±1 (974)
Anti-Thy-1.1+C'	48±1.0 (614)	18±3 (270)	16±1 (142)

30×10^6 normal AKR spleen cells (H-2^k, Thy-1.1, Ly-1.2, 2.1, 6.2) were treated with various alloantisera, used at plateau levels of cytotoxicity, and rabbit serum, absorbed with mouse spleen cells, as a source of complement. The cells remaining after a two-stage cytotoxicity procedure were adjusted to 10×10^6 cells per ml and incubated with 3×10^6 irradiated (2,000 rad) BALB/c (H-2^d) spleen cells, for 6 d in a Marbrook tissue culture chamber. The cells were collected and tested for their ability to lyse chromium labelled P815 (H-2^d) mastocytoma cells in a 4-h assay. A peak killer target ratio of 20 : 1 was used, with serial dilutions of the effector cells (not shown). The data in each experiment shown for the 20 : 1 ratio are also valid for each of the serial dilutions. The % isotope release was calculated using the formula

$$\% \text{ isotope release} = \frac{\text{Experimental value} - \text{medium background}}{\text{Detergent release} - \text{medium background}} \times 100$$

Total activity = % viable cell yield \times % isotope release at 20 : 1 K:T ratio. Values are mean \pm s.e.m. ND, Not determined.

all of the precursor cells. This is not surprising as the actual precursors are likely to be heterogeneous with regard to the amount of antigen expressed on the cell surface and as some might escape antiserum treatment. This might be expected in the case of weak alloantisera where the effective antibody is in low concentration. An alternative view is that if only a small percentage of precursors escape kill these may still give a surprisingly high response because feedback controls provided by the missing cells are now lacking.

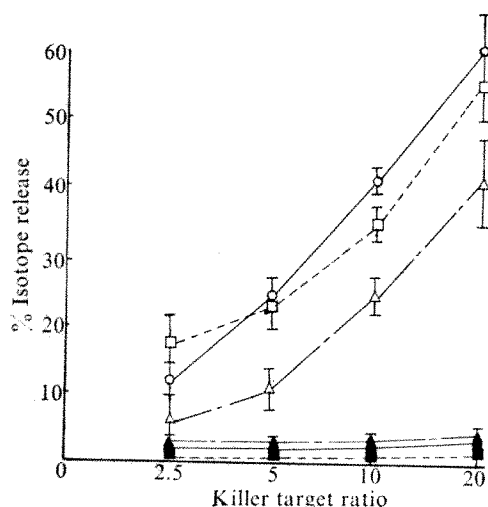
Figure 2 shows the results of experiments in which cytotoxic effector cells were generated from anti-Ly-6.2 treated spleen cells. The killer cells were then retested for phenotype using the various alloantisera. It is clear that the phenotype Thy-1⁺ Ly-1⁺ 2⁺ 6⁺ is identical to killer cells generated from untreated spleen cells. This suggests that only one pathway is open for the maturation of killer cells, and that at least for the allogeneic system, only one type of cytotoxic cell can be generated in that they are all Ly-6⁺. Replicate experiments have given similar results.

Mouse strains used for producing anti-Ly-6.2 also differ at Ala-1, hence a theoretical possibility exists that Ala-1.2

contaminates our anti-Ly-6.2, although the former alloantisera is produced by immunisation with mitogen-activated cells, and the latter with resting spleen and lymph node cells, which should not carry the Ala-1 antigens. The results of an experiment designed to answer this question are shown in Table 2. After five absorptions of anti-Ly-6.2 with 129 mouse spleen cells (Ly-6.2, Ala-1.1) the ability to lyse killer cells was essentially gone indicating that the major cytotoxic activity of anti-Ly-6.2 is directed against that alloantigen, and that any anti-Ala activity present is at best very weak. The reduction of activity in anti-Ly-6 absorbed with CBA spleen cells probably represents some dilution as well as passive absorption, to be expected when multiple absorptions are performed.

In addition to the implications with regard to the diversity of T cells, our findings suggest that Ly-6 is a differentiation antigen for cytotoxic effector cells. Whether this holds true for other functional T cells such as suppressor cells and helper cells is under study. If Ly-6 is a differentiation antigen for effector T cells one might wonder why it is cytotoxic for such a large number of peripheral T cells. It could well be that the majority of peripheral cells are in fact memory cells for helper, suppressor or killer populations awaiting reactivation with antigen and hence might be susceptible to Ly-6 treatment. One might predict that Ly-1⁺ helper cells¹⁰, Ly-2⁺ 3⁺ suppressor cells¹¹ and Ly-1⁺ 2⁺ 3⁺ killer cells¹², will be sensitive to anti-Ly-6.2 in contrast to their precursors.

Fig. 2 Treatment of cytotoxic effector cells generated from anti-Ly-6.2 treated spleen with various alloantisera. 120×10^6 normal AKR (H-2^k, Thy-1.1, Ly-1.2, Ly-2.1, Ly-6.2) spleen cells were treated with anti-Ly-6.2 and complement. The viable cells (10×10^6 per ml) were incubated with 3×10^6 per ml irradiated (2,000 rad) BALB/c (H-2^d) spleen cells for 6 d in Marbrook tissue culture flasks. The cells were collected and aliquots placed in tubes containing 2×10^6 viable cells. Treatment with the various alloantisera and assay for cytotoxic activity were as for Fig. 1. Symbols as in Fig. 1.

**Table 2** Ability of anti-Ly-6.2 to lyse cytotoxic effector cells after absorption with spleen cells from various mouse strains

Alloantiserum	Spleen cell absorption	C'	(Ly-6.2 ⁺ Ala-1.2 ⁺) effector cells	% Isotope release
—	—	—	3×10^6	$52 \pm 5^*$
—	—	+	3×10^6	$53 \pm 1^*$
Anti-Ly-6.2	—	+	3×10^6	1±1
Anti-Ly-6.2	CBA (Ly-6.1, Ala-1.1)	+	3×10^6	30 ± 1
Anti-Ly-6.2	C57BL (Ly-6.2, Ala-1.2)	+	3×10^6	$52 \pm 1^*$
Anti-Ly-6.2	129 (Ly-6.2, Ala-1.1)	+	3×10^6	$44 \pm 1^*$
Anti-Thy-1.1	—	+	3×10^6	1±1

Aliquots of 0.5 ml of a 1/10 dilution of anti-Ly-6.2 were absorbed five times with packed spleen from various mice. Each absorption used 10 volume % packed spleen cells, incubating with the antiserum for 45 min at 37 °C, followed by centrifugation to remove the cells and repeating the process. After the final absorption each serum was incubated with 3×10^6 cytotoxic C57BL (H-2^b, Ly-6.2, Ala-1.2) effector cells for 30 min at 37 °C. The cells were washed and incubated for a further 45 min with 0.5 ml of spleen absorbed rabbit complement diluted to 10%. After washing, the cells were resuspended to equal volumes and serially diluted in microtitre plates. The assay for cytotoxic activity was identical to that described in Fig. 1.

*Indicates that samples are not significantly different ($P > 0.05$).

I thank Drs I. F. C. McKenzie and A. Ahmed for preparing and donating the antisera used in this study. This work was supported in part by the US Navy Research and Development Command Work Unit No. MR041.02.01. 0012BJC.

JAMES N. WOODY*

ICRF Tumour Immunology Unit,
Department of Zoology,
University College London,
Gower Street, London WC1, UK

Received 8 June; accepted 7 July 1977.

* Present address: Cellular Immunology Division, Naval Medical Research Institute, Bethesda, Maryland 20014.

- ¹ Takahashi, T., Old, L. J. & Boyse, E. A. *J. exp. Med.* **131**, 1325–1341 (1970).
- ² Feeney, A. J. & Hammerling, U. in *Progress in Differentiation Research* (ed. Muller-Berat, N.) 551–557 (North Holland, Amsterdam, 1976); *Immunogenetics* **3**, 369–379 (1976).
- ³ Beverley, P. C. L., Woody, J., Dunkley, M., Feldmann, M. & McKenzie, I. F. C. *Nature* **262**, 495–497 (1976).
- ⁴ Feldmann, M., Beverley, P. C. L., Woody, J. & McKenzie, I. F. C. *J. exp. Med.* **145**, 793–801 (1977).
- ⁵ Kontiainen, S. & Feldmann, M. *Eur. J. Immun.* (in the press).
- ⁶ McKenzie, I. F. C., Sutton, T., Cherry, M. & Snell, G. G. *J. Immun.* **118** (in the press).
- ⁷ Woody, J. N., Feldmann, M., Beverley, P. C. L. & McKenzie, I. F. C. *J. Immun.* **118**, 1739–174x (1977).
- ⁸ Beverley, P. C. L. in *Immunologic Aspects of Cancer* (ed. Castro, J. E.) (MTP, Lancaster, UK, in the press).
- ⁹ Shiku, H. *et al. J. exp. Med.* **141**, 227–241 (1975).
- ¹⁰ Kisielow, P. *et al. Nature* **253**, 219–220 (1975).
- ¹¹ Feldmann, M., Beverley, P. C. L., Dunkley, M. & Kontiainen, S. *Nature* **258**, 614–615 (1975).
- ¹² Cantor, H. & Boyse, E. A. *J. exp. Med.* **141**, 1376–1389 (1975).

Regulation of requirements for anchorage-independent growth of Syrian hamster fibroblasts by somatic mutation

THE ability of mammalian cells to form colonies following suspension in semisolid agar, termed 'anchorage independence', is a phenotypic characteristic associated with cells transformed by oncogenic viruses and chemical carcinogens^{1–3}. Because this phenotypic characteristic has been correlated with tumorigenicity^{2–5}, colony formation in semisolid agar is used frequently as a quantitative *in vitro* assay for neoplastic transformation, especially in studies in Syrian hamster cell systems^{4,5}. It is therefore important to understand the molecular and physiological basis of this phenotype. If the expression of this phenotype can be altered by somatic mutation of identifiable genes, information can be gained concerning the mechanism of anchorage-independent growth *in vitro* and its exact relationship to tumorigenicity and malignancy *in vivo*. We have investigated the ability of cells of the highly tumorigenic Syrian hamster fibroblast line, BP6T (ref. 9) to form colonies when suspended in semisolid agar. Cells of this line produce fibrosarcomas in 100% of newborn Syrian hamster inoculated with ten cells. In addition to anchorage-independent growth, BP6T cells also exhibit other *in vitro* characteristics of neoplastic transformed cell lines, such as increased plating efficiency in liquid medium at low cell densities, and enhanced fibrinolytic activity⁶. In this communication, we report that the ability of this Syrian hamster line to form colonies in semisolid agar is dependent on its utilisation of exogenous purines by way of the hypoxanthine phosphoribosyltransferase (HPRT, E.C.2.4.2.8.)- and adenine phosphoribosyltransferase (APRT, E.C.2.4.2.7.)-mediated salvage pathways. Furthermore, this anchorage-independent property can be inhibited by a somatic mutation at the HPRT locus.

Supplementation of semisolid agar medium with either bactopectone or bactotryptose is a routine procedure when semisolid agar is used as a test medium for anchorage-independent growth^{1–6}. We found that in the absence of bactopectone, the ability of BP6T to produce colonies in semisolid agar was greatly influenced by the density at which the cells were suspended in the agar-containing

medium. At plating densities of less than 10⁴ cells per plate, colony formation was less than 0.1%. As shown in Table 1, however, supplementation of the agar suspension with 0.12% bactopectone permitted a colony-forming efficiency of 80% when 10–100 total cells were plated in each dish. With bactopectone, when the plating inoculum was increased above 10² cells, the efficiency of colony formation declined, decreasing to a value of approximately 30% at an inoculum of 10³ cells (Table 1). This reduction in the efficiency of colony formation with increasing cell density may have resulted both from the convergence of colonies and the competition for growth factors contained in the foetal calf serum and bactopectone supplements.

Since bactopectone has been reported to contain both hypoxanthine, and the unusual purine, 6,8-dihydroxy-purine^{12,13}, we examined the ability of hypoxanthine and other purines to replace the bactopectone requirement. Table 2 demonstrates that hypoxanthine (4 × 10⁻⁵ M) was effective in permitting colony-forming efficiency of 30–42%, whereas guanine (4 × 10⁻⁵ M), was only partially effective, giving a colony-forming efficiency of approximately 20%. Xanthine, which is an inefficient substrate for hamster HPRT (ref. 7), did not support colony formation in agar. These results indicated that hypoxanthine or guanine could replace the bactopectone requirement for efficient colony formation in semisolid agar.

Since the utilisation of hypoxanthine and guanine is catalysed by the salvage enzyme HPRT, we examined the ability of several independently isolated HPRT-deficient strains of BP6T to form colonies in bactopectone-supplemented agar. Three spontaneously occurring (subclones 1–3) and three *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced (subclones 4–6) HPRT-deficient colonies were isolated from BP6T following independent selections in 1–5 µg ml⁻¹ 6-thioguanine. Each of these subclones of BP6T contained less than 1% of the parental HPRT activity, as assayed by the ability of intact cells to incorporate ¹⁴C-hypoxanthine into an acid-precipitable form¹⁰. Moreover, these strains maintained their HPRT-deficient phenotype for at least 40–50 population doublings in the absence of 6-thioguanine, with a reversion frequency less

Table 1 Effect of cell density and bactopectone supplement on colony formation efficiency in semisolid agar

Condition	BP6T cell number seeded	Colony number	Colony formation efficiency
With bactopectone 0.12%	10	9	90
	100	80	80
	200	103	50
	500	186	37
	1,000	260	26
Without bactopectone	100	0	0
	1,000	0	0
	10,000	> 300	> 3

The cell culture medium used was IBR modified Dulbecco's Eagle reinforced medium (ERM) (Biolabs, Northbrook, Illinois) supplemented with 0.22 g% NaHCO₃ and 10% Reheatin F.S. foetal bovine serum (RFS) (Reheis Chemical, Kankakee, Illinois) without antimicrobial agents. Cells were transferred by a gentle trypsinisation with 0.1% trypsin solution (1:250, Gibco, Grand Island, New York) for 5 min at 36.5 °C. Agar cultures contained 3 ml base agar (0.6% agar dissolved in the above tissue culture medium) and 4 ml semisolid agar (0.3–0.4% agar dissolved tissue culture medium) in 35-mm or 60-mm diameter plastic Petri dishes. Before suspension in agar, cells were trypsinised and diluted into tissue culture medium. The cell number in the suspension was counted using a Coulter counter; a pre-determined number of cells was then suspended in the semisolid agar solution at temperatures between 37 and 40 °C, and the agar solution poured just before solidification. Agar cultures were incubated in a 37 °C incubator in a 5% CO₂ atmosphere at 90% relative humidity. At the end of 12–14 d, the number of colonies was counted under a light microscope. Each assay condition was measured in triplicate and the averaged results reported.

Table 2 Effect of purines on colony formation efficiency in semisolid agar by wild-type HPRT⁺ strains of BP6T cells

Cell strain	Agar supplement	Cell number seeded	Colony number	Colony formation efficiency (%)
BP6T (wild-type)	None	1,000	0	0
	Hypoxanthine 4×10^{-5} M	250	106	42
	Hypoxanthine 4×10^{-5} M	600	223	37
	Hypoxanthine 4×10^{-5} M	1,000	> 300	> 30
	Guanine 4×10^{-5} M	500	96	19
	Guanine 4×10^{-5} M	1,000	212	21
	Xanthine 4×10^{-5} M	500	0	0
	Xanthine 4×10^{-5} M	1,000	2	< 1
	Adenine 4×10^{-5} M	500	234	47
	Adenine 4×10^{-5} M	1,000	300	> 30
BP6T subclone 4 (HPRT ⁻)	Bactopeptone 0.12%	500	0	0
	Bactopeptone 0.12%	1,000	0	0
	Hypoxanthine 4×10^{-5} M	500	0	0
	Hypoxanthine 4×10^{-5} M	1,000	0	0
	Adenine 4×10^{-5} M	500	159	32
	Adenine 4×10^{-5} M	1,000	254	25
*BP6T subclone 5 (HPRT ⁻)	Bactopeptone 0.12%	550	0	0
	Bactopeptone 0.12%	1,000	< 1	< 1
	Bactopeptone 0.12%			
	+ adenine 4×10^{-5} M	550	142	26
	+ adenine 4×10^{-5} M	1,000	268	27

than 2×10^{-7} (as measured by their sensitivity to medium containing hypoxanthine $13.6 \mu\text{g ml}^{-1}$, amethopterin $0.19 \mu\text{g ml}^{-1}$ and thymidine $3.9 \mu\text{g ml}^{-1}$ (ref. 11).

The ability of these HPRT-deficient strains of BP6T to form colonies in bactopeptone-supplemented agar (Table 3) was either greatly diminished or totally lacking at

colonies (subclones 2, 3 and 6). This represents minimally a 50–300-fold decrease in colony-forming efficiency in the HPRT-deficient strains compared with the wild-type BP6T strain.

When the wild-type BP6T and the HPRT-deficient strains were assayed by the same procedure¹⁰ for ¹⁴C-adenine incorporation by way of the APRT pathway, all the HPRT-deficient strains incorporated adenine into acid-precipitable material at a level comparable with the parental BP6T wild-type strain. This result indicated that the HPRT-deficient strains possessed a functional adenine salvage pathway. Since HPRT-deficient strains of BP6T cells retained the ability to salvage adenine and consequently the ability to convert AMP to other purine nucleotides (IMP and GMP), we examined the ability of adenine to support colony formation by these deficient cells in bactopeptone-supplemented agar. Table 2 shows that HPRT-deficient strains (subclones 4 and 5), which did not form colonies in semisolid agar supplemented with either bactopeptone or hypoxanthine, regained the ability to form colonies in agar at 24–34% efficiency after the addition of adenine 4×10^{-5} M). Adenine was also found to support colony formation by the wild-type BP6T strain in unsupplemented agar, as a replacement for bactopeptone (Table 2).

These results indicate that the malignant Syrian hamster fibroblast cell line, BP6T, requires exogenous purines and appropriate functional salvage enzymes to form colonies efficiently in semisolid agar. Other *in vitro* properties of this cell line, such as cloning efficiency in liquid medium and enhanced fibrinolytic activity were essentially

Table 3 Effect of HPRT deficiency on colony formation in semisolid agar*

BP6T strain	Cell number seeded	Colony number	Colony formation efficiency %
BP6T (wild type)	200	103	50
	500	186	37
	1,000	260	26
BP6T subclone 1 (HPRT ⁻)	500	0	0
BP6T subclone 2 (HPRT ⁻)	550	6	1
BP6T subclone 3 (HPRT ⁻)	500	3	< 1
BP6T subclone 4 (HPRT ⁻)	1,000	1	< 1
BP6T subclone 5 (HPRT ⁻)	1,000	1	< 1
BP6T subclone 6 (HPRT ⁻)	1,000	11	1

* The semisolid agar media were all supplemented with bactopeptone, 0.12%.

plating densities of less than 10^4 cells. After 14 d incubation, 200–500 BP6T cells produced colonies at an efficiency of 30–50% (Table 3), whereas 500–1,000 HPRT-deficient cells from six individual clones produced either one or no colonies (subclones 1, 4 and 5) or from three to eleven

Table 4 Summary of *in vitro* properties of Syrian hamster cell lines

Cell type	Genotype	CE*	Fibrinolysis†	Colony-forming efficiency‡ in semisolid agar	Tumorigenicity§
Syrian hamster embryo cells	HPRT ⁺	2–6%	(–)	0%	–
BP6T	HPRT ⁺	80%	+	50–80%	+
BP6T subclone 5	HPRT ⁻	41%	+	< 1%	+
BP6T subclone 6	HPRT ⁻	61%	+	1%	ND

* CE is cloning efficiency in liquid medium.

† Fibrinolytic activity was measured both in the extracellular medium and in cell lysates by procedures described previously⁹.

‡ The semisolid agar media were supplemented with 0.12% bactopeptone only. Values for the BP6T derivatives were taken from Table 3. Syrian hamster embryo cells exhibited no colonies when 10^6 cells were tested.

§ Tumorigenicity was measured by subcutaneously injecting 0.1 ml complete medium containing varying concentrations of cells into non-immunosuppressed neonatal littermates (1–3-d-old) of outbred Syrian hamsters (Lakeview). Injection of 10^7 Syrian hamster embryo cells produced no tumours, whereas injection of 10^2 cells of BP6T and BP6T subclone 5 produced tumours.

unaltered by the forward mutation at the HPRT locus (Table 4). Additionally, the population-doubling times and cell saturation densities were unchanged (data not shown). It would be interesting to revert HPRT-deficient strains to HPRT⁺ to see if growth in agar supplemented only with hypoxanthine is regained. To date, however, attempts to isolate HPRT⁺ revertants of these strains have been unsuccessful.

Previous studies have shown that virally transformed derivatives of BHK 21 clone 13 (an established cell line of Syrian hamster origin) also require exogenous purines to support colony formation in agar, although not in liquid medium^{6,7}. Furthermore, 'normal' BHK cells (which induce tumours with high numbers of injected cells⁴) can be stimulated to form colonies in semisolid agar if thymidine is supplied in addition to the required purine supplement⁸. In contrast to our findings with BP6T, we observed no dependence on purines or purine salvage enzymes for anchorage-independent growth by HPRT- and APRT-deficient mouse fibroblast strains (data not shown). This finding suggests that *in vitro* requirements for anchorage-independent growth may vary between cells of different species origin.

Recently, stable variants which have lost certain *in vitro* phenotypes often associated with neoplasia have been isolated after treatment with mutagens^{4,14,15}. Such studies frequently rely on assumed correlations between demonstrated cellular alterations *in vitro* and tumorigenicity *in vivo*. Our results demonstrate that somatic mutation in tumorigenic Syrian hamster cells can lead to altered anchorage requirements for cellular growth, although the tumorigenic potential of these cells need not be lost (Table 4). Therefore, alterations in requirements for anchorage-independent growth *in vitro* may not necessarily correlate with malignancy.

This work was supported by the National Science Foundation. J.C.L. was a postdoctoral fellow of the National Cancer Institute.

J. C. LEAVITT
B. D. CRAWFORD
J. C. BARRETT
P. O. P. TS'O

Department of Biochemical and Biophysical Sciences,
School of Hygiene and Public Health,
Johns Hopkins University,
Baltimore, Maryland 21205

Received 23 November 1976; accepted 24 June 1977.

- 1 MacPherson, I. & Montagnier, L. *Virology* **23**, 291-294 (1964).
- 2 Freedman, V. H. & Shin, S. *Cell* **3**, 355-359 (1974).
- 3 diMayorca, G., Greenblatt, M., Tranthen, T., Soller, A. & Giordano, R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 46-49 (1973).
- 4 Bouck, N. & diMayorca, G. *Nature* **264**, 722-727 (1976).
- 5 Kakunaga, T. & Kamahara, J. *Biken's J.* **11**, 313-332 (1968).
- 6 Montagnier, L. *C. r. hebdo. Séanc. Acad. Sci., Paris, Serie D*, **268**, 2218-2221 (1969).
- 7 Clarke, G. D. & Smith, C. J. *Cell Physiol.* **81**, 125-132 (1972).
- 8 Taylor, J. C., Hill, D. W. & Rogolsky, M. *Expl Cell Res.* **90**, 468-471 (1975).
- 9 Barrett, J. C., Crawford, B. & Ts'o, P. O. P. *Cancer Res.* **37**, 1182-1185 (1977).
- 10 Sharp, J. D., Cappechi, N. E. & Cappechi, M. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3145-3149 (1973).
- 11 Szybalski, W., Szybalska, E. H. & Ragni, G. *Natn. Cancer Inst. Monogr.* **7**, 75-80 (1962).
- 12 Yamane, I. & Murakami, O. *Biochem. biophys. Res. Commun.* **45**, 1431-1436 (1971).
- 13 Yamane, I. & Murakami, O. *J. Cell Physiol.* **81**, 281-284 (1973).
- 14 Miyashita, K. & Kakunaga, T. *Cell* **5**, 131-138 (1975).
- 15 Vogel, A. & Pollack, R. *Methods in Cell Biology* **8** (ed. Prescott, D. M.) 75-92 (Academic, New York, 1974).

Rapid induction of amphotericin B sensitivity in L1210 leukaemia cells by liposomes containing ergosterol

AMPHOTERICIN B is a polyene antibiotic whose clinical use has been limited to the treatment of fungal infections. The mode of action of amphotericin B is believed to involve damage to cell membranes containing certain sterols^{1,2}. One sterol of primary importance for this membrane interaction is ergosterol³⁻⁵, and it is believed that its presence in

cell membranes of fungi confers sensitivity to amphotericin B². Studies with phospholipid vesicles indicate that it is possible to alter the lipid content of cultured mammalian cells after short incubations with liposomes of defined content^{6,7}. We report here that brief treatment with liposomes containing ergosterol can sensitise L1210 murine leukaemia cells to the subsequent action of amphotericin B.

Liposomes containing sterol were prepared using methods described by Weissmann⁸ and Cooper⁹. Egg yolk lecithin (EYL, Sigma) 40 mg alone or EYL 40 mg and ergosterol (Sigma) 80 mg, were dissolved in chloroform to assure complete mixing. The mixture of sterol and phospholipid was taken to dryness under nitrogen and 10 ml of buffer (100 mM NaCl, 15 mM KCl, 5 mM TES (N-Tris[hydroxymethyl]methyl-2-aminoethane sulphonic acid), 10 mM dextrose, 0.1 mM EDTA; pH 7.4) were added. This mixture was sonicated in a Branson Sonifier (Heat Systems Inc.), at an output setting of 60 W in a nitrogen atmosphere for 1 h at 3 °C. After sonication, the suspension was centrifuged at 30,000g for 10 min to remove non-dispersed lipids. Final phospholipid concentration was measured by the method of Bartlett¹⁰. Final ergosterol concentration was determined by the cholesterol method of Rudel and Morris¹¹, using ergosterol for the standard curve. Before incubation with cells, liposomes were diluted with buffer and medium to yield a final phospholipid concentration of 0.4 mg ml⁻¹ and a final ergosterol concentration of 0.05 mg ml⁻¹. Thin layer chromatography of the liposome preparation revealed the expected amount of phospholipid and sterol with less than 2% lysophosphatidylcholine contamination.

L1210 mouse leukaemia cells which had been in stationary phase for 5 h were adjusted to 7×10^5 cells per ml with fresh medium. Aliquots of this suspension were placed in 75-cm² Falcon flasks, to which (1) buffer without liposomes, (2) EYL liposomes in buffer, or (3) EYL liposomes containing ergosterol in buffer, were added to yield a final cell concentration of 5.5×10^5 per ml. Cells were incubated for 1 h at 37 °C, collected by centrifugation at 300 g for 5 min, and washed twice with fresh medium. They were resuspended at 1×10^5 cells per ml in fresh medium (10 ml) containing amphotericin B or the solvent, DMSO, in 25 cm² Corning flasks. Cells were incubated for 2 h at 37 °C, washed twice more and resuspended in fresh medium.

After treatment and washing, one or more of the following techniques were used to assess cell survival. (1) Cell number was determined immediately after incubation in medium containing amphotericin B. (2) Trypan blue exclusion—washed cells in 0.5 ml medium were mixed with 0.1 ml 0.4% trypan blue in normal saline—after 5 min, 200 cells were counted and the number of cells excluding stain determined. (3) Cell number was determined after resuspension in fresh medium and 45 h additional incubation at 37 °C.

L1210 cells which were first incubated for 1 h with EYL liposomes containing ergosterol, and then treated for 2 h with amphotericin B at concentrations of 4–8 µg ml⁻¹ were reduced in number when counted immediately after the incubation period with amphotericin B (Fig. 1). These cell populations were unable to exclude Trypan blue effectively (Table 1), and phase microscopic examination revealed many cells undergoing lysis and much cellular debris. Treatment of cells with buffer alone or EYL liposomes without ergosterol did not confer such sensitivity to the action of amphotericin B.

Cells first treated with EYL liposomes containing ergosterol, exposed to amphotericin B, then incubated without ergosterol or amphotericin B for a further 45 h, exhibited growth inhibition and/or continuing diminution of cell number (Fig. 1). This effect was dose dependent over an amphotericin B concentration range of 2–8 µg ml⁻¹. Liposome treatment without subsequent amphotericin B

exposure was without effect. Amphotericin B treatment of cell populations previously incubated with buffer or EYL liposomes did not result in such reduction in cell number after 45 h further incubation (Fig. 1).

All cell populations examined immediately after the 1-h incubation period with buffer or liposome preparations excluded Trypan blue effectively. There was no change in cell number when these populations were compared with untreated cells. No cells were found adherent to vessel surfaces.

We have demonstrated that brief exposure to liposomes containing ergosterol can confer amphotericin sensitivity to one type of murine leukaemia cell. Other workers have demonstrated that *Tetrahymena pyriformis* cells grown for 36–40 h in culture medium containing ergosterol will incorporate ergosterol into their cell membranes and then become sensitive to the action of amphotericin B⁴. In an earlier communication¹² it was reported that 22 h of growth in culture medium containing ergosterol was required for L1210 cells to become markedly affected by amphotericin B.

The mechanisms of ergosterol and amphotericin B action are not fully understood. Ergosterol may nonspecifically prepare the cell membrane for optimum interaction with amphotericin B by making the membrane more rigid or ordered¹³. Alternatively ergosterol could uniquely combine with amphotericin B and change membrane configuration so that membrane disruption occurs and cell lysis ensues^{14,15}.

Liposomes have been shown to interact with a variety of mammalian cell types^{7,8}. Although the precise nature of the interaction is not clear, the liposomes can transfer large amounts of lipids to cells and alter cellular lipid content⁶. Since sensitisation to amphotericin B might require the presence of a specific sterol at the membrane level, we presume that sensitisation observed after treat-

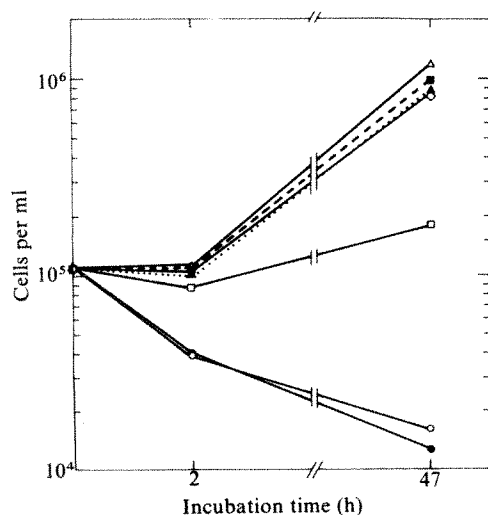


Fig. 1 Time course of effect of amphotericin B on cell number. L1210 cells (from Dr V. Bono Jr, NCI) were incubated for 1 h in the buffer, EYL liposomes or EYL liposomes containing ergosterol. Cells were washed, treated for 2 h with amphotericin B or dimethylsulphoxide (DMSO), then washed again. One aliquot of cells was counted and another was resuspended in fresh medium and incubated for a further 45 h and then counted. Cells treated with EYL liposomes containing ergosterol followed by treatment with DMSO (Δ), or amphotericin B at $2 \mu\text{g ml}^{-1}$ (\diamond), $4 \mu\text{g ml}^{-1}$ (\square), $6 \mu\text{g ml}^{-1}$ (\circ) or $8 \mu\text{g ml}^{-1}$ (\bullet). Cells treated with EYL liposomes then amphotericin B $6 \mu\text{g ml}^{-1}$ (\blacksquare); cells treated with buffer (without any liposomes) then amphotericin B $6 \mu\text{g ml}^{-1}$ (\blacktriangle). RPMI 1630 medium was from NIH Media Unit. In all experiments media were supplemented with 20% foetal calf serum (Flow) and gentamicin $40 \mu\text{g ml}^{-1}$ (Schering). Foetal calf serum was heat inactivated at 56°C for 30 min. Cell number was estimated with a Coulter Counter. Amphotericin B (Calbiochem) was freshly prepared in DMSO to give a 20 mg ml^{-1} stock solution. The DMSO concentration in incubation mixtures did not exceed 0.1% (v/v).

Table 1 Effect of treatment with liposomes containing ergosterol on Trypan blue exclusion

Amphotericin B ($\mu\text{g ml}^{-1}$)	Cells excluding Trypan blue (% of buffer control)	
	EYL liposomes containing ergosterol	EYL liposomes alone
0	94	94
2	73	85
4	44	78
6	15	85

L1210 cells were incubated for 1 h with buffer, EYL liposomes or EYL liposomes containing ergosterol. Cells were washed, treated for 2 h with amphotericin B, washed again, and resuspended in fresh medium. 0.5 ml of cells in fresh medium was mixed with 0.1 ml 0.4% Trypan blue in normal saline. After 5 min, 200 cells were counted and number excluding trypan blue determined.

ment with liposomes containing ergosterol was due to addition of this specific sterol to the membrane region.

As drug carriers, liposomes offer several advantages over conventional modes of drug administration. It has been suggested that they can transport high concentrations of otherwise toxic agents and perhaps can be directed to selected tissue targets^{16–19}. Our data indicate that in addition, specifically constituted liposomes may sensitise certain cell types to chemotherapeutic agents to which they were previously resistant. This study demonstrates a novel use of liposomes for the purpose of functionally altering the membranes of mammalian cells.

We thank Dr Marco Rabinovitz for his guidance and support, Ms Joyce Fisher for her technical assistance, and Ms Sylvia Rose and Ms Charlotte Thomas for secretarial help.

FRED J. SCHIFFMAN

Laboratory of Medicinal Chemistry
and Biology,

IRWIN KLEIN

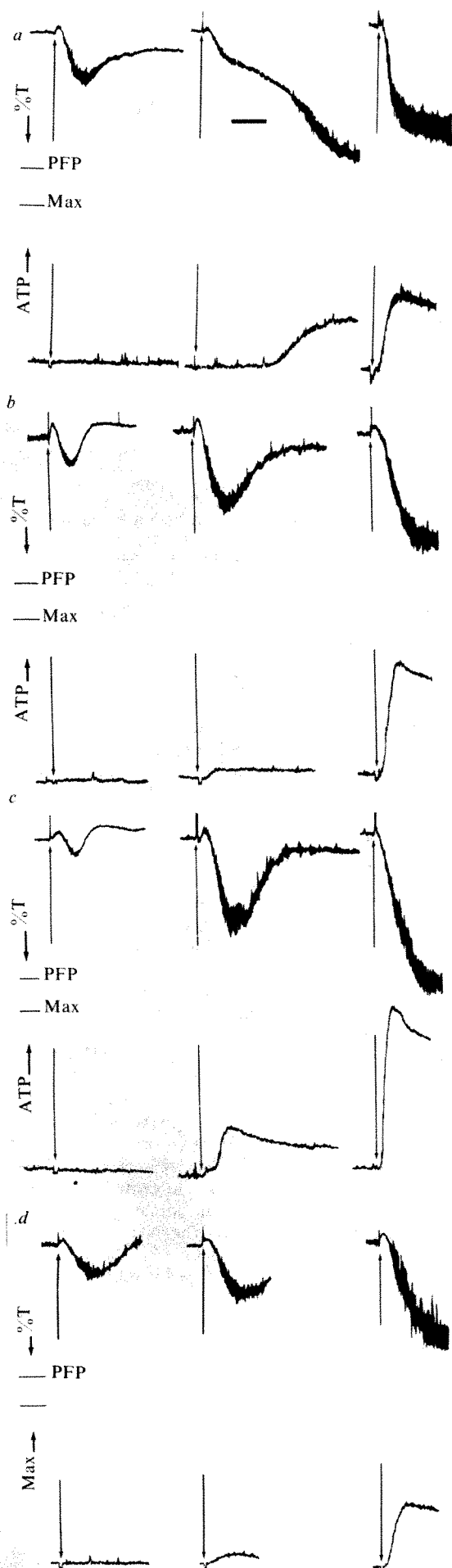
Laboratory of Molecular Biology,
National Cancer Institute,
Bethesda, Maryland 20014

Received 4 May; accepted 11 July 1977.

- Kinsky, S. C. *J. Bact.* **82**, 889–897 (1961).
- Hamilton-Miller, J. M. T. *Bact. Rev.* **37**, 166–196 (1973).
- Woods, R. A., Bard, M., Jackson, I. E. & Drutz, D. J. *J. infect. Dis.* **129**, 53–58 (1974).
- Conner, R. L. *et al. Biochem. biophys. Res. Commun.* **44**, 995–1000 (1971).
- HsuChen, C. C. & Feingold, D. S. *Antimicrob. Ag. Chemother.* **4**, 316–319 (1973).
- Huang, L. & Pagano, R. E. *J. Cell Biol.* **67**, 38–48 (1975).
- Paphadjopoulos, D., Mayhew, E., Poste, G. & Smith, S. *Nature* **252**, 163–166 (1974).
- Sessa, G. & Weissmann, G. *J. Lipid Res.* **9**, 310–318 (1968).
- Cooper, R. A., Arner, E. C., Wiley, J. S. & Shattil, S. J. *J. clin. Invest.* **55**, 115–126 (1975).
- Bartlett, G. R. *J. biol. Chem.* **234**, 466–468 (1959).
- Rudel, L. L. & Morris, M. D. *J. Lipid Res.* **14**, 364–366 (1973).
- Schiffman, F. J., Fisher, J. M. & Rabinovitz, M. *Biochem. Pharmacol.* **26**, 177–180 (1977).
- HsuChen, C. C. & Feingold, D. S. *Biochem. biophys. Res. Commun.* **51**, 972–978 (1973).
- Andreoli, T. E. *Ann. N. Y. Acad. Sci.* **235**, 448–468 (1974).
- Holz, R. W. *Ann. N. Y. Acad. Sci.* **235**, 469–479 (1974).
- Gregoriadis, G. & Neerunjun, E. D. *Biochem. biophys. Res. Commun.* **65**, 537–544 (1975).
- Magee, W. E. & Miller, O. V. *Nature* **235**, 339–340 (1972).
- Jonah, M. M., Cerny, E. A. & Rahman, Y. E. *Biochim. biophys. Acta* **401**, 336–348 (1975).
- Weissmann, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 88–92 (1975).

Prostaglandin endoperoxides and thromboxane A₂ can induce platelet aggregation in the absence of secretion

PLATELET aggregation and secretion are widely studied not only because they are believed to reflect the major *in vivo* platelet function, formation of the primary haemostatic plug, but also because they involve fundamental cellular regulatory mechanisms. The synthesis of biologically active prostaglandin (PG) intermediates (endoperoxides and thromboxanes) seems to be an important step in the



regulation of platelet function^{1,2}, but the physiological roles and mechanism(s) of these compounds are unknown. It is claimed by Malmsten *et al.*³ and Samuelsson *et al.*⁴ that PG endoperoxides and thromboxane A₂ cause platelet aggregation only by inducing platelets to secrete ADP that in turn causes aggregation. In view of observations that platelets deficient in releasable ADP (storage pool-deficient platelets or platelets depleted of storage granules) still aggregate in response to arachidonic acid⁵⁻⁷, the precursor of the intermediates, the validity of this claim has been open to question. We have investigated platelet stimulation by PG endoperoxides (PGG₂ and PGH₂), an endoperoxide analogue ((15S)-hydroxy-11 α , 9 α -(epoxy-methano) prosta-5Z, 13E-dienoic acid; U-46619), thromboxane A₂-like material and arachidonic acid, using a new instrument that simultaneously monitors aggregation and secretion in the same platelet suspension⁸, permitting careful analysis of the relationships between aggregation and secretion. We report here that although high concentrations of the endoperoxide analogue, the natural endoperoxides and thromboxane A₂ induce both platelet aggregation and secretion, low concentrations cause aggregation without detectable secretion.

Secretion was monitored by recording luminescence resulting from the interaction of released ATP (secreted simultaneously with ADP) with firefly luciferase and

Fig. 1 Simultaneous recording of aggregation and secretion induced by (a) PG endoperoxide analogue (U-46619), (b and c) natural endoperoxides (PGH₂ and PGG₂) and (d) thromboxane A₂ added to stirred platelet-rich plasma. These experiments represent typical results obtained with low, intermediate and saturating (maximal aggregation and secretion of ATP) concentrations of each stimulus. Secretion of ATP (lower traces) was measured by the firefly luminescence assay, with the gain of the instrument set so that maximum release with a high concentration of thrombin gave half-chart deflection (max), which corresponds to about 5 μ M ATP or 1 nmol per 10⁸ platelets. In the three experiments where the lowest concentrations of the analogue, PGH₂ or PGG₂ were added, the gain was doubled to detect even trace secretion of ATP. The sensitivity of the measurement in these conditions is such that a pen deflection of twice the noise amplitude is caused by less than 50 nM ATP. Since others^{19,10} have shown that ATP and ADP are secreted in parallel with the ATP/ADP ratio between 0.5 and 1.0, we would detect secretion of 0.1 μ M ADP. The decrease in the signal seen at the end of some curves does not represent uptake or metabolism of ATP by platelets, but is due to the well-known property of the assay system to show a decay in luminescence^{21,22}. Aggregation (upper traces) was measured simultaneously in the same cuvette, with the limit set with platelet-free plasma (PFP) as indicated. Reactions were initiated by addition of the stimulating agents to stirred platelet-rich plasma, except for experiments with PGH₂ and PGG₂, where the reaction was initiated by addition of platelet-rich plasma to a cuvette to which the endoperoxide had previously been added and the solvent (acetone) evaporated with a gentle stream of nitrogen. Thromboxane A₂-like material was generated by incubation of dog platelet-rich plasma with 0.5 mM arachidonic acid, as described by Chignard and Vargaftig¹². Aliquots of the dog platelet-rich plasma containing thromboxane A₂-like material were transferred directly to human platelet-rich plasma that had been pre-incubated with indomethacin (15 μ M for 30 s) to prevent stimulation by arachidonic acid. The concentration of thromboxane A₂-like material was varied by transferring different volumes or by varying the time of incubation with arachidonic acid. Control dog platelet-rich plasma had no effect on human platelets, and we confirmed the observations of Chignard and Vargaftig¹² that dog platelets neither aggregate nor secrete in response to arachidonic acid, although they do metabolise arachidonic acid to PGs and thromboxanes. All experiments were carried out with the cell compartment at 37°C, and the chart speed was 60 s inch⁻¹ (time bar in top trace). Each experiment was carried out on platelet-rich plasma (counts not standardised) from at least three donors. Although there were some quantitative differences between donors, the pattern of responses was consistent and the data shown are from a single donor, so that comparisons between stimuli are valid. The concentrations of stimuli (for low, intermediate and high concentrations) were: endoperoxide analogue 0.5, 1.2 and 2.2 μ M; PGH₂ 0.5, 0.8 and 9.4 μ M; PGG₂ 0.3, 0.5 and 2.8 μ M. Exact concentrations of thromboxane A₂-like material were not determined.

luciferin as described previously^{9,10}. The reaction was carried out in a new cell compartment designed to permit simultaneous measurement of platelet aggregation and secretion. Details of this technique have been described previously⁸. PGG₂ and PGH₂ were prepared essentially as described elsewhere¹¹. Final concentrations quoted are based on the specific activity of the radioactive arachidonic acid used in their biosynthesis. Thromboxane A₂-like material was generated by incubation of dog platelet-rich plasma with arachidonic acid¹². Although dog platelets do not aggregate or secrete nucleotides or other contents of dense bodies in response to arachidonic acid, they do synthesise PG endoperoxides and thromboxane A₂, which will aggregate human platelets. Since Smith *et al.*¹³ have shown that in the conditions used (incubation in plasma at 37 °C), thromboxane A₂ activity persists after complete disappearance of the endoperoxides, we refer to the active component as thromboxane A₂. Human platelet-rich plasma, prepared from whole blood collected in 0.1 volume 3.8% sodium citrate¹⁴, was kept at room temperature and was used within 3 h. Experiments were at 37 °C. All donors denied taking any drug for at least 10 d before venipuncture.

The effects of the unstable PG intermediates and the stable endoperoxide analogue on platelets were observed by recording the precise temporal relationships of aggregation and secretion (Fig. 1). Each compound was tested over a range of concentrations from an amount just sufficient to cause an observed effect to an amount greater than that required for the maximal effect. Individual donors varied in their responsiveness to the endoperoxides, but PGG₂ was consistently more potent than PGH₂. Examples of typical results for low, intermediate and saturating concentrations of each compound are shown in Fig. 1.

At low concentrations, each compound induced aggregation in the complete absence of detectable secretion (that is, primary aggregation), directly refuting the claim^{3,4} that the endoperoxides induce platelet aggregation only through release of ADP.

At intermediate concentrations, some difference was observed between the stable endoperoxide analogue and the unstable PG intermediates. The analogue induced biphasic aggregation, with secretion beginning only after a long lag and in parallel with the second wave of aggregation. This is characteristic of the response of platelets to other primary aggregating agents such as ADP and adrenaline^{14,15}. In contrast, when the stimulating agent was PGH₂ or PGG₂ we never observed biphasic aggregation or a long lag before secretion; instead intermediate concentrations of these compounds have consistently induced completely reversible aggregation accompanied by secretion. (Such readily reversible aggregation in platelets undergoing secretion is unusual.) This difference indicates that the synthetic endoperoxide analogue may not exactly mimic the platelet stimulating properties of the natural endoperoxides. Thromboxane A₂-like material induced aggregation and secretion with a temporal relationship similar to that for the naturally occurring endoperoxides.

Addition of increasingly higher concentrations of each stimulus resulted in more rapid and extensive aggregation-secretion curves until a maximal response was attained. In the case of maximal response, aggregation and secretion proceeded as temporally parallel events. With each stimulus, the secretion curves resulting from high concentrations of PGG₂ were nearly identical to those obtained previously with other potent stimuli (for example, thrombin or A23187) (ref. 15), with no evidence of the extremely fast secretion reported by Malmsten¹⁶ and Corey *et al.*¹⁷, who observed complete release within 5 s.

Arachidonic acid also induced aggregation and secretion, probably because it is the substrate for PG and thromboxane synthesis¹⁸. Arachidonic acid differed from the other compounds tested in that aggregation was always

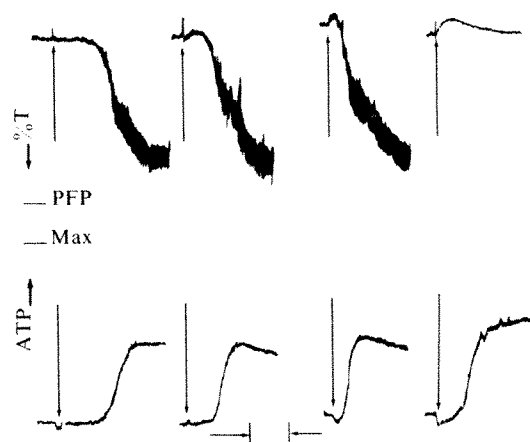


Fig. 2 Simultaneous recording of aggregation and secretion induced by arachidonic acid. The experiments were as described in Fig. 1. The fourth trace was done in the absence of stirring. Note that the higher concentrations of arachidonic acid lead to progressively shorter lag times and more rapid kinetics of secretion, but the total ATP secreted does not increase significantly. Lower concentrations of arachidonic acid also induced aggregation and secretion in parallel, with increasingly longer lags, until a threshold concentration was reached below which there was neither aggregation nor secretion (approximately 100 μM in the experiment shown). Sodium arachidonate concentrations (from left to right): 110, 190, 640, 640 μM.

accompanied by secretion (Fig. 2). (This is not inconsistent with a report⁵ that arachidonic acid causes aggregation of platelets that have been depleted of their secretory granules by treatment with thrombin, but suggests that aggregation and secretion may be independent, parallel responses of platelet activation¹⁵.) This difference in activity of the PG intermediates and arachidonic acid suggests that there may be a difference between the effects of extracellular intermediates and the same compounds generated intracellularly. That is, primary aggregation may be due to interactions of the intermediates with the outer membrane, whereas activation by arachidonic acid may involve an intracellular action of the same intermediates.

We also observed that at higher concentrations, each stimulus, as shown in Fig. 2 for arachidonic acid, is able to induce secretion in the virtual absence of aggregation (unstirred suspension), indicating that secretion is not dependent on aggregation, as shown for other stimuli, such as thrombin¹⁵.

We conclude that endoperoxides and thromboxane A₂ can induce aggregation without secretion of nucleotides, that higher concentrations of endoperoxides and thromboxanes can induce secretion independent of aggregation, and that human platelet aggregation can spontaneously reverse even when accompanied by secretion. This is the first direct evidence that PG intermediates can cause primary aggregation of normal human platelets.

This work was supported by the NIH. We thank Dr B. Matanic for veterinarian services.

ISRAEL F. CHARO
RICHARD D. FEINMAN
THOMAS C. DETWILER

Department of Biochemistry,
SUNY Downstate Medical Center,
Brooklyn, New York 11203

J. BRYAN SMITH
CAROL M. INGERMAN
MELVIN J. SILVER

Cardeza Foundation,
Thomas Jefferson University,
Philadelphia, Pennsylvania 19107

Received 3 May; accepted 5 July 1977.

¹ Hamberg, M., Svensson, J., Wakabayashi, T. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* 71, 345 (1974).

- ² Hamberg, M., Svensson, J. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975).
- ³ Malmsten, C., Hamberg, M., Svensson, J. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1446 (1975).
- ⁴ Samuelsson, B., Hamberg, M., Malmsten, C. & Svensson, J. in *Advances in Prostaglandin and Thromboxane Research 2* (eds Samuelsson, B. & Paoletti, R.) 737 (Raven, New York, 1976).
- ⁵ Kinlough-Rathbone, R. L., Reimers, H. J., Mustard, J. F. & Packham, M. A. *Science* **192**, 1011 (1976).
- ⁶ Smith, J. B., Ingberman, C. M. & Silver, M. J. in *Advances in Prostaglandin and Thromboxane Research 2* (eds Samuelsson, B. & Paoletti, R.) 747 (Raven, New York, 1976).
- ⁷ Weiss, H. J., Willis, A. L., Kuhn, D. & Brand, H. *Br. J. Haemat.* **32**, 257 (1976).
- ⁸ Feinman, R. D., Lubowsky, J., Charo, I. & Zabinski, M. *J. lab. clin. Med.* **90**, 125 (1977).
- ⁹ Detwiler, T. C. & Feinman, R. D. *Biochemistry* **12**, 2462 (1973).
- ¹⁰ Charo, I. F., Lubowsky, J., Feinman, R. D. & Detwiler, T. C. in *Platelet Function-Testing* (eds Day, H. J., Holmsen, H. & Zucker, U. B.) (DHEW-Publ.-NTH-77-108, US Government Printing Office, Bethesda, in the press).
- ¹¹ Nugteren, D. H. & Hazelhof, E. *Biochim. biophys. Acta* **326**, 448 (1973).
- ¹² Chignard, M. & Vargaftig, B. *Eur. J. Pharmac.* **38**, 7 (1976).
- ¹³ Smith, J. B., Ingberman, C. & Silver, M. J. *J. clin. Invest.* **58**, 1119 (1976).
- ¹⁴ Smith, J. B. & Macfarlane, D. E. in *The Prostaglandins 2* (ed. Ramwell, P. W.) 293 (Plenum, New York, 1974).
- ¹⁵ Charo, I. F., Feinman, R. D. & Detwiler, T. C. *J. clin. Invest.* (in the press).
- ¹⁶ Malmsten, C. *Life Sci.* **18**, 169 (1976).
- ¹⁷ Corey, E. J., Nicolaou, K. C., Yoshimasa, M., Malmsten, C. L. & Samuelsson, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3355 (1975).
- ¹⁸ Silver, M. J., Smith, J. B., Ingberman, C. & Kocsis, J. J. *Prostaglandins* **4**, 864 (1973).
- ¹⁹ Holmsen, H., Day, H. J. & Setkowsky, C. A. *Biochem. J.* **129**, 67 (1972).
- ²⁰ Mills, D. C. B., Robb, I. A. & Roberts, G. C. K. *J. Physiol. Lond.* **195**, 715 (1968).
- ²¹ DeLuca, M. & McElroy, W. D. *Biochemistry* **13**, 921 (1974).
- ²² Lundin, A., Rickardsson, A. & Thore, A. *Analyt. Biochem.* **75**, 611 (1976).

New polymorphism of platelet membrane glycoproteins

THE surface of the human blood platelet is rich in carbohydrate and at least four glycoprotein components have been resolved by gel electrophoresis of isolated platelet plasma membranes¹. Two platelet disease states are characterised by a diminution or absence of certain of these glycoproteins²⁻⁵: in the case of Bernard-Soulier disease, glycoprotein I (molecular weight (MW) 150,000) is lacking while platelet membranes isolated from patients suffering from Glanzmann's thrombasthenia lack glycoprotein II (MW 120,000). While screening several patients with ill-defined but clinically evident haemostatic problems, we have now identified two unrelated individuals who show an abnormal polymorphic pattern of glycoprotein distribution within their platelet membranes and a concomitant reduction in the ability of the intact platelets to bind thrombin.

Polyacrylamide gel electrophoresis of normal platelet plasma membranes gives a pattern of four major bands which stain with the periodate-Schiff reagent¹ (Fig. 1a). These bands have been designated glycoproteins I, II, III and IV and have electrophoretic mobilities corresponding to molecular weights of 150,000, 120,000, 98,000 and 87,000, respectively. These four peaks are in the ratio of 55:28:6:11, averaged from 37 samples, and show no age or sex-related differences.

In the case of platelets from two individuals identified

Fig. 1 Glycoprotein patterns from normal (a) and abnormal (b) platelets. A platelet membrane fraction was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and β -mercaptoethanol followed by staining with periodate-Schiff reagent, as previously described¹.

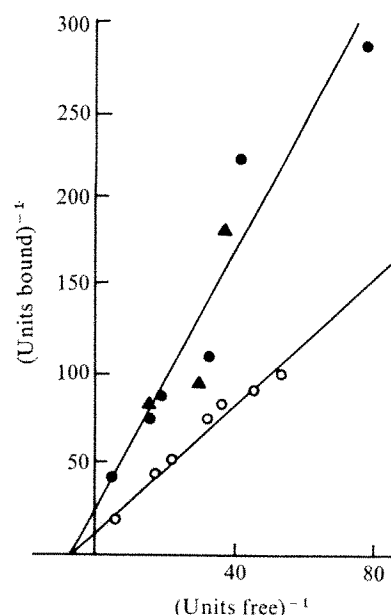
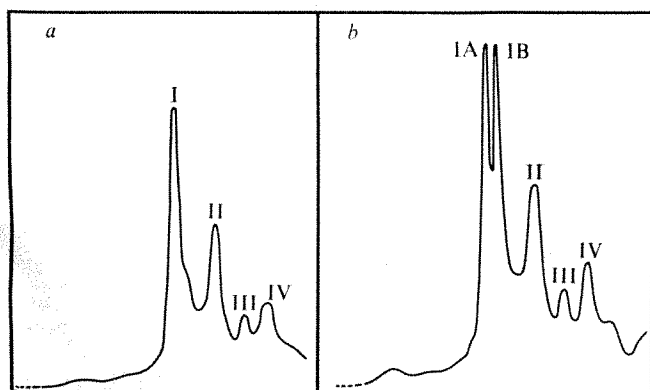


Fig. 2 Thrombin binding to normal and abnormal platelets. Control, \circ : patient 1, Δ : patient 2, \bullet . Binding studies were performed by the Majerus technique using cacodylate buffer¹² and highly purified human thrombin¹⁴ (2,850 NIH units mg^{-1}) (ref. 14) iodinated with ¹²⁵I and were corrected for nonspecific binding.

during these experiments we found a pattern which differed from normal in that there was a double band in the region of glycoprotein I (Fig. 1). The first band corresponded in electrophoretic mobility to glycoprotein I while the mobility of the second band indicated a molecular weight 10,000–12,000 lower. Each of these components was present in about one-half the amount of glycoprotein I in normal platelets. Combining the values for peaks IA and IB gives ratios (IA+B:II:III:IV) of 63:23:7:7 and 63:18:9:10, respectively, for the two patients which are ratios similar to those found in normal controls.

Several different types of experiment were carried out to determine whether this polymorphism was an intrinsic property of the membrane glycoproteins in these two cases or could have arisen from extraneous causes. Since glycoprotein I is known to be particularly sensitive to proteolytic digestion^{1,3} the possibility of increased proteolytic activity in the patients' serum was investigated by incubating normal platelets with samples of plasma from the two subjects for 8 h at 37 °C; this did not result in the appearance of a double band in the membranes subsequently isolated from the treated platelets indicating that the double band did not result from extraneous proteolysis, nor was there any appearance of the second band on prolonged incubation of the platelets themselves indicating that it was not due to protease activity in the abnormal platelets. Peaks IA and IB were equally susceptible to added trypsin in intact platelets and a single PAS-positive band was observed in the soluble supernatant with an electrophoretic mobility indistinguishable from that of the macroglycopeptide obtained from normal platelets⁶. These observations suggested that differences in the electrophoretic mobility of peaks IA and IB probably were not due to structural differences in the macroglycopeptide portion of the two molecules.

Glycoprotein I seems to exist both in membrane-bound forms and in a form, glycocalicin, which is loosely bound within the platelet glycocalyx and is obtained in soluble form following platelet homogenisation¹. The relationship between glycoprotein I and glycocalicin is not clear, however, and they differ in their pattern of labelling with surface probes¹. Glycocalicin has been isolated and characterised⁷ and has been shown to be an inhibitor of

Table 1 Clinical laboratory studies

	Normal values	Subject 1 (female, aged 22 yr)	Subject 2 (female, aged 28 yr)
Platelet count (mm ⁻³)	150,000–450,000	400,000	290,000
Bleeding time (min)	4–10	6	5
Platelet retention (%)	> 35	23	10
Prothrombin time (s)	12–14	13.2	13.0
Activated PTT (s)	< 35	32	35
Aggregation (%)			
Collagen (2 µg)	> 65	35	75
Adrenaline (2.5 µM)	> 70	6	85
ADP (10 µM)	> 70	39	90
Ristocetin (1.5 mg ml ⁻¹)	> 80	100	88
Thrombin (0.2 units ml ⁻¹)	> 65	74	84
VIII _{VWF} (%)	55–120	86	81
VIII _{AHF} (%)	50–200	100	115
PF-3 (s)	60	72	ND

Platelets were counted by phase microscopy. Platelet adhesiveness was measured by a modified Salzman technique¹⁶, bleeding times by the template technique of Mielke¹⁷ and prothrombin times, activated partial thromboplastin times (PTT) and one stage Factor VIII bioassay by standard methods. VIII_{VWF} assays were performed by the quantitative technique of Weiss¹⁸, platelet factor 3 (PF 3) availability was performed by the kaolin technique¹⁹ and platelet aggregation by the turbidimetric method of Born²⁰. ND, Not determined.

thrombin-induced platelet aggregation⁸ and to have thrombin-binding activity associated with the (nonglyco) peptide 'tail' portion of the molecule (T.O. and G.A.J., submitted for publication). Although there was no resolution into two electrophoretic peaks, glyocalicin present in the soluble portion of the platelet homogenate in these two cases showed a broader distribution of electrophoretic mobilities as compared with controls and the amount present was in the low-normal range.

Quantitative studies of thrombin-binding were carried out by the filtration technique of Majerus⁹ in the high affinity range (0.020–0.080 units) considered to be significant for thrombin action under physiological conditions^{9–11}. Binding studies were carried out in cacodylate buffer which gives a 10-fold increase in binding efficiency¹². The number of thrombin-binding sites per platelet were calculated from double reciprocal plots according to Steck and Wallach¹³ using computer-assisted analysis to obtain best fit (Fig. 2). Data calculated in this way gave a value of 5,400 thrombin binding sites per platelet for normal controls but only one half that, 2,400 per platelet, for the two patients showing the glycoprotein abnormality. The reciprocal plots intersected at the abscissa indicating that there is no difference in the affinity for thrombin between the patients and controls: a value of 0.35 units ml⁻¹ was determined for half saturation of thrombin binding sites.

Although both the glycoprotein polymorphism and the decrease in thrombin binding were found consistently in these patients it was not possible to correlate these biochemical observations with clinical laboratory data (Table 1). In one case (subject 2) all values were within the normal range whereas in the other case (subject 1) there seemed to be an associated release defect. Associated release defects have also been noted in other cases of abnormal haemostasis¹⁵. It may be noted that thrombin-induced aggregation was normal in both patients. Binding of thrombin to platelets is kinetically complex^{9–11}, however, and it is not possible to predict a relationship between the number of thrombin receptors present and the extent of aggregation to be expected.

No evidence for a genetic basis for the polymorphism could be obtained within the limited family groups available: subject 1 gave no history of family bleeding problems while subject 2 gave a family history of easy bruising in her father, sister and daughter but without the more marked bleeding episodes of her late mother and herself. Examination of the three living family members failed to reveal any glycoprotein abnormality and all gave normal values for VIII_{AHF}, VIII_{VWF}, in routine coagulation and aggregation studies, and in thrombin binding.

Although it is not known whether this is the primary cause of the bleeding defects observed in these two individuals, the two cases described here show identical patterns of glycoprotein polymorphism and decreased thrombin binding capacity. Since the macroglycopeptide portions of the two components seem to be identical on the basis of electrophoretic mobility, and since the thrombin-binding site of glycoprotein I/glyocalicin is present in the peptide 'tail' (MW 45,000) of the molecule, these suggest that the lower molecular weight of the abnormal form of glycoprotein I may result from a deletion of structures associated with thrombin binding in that region. Direct correlation of the electrophoretic and functional data will require isolation and comparison of the two forms of glycoprotein I manifest in these individuals.

R. B. BOLIN
T. OKUMURA
G. A. JAMIESON

American National Red Cross,
Blood Research Laboratory,
Bethesda, Maryland 20014

Received 28 March; accepted 12 July 1977.

- Okumura, T. & Jamieson, G. A. *J. Biol. Chem.* **251**, 5944–5949 (1976).
- Nurden, A. T. & Caen, J. P. *Br. J. Haemat.* **28**, 253–260 (1974).
- Nurden, A. T. & Caen, J. P. *Nature* **255**, 720–722 (1975).
- Phillips, D. R., Jenkins, C. S. P., Luscher, E. F. & Larrieu, M. J. *Nature* **257**, 599–600 (1975).
- Jenkins, C. S. P. *et al. J. clin. Invest.* **57**, 112–124 (1976).
- Pepper, D. S. & Jamieson, G. A. *Biochemistry* **9**, 3706–3713 (1970).
- Okumura, T., Lombart, C. & Jamieson, G. A. *J. Biol. Chem.* **251**, 5950–5955 (1976).
- Okumura, T. & Jamieson, G. A. *Thromb. Res.* **8**, 701–705 (1976).
- Tollefsen, D. M., Feagler, J. R. & Majerus, P. W. *J. Biol. Chem.* **249**, 2646–2656 (1974).
- Martin, B. M., Wasiewski, W. W., Fenton, J. W. & Detwiler, T. C. *Biochemistry* **15**, 4886–4893 (1976).
- Sonnichsen, W. J. & Ganguly, P. *Br. J. Haemat.* **34**, 291–301 (1976).
- Shulman, M. A. & Majerus, P. W. *J. clin. Invest.* **56**, 945–950 (1975).
- Steck, T. L. & Wallach, D. F. H. *Biochim. biophys. Acta* **97**, 510–522 (1965).
- Kang, E. P. & Triantaphyllopoulos, D. C. *Biochim. biophys. Acta* (in the press).
- Weiss, H. J. *New Engl. J. Med.* **293**, 531–541; 580–588 (1975).
- Bowie, E. J. W. & Owen, C. A. *Am. J. Clin. Pathol.* **60**, 302–308 (1973).
- Mielke, C. H., Kaneshiro, M. M. & Maler, I. A. *Blood* **34**, 204–215 (1969).
- Weiss, H. J., Hoyer, L. W., Rickles, F. R., Varma, A. & Rogers, J. J. *clin. Invest.* **52**, 2708–2716 (1973).
- Weiss, H. J. & Rogers, J. *Blood* **39**, 187–196 (1972).
- Born, G. V. R. & Cross, M. J. *J. Physiol.* **168**, 178–195 (1963).

Interaction of colchicine with phosphatidylcholine membranes

EXPERIMENTAL results have suggested that the alkaloid drug colchicine affects the lateral mobility of membrane components^{1–5}. To explain these observations, Wunderlich *et al.*⁶ have proposed two alternative hypotheses: the lipophilic

drug intercalates into the lipid bilayer in a manner analogous to cholesterol; or colchicine acts by binding to membrane protein. It is well documented that in lipid bilayers of both model and biological membranes, the methylene groups of the lipid fatty acyl chains are more mobile near the chain terminus than near the glycerol backbone of the phospholipid, resulting in a gradient of increasing fluidity in the non-polar phase of the bilayer interior⁷⁻¹². From nuclear magnetic resonance (NMR) and spin-label electron spin resonance (ESR) studies it has been found that cholesterol, by intercalating between the phospholipid fatty acyl chain, markedly increases the rigidity of the hydrocarbon phase of the bilayer¹³⁻¹⁹. We present here the results of a detailed spin-label study using phosphatidylcholine (PC) liposomes to show that, in contrast with cholesterol, colchicine does not detectably change the rigidity of PC bilayers.

The spin labels used (see Fig. 1), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), 2-(3-carboxypropyl)-4,4 dimethyl-2-tridecyl-3-oxazolidinyloxy (C_5 nitroxide-labelled stearic acid), 2-(10-carboxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxy (C_{12} nitroxide-labelled stearic acid) and 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxy (C_{16} nitroxide-labelled stearic acid) are convenient probes for the measurement of the effects of a perturbant on the hydrophobic phase of a lipid bilayer. An increase in the rigidity of the matrix of the fatty acyl chains is reflected by an increase in the hyperfine splitting ($2A'_{zz}$) in the ESR spectrum of the stearic acid spin labels and by a decrease in the parameter f of the TEMPO ESR spectrum²⁰ (see Fig. 1).

The results of replicate experiments indicate that on increasing the concentration of colchicine there was no change in the stearic acid spin label ESR spectra and no change in the f -value of TEMPO (ref. 20). As an example, ESR spectral parameters for 0 and 30 mg ml⁻¹ colchicine are listed in Table 1. However, an increase in cholesterol to phospholipid molar ratios over the range of the colchicine to phospholipid molar ratios (0.0-1.0) used in this experiment greatly increases the rigidity of the fatty acid acyl chains¹³⁻¹⁹.

From these results, there is no evidence to suggest that colchicine, like cholesterol, decreases the fluidity of the lipid

Fig. 1 ESR spectra from C_5 , C_{12} , C_{16} and TEMPO-labelled unsonicated lecithin dispersions containing 30 mg ml⁻¹ colchicine. For the C_5 and C_{16} spectra, $2A'_{zz}$ is defined by the horizontal distances indicated. From the TEMPO spectrum, the quantity f is defined as $h_L/(h_L + h_W)$, where h_L and h_W to a first approximation are proportional to the fraction of TEMPO in the lipid and water phases, respectively. H is the applied magnetic field.

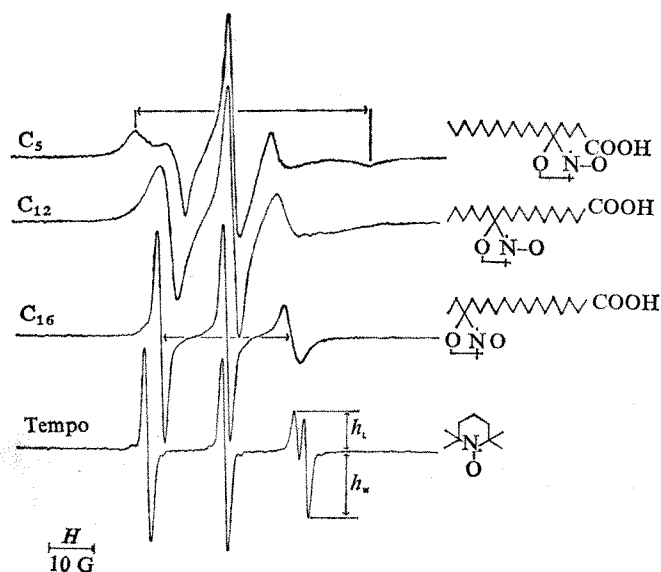


Table 1 ESR data summary*

Label	Spectral parameter	
	Colchicine (0 mg ml ⁻¹)	Colchicine (30 mg ml ⁻¹)
TEMPO	$f = 0.38 \pm 0.01$	$f = 0.38 \pm 0.01$
C_5	$A'_{zz} = 26.0 \pm 0.2$ G	$A'_{zz} = 26.0 \pm 0.2$ G
C_{12}	Spectra superimposable; A'_{zz} is difficult to extract	
C_{16}	$A'_{zz} = 14.3 \pm 0.2$ G†	$A'_{zz} = 14.3 \pm 0.2$ G†

Phosphatidylcholine was extracted from egg yolks by the method of Singleton *et al.*²³ with the exception that the acetone, used for precipitation of the crude phosphatides, was used at dry ice temperature under N₂. This speeded precipitation and retarded atmospheric oxidation of the crude phosphatides, as determined by thin-layer chromatography. TEMPO was prepared by the method of Briere *et al.*²⁴. The nitroxide-labelled stearic acids were purchased from Syva. PC and the desired spin label in 1:200 molar ratio with PC were dissolved in N₂-saturated distilled chloroform and dried to a film using a rotary evaporator. The last traces of chloroform were removed under vacuum (about 6.0×10^{-5} atm) for 2 h. N₂-saturated buffer, 10 mM Tris, 5 mM KCl, pH 7.4, was added to the mixture to give a 100 mg ml⁻¹ PC dispersion. The slurry was agitated until no PC was seen adhering to the walls of the flask. Colchicine (Sigma), in buffer solution, was added to 0.5-ml aliquots of the PC dispersion to give a final PC concentration of 50 mg ml⁻¹ and colchicine concentrations ranging from 0 to 30 mg ml⁻¹. The PC-colchicine solutions were shaken on a vortex mixer for 1 min and allowed to equilibrate for at least 2 h at room temperature under N₂ before spectra were taken. The mixtures were vortexed again immediately before the taking of spectra. A parallel series of experiments was done with lipid dispersions sonicated as described by Godici and Landsberger for 25 min. Colchicine was added to these sonicated dispersions as described above. In one series of experiments, PC and colchicine in concentrations of 0 and 30 mg ml⁻¹ were co-sonicated in conditions previously described⁸. ESR spectra were obtained with Varian E-4 and E-12 spectrometers.

*Average values of replicate experiments using unsonicated dispersions.

† A'_{zz} is one-half the distance between the base-line crossings of the high and low magnetic field peaks of the ESR spectrum.

fatty acyl chains. The data also rule out any detergent-like interaction between colchicine and lipid membranes. Although the possibility is not rigorously precluded that colchicine inhibits lateral diffusion of lipids, as has been described for cholesterol^{21,22}, and hence that of membrane protein, this seems extremely unlikely. Colchicine, in concentrations similar to these cholesterol studies^{21,22} and up to 10⁴ times greater than those known to affect the lateral mobility of membrane components¹⁻⁵, does not affect the motional freedom of the lipid hydrocarbon chains.

Thus, we have tried to differentiate between the two hypotheses suggested by Wunderlich *et al.*⁶. We conclude that colchicine does not interact with the lipid bilayer in a manner analogous to that of cholesterol and that it seems that colchicine might interact with membrane and/or microtubular protein¹⁻⁶.

This research was supported by a US NSF grant. L.D.A. is a predoctoral fellow on a NCI training grant. F.R.L. is an Andrew W. Mellon Foundation Fellow.

LAWRENCE D. ALTSTIEL
FRANK R. LANDSBERGER

The Rockefeller University,
New York, New York 10021

Received 31 May; accepted 11 July 1977.

- Berlin, R. D., Oliver, J. M., Ukena, T. E. & Yin, H. H. *Nature* **247**, 45-46 (1974).
- Berlin, R. D. & Ukena, T. E. *Nature new Biol.* **238**, 120-122 (1972).
- de Petris, S. *Nature* **250**, 54-56 (1974).
- Edelman, G. M., Yahara, I. & Wang, J. L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1442-1446 (1973).
- Furcht, L. T. & Scott, R. E. in *Experimental Cell Research* (eds Caspersson, T., Gustafson, T., Mazia, D. & Ringertz, N. R.), 271-282 (Academic, New York and London, 1975).
- Wunderlich, F., Müller, R. & Speth, V. *Science* **182**, 1136-1138 (1973).
- Chan, S. I., Seiter, C. H. A. & Feigenson, G. W. *Biochem. biophys. Res. Commun.* **46**, 1488-1492 (1972).
- Godici, P. E. & Landsberger, F. R. *Biochemistry* **14**, 3927-3933 (1975).
- Horwitz, A. F., Horsley, W. J. & Klein, M. P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 590-593 (1972).
- Hubbell, W. L. & McConnell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **64**, 20-27 (1969).

- ¹¹ Jost, P., Libertini, L. J., Hebert, V. C. & Griffith, O. H. *J. molec. Biol.* **59**, 77-98 (1971).
- ¹² Levine, Y. K., Birdsall, N. J. M., Lee, A. G. & Metcalfe, J. C. *Biochemistry* **11**, 1416-1421 (1972).
- ¹³ Darke, A., Finer, E. G., Flook, A. G. & Phillips, M. C. *J. molec. Biol.* **63**, 265-279 (1972).
- ¹⁴ Gent, M. P. N. & Prestegard, J. H. *Biochemistry* **13**, 4027-4033 (1974).
- ¹⁵ Keough, K. M., Oldfield, E. & Chapman, D. *Chem. Phys. Lipids* **10**, 37-50 (1973).
- ¹⁶ Lee, A. G., Birdsall, N. J. M., Levine, Y. K. & Metcalfe, J. C. *Biochim. biophys. Acta* **255**, 43-56 (1972).
- ¹⁷ McConnell, H. M. & McFarland, B. G. *Ann. N.Y. Acad. Sci.* **195**, 207-217 (1972).
- ¹⁸ Mendelsohn, R. *Biochim. biophys. Acta* **290**, 15-21 (1972).
- ¹⁹ Saito, H., Schreier-Mucillo, S. & Smith, I. C. P. *FEBS Lett.* **33**, 281-285 (1973).
- ²⁰ McConnell, H. M., Wright, K. L. & McFarland, B. G. *Biochem. biophys. Res. Commun.* **47**, 273-281 (1972).
- ²¹ Devaux, P. & McConnell, H. M. *J. Am. chem. Soc.* **94**, 4475-4481 (1972).
- ²² Lee, A. G., Birdsall, N. J. M. & Metcalfe, J. C. *Biochemistry* **12**, 1650-1659 (1973).
- ²³ Singleton, W. S., Gray, M. S., Brown, L. N. & White, J. L. *J. Am. Oil Chem. Soc.* **42**, 53-56 (1965).
- ²⁴ Brière, R., Lemaire, H. & Rassat, A. *Bull. Soc. Chim. Fr. Ser. 5*, **31**, 3273-3283 (1965).

Are benzodiazepines GABA antagonists?

ALTHOUGH benzodiazepines are widely used in routine medical practice their mechanism of action remains controversial^{1,2}. Several authors have suggested that the action of benzodiazepines is to potentiate GABA-ergic processes in the central nervous system³⁻⁶. In addition, a number of studies have shown the effect of benzodiazepines to be antagonised by γ -aminobutyric acid (GABA) antagonists^{6,7}. But, Steiner and Felix⁸ and Gähwiler⁹ have adopted the opposite view and reported that in the cerebellum and Deiters nucleus benzodiazepines antagonise the inhibitory action of GABA. On the other hand, Dray and Straughan⁶ have shown microiontophoretic applications of benzodiazepines to be inhibitory on the spontaneous and glutamate activity of neurones of the brain stem and were unable to confirm that benzodiazepines antagonise the action of GABA. Curtis *et al.*¹⁰ were also unable to confirm the results of Steiner and Felix⁸ on the neurones of the cerebellum and spinal cord. In the recent paper by Squires and Braestrup¹¹ a direct interaction of benzodiazepines with GABA-receptors has been discarded. We have investigated the interaction between chlordiazepoxide (Librium) and GABA by observing the effects of simultaneous microiontophoretic application of both agents on the spontaneous and glutamate-evoked firing of single neurones of sensorimotor cortex of the rabbit and here we report that the actions of the two are synergistic.

The experiments were performed on seven adult rabbits anaesthetised with hexobarbitone sodium and artificially ventilated. Microiontophoresis and recording was from five to seven barrelled micropipettes of 4-6 μ m overall tip diameter. The central recording barrel was filled with 2M NaCl and only those neurones with extracellular action potentials of 1 mV or greater

Fig. 1 The dose-dependent action of microiontophoretically applied chlordiazepoxide hydrochloride (CDP) on the glutamate-evoked firing of a sensorimotor neurone. The iontophoretic application and the current passed in nA (1×10^{-9} A) are indicated by the horizontal bar and the figures above them. The rate meter was reset every 3 s. Retaining currents in all the experiments were between 15 and 45 nA.

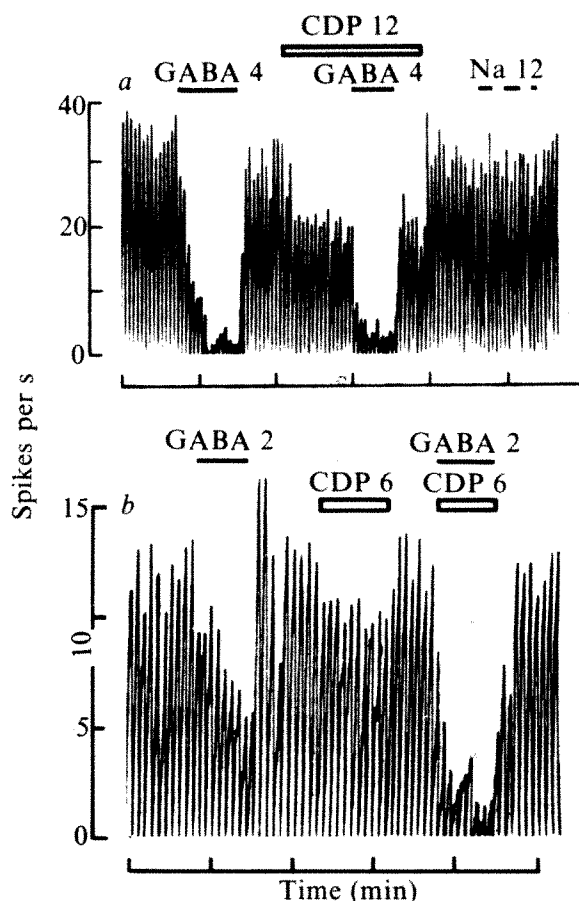
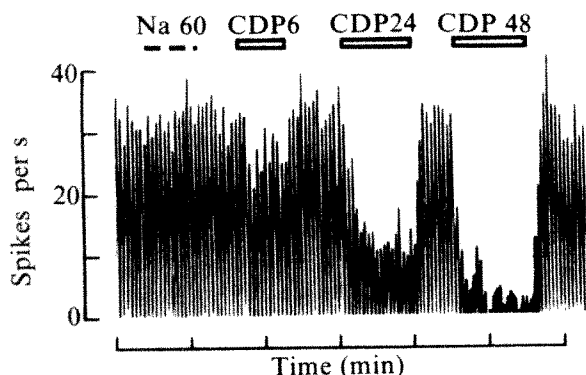


Fig. 2 Synergism between chlordiazepoxide and GABA applied microiontophoretically to the same neurone. *a*, The failure of CDP to antagonise the inhibitory action of GABA. *b*, The summation of submaximal inhibition evoked by GABA and CDP. In (*a*) the activity of the neurone was maintained by the application of glutamate (3 nA) and the rate meter was reset every 3 s in (*a*) and 5 s in (*b*).

were studied. The concentration of chlordiazepoxide was 0.2M ($pH = 3.0$), and the other barrels contained sodium glutamate (1 M; $pH = 7.5$), GABA (1 M; $pH = 3.0$) and NaCl (3 M). GABA was applied with the minimum microiontophoretic ejecting current required to induce a marked and stable inhibitory effect of the agent on the spontaneous or glutamate-evoked activity of a given neurone. The effects of current flow on neuronal activity was minimised using standard current balancing techniques.

Figure 1 shows the marked reduction in activity evoked by a microiontophoretic application of chlordiazepoxide to a neurone of the sensorimotor cortex. This inhibitory action of chlordiazepoxide increased with the applied current and this effect was found on all 22 cells studied; in no trial did chlordiazepoxide increase neuronal activity.

Figure 2a shows the failure of chlordiazepoxide to antagonise the action of GABA. Similar results were obtained on 14 neurones and no antagonism between chlordiazepoxide and GABA was observed during the application of chlordiazepoxide with currents between 2 and 120 nA. Similar results were obtained in two additional experiments in which anaesthesia was induced and maintained with urethane or chloralose.

In complete contradiction to the suggestion that chlordiazepoxide antagonises the action of GABA we have found the inhibitory action of chlordiazepoxide to be synergistic with that of GABA. In Fig. 2b, for instance, small applications of both GABA and chlordiazepoxide which evoked only small degrees of inhibition combine to induce almost complete inhibition. Since in previous studies we observed systematically administered diazepam to potentiate the action of GABA on the spontaneous

activity of neurones in the sensorimotor cortex¹², we suggest that the actions of benzodiazepines and GABA are synergistic.

S. N. KOZHECHKIN
R. U. OSTROVSKAYA

Institute of Pharmacology,
Academy of Medical Sciences of the USSR,
Moscow, USSR

Received 16 January; accepted 30 May 1977.

- ¹ Haefely, W., Pieri, L., Polc, P. & Schaffner, R. *Nature* **263**, 173–174 (1976).
- ² Steiner, F. A. & Felix, D. *Nature* **263**, 174 (1976).
- ³ Costa, E., Guidotti, A., Mao, C. C. & Suria, A. *Life Sci.* **17**, 167–186 (1975).
- ⁴ Haefely, W. et al. in *Mechanism of Action of Benzodiazepines* (eds Costa, E. & Greengard, P.) 131–151 (Raven, New York, 1975).
- ⁵ Zakusov, V. V. et al. *Archs int. Pharmacodyn. Ther.* **214**, 188–205 (1975).
- ⁶ Dray, A. & Straughan, D. W. J. *Pharm. Pharmac.* **28**, 314–315 (1976).
- ⁷ Ostrovskaya, R. U. & Molodavkin, G. M. *Bull. exp. Biol. Med. U.S.S.R.* **82**, 1073–1076 (1976).
- ⁸ Steiner, F. A. & Felix, D. *Nature* **260**, 346–347 (1976).
- ⁹ Gähwiler, B. H. *Brain Res.* **107**, 176–179 (1976).
- ¹⁰ Curtis, D. R., Lodge, D., Johnston, G. A. R. & Brand, S. J. *Brain Res.* **118**, 344–347 (1976).
- ¹¹ Squires, R. F. & Braestrup, C. *Nature* **266**, 732–734 (1976).
- ¹² Kozhechkin, S. N., Ostrovskaya, R. U. *Bull. exp. Biol. Med. U.S.S.R.* **82**, 1448–1450 (1976).

Agonist-antagonist properties of *N*-allyl-[D-Ala]²-Met-enkephalin

RECEPTOR displacement of the opiate antagonist naloxone requires concentrations of morphine and other opiate alkaloids similar to those required to elicit analgesia^{1–3} or inhibit ileal contraction^{4–5}. A new class of opiates with structures based on the discovery of endogenous, morphine-like pentapeptide—enkephalin⁶—also displace opiate receptor binding with potencies paralleling *in vivo* activities^{7–10}. Intrinsic activity (agonist or antagonist quality), a different dimension of opiate alkaloid effects *in vivo*, is closely correlated with the *in vitro* effects of sodium ion in the incubation medium: while antagonist binding to opiate receptors labelled by radioactive naloxone is almost unaffected by sodium ion addition, opiate alkaloid agonists become 10–60-fold weaker and mixed agonist-antagonists have intermediate downward shifts in apparent affinity after sodium ion addition^{1–11}. We present here an analysis of the sodium sensitivities of the major opiate peptides which have all been identified as 'agonists' thus far. In addition, we describe the synthesis, behavioural and *in vitro* receptor analysis of *N*-allyl-[D-Ala]²-Met-enkephalin (Fig. 1), a novel peptide whose structure was designed in analogy¹² with that of the alkaloid opiate antagonists¹³ in order to obtain a peptide opiate antagonist, if possible.

The sodium ion dependence of the receptor interactions of morphine, *N*-allyl-[D-Ala]²-Met-enkephalin and three opiate peptides is shown in Fig. 2. Like the prototype opiate agonist morphine, [D-Ala]²-Met-enkephalinamide (an enzyme-resistant enkephalin)¹⁴ and the C fragment of β -lipotropin (61–91) (refs 15–17) show large 'sodium shifts' of 25 and 17 respectively. This is consistent with recent reports which indicate that these peptides are morphine-like agonists as analgesics^{9,14,18,19}, and in the guinea pig ileum^{10,16}. It should be noted that Bradbury *et al.*¹⁵ reported a 'sodium shift' for the C-fragment of only 1.3. In



Fig. 1 Structure of *N*-allyl-[D-Ala]²-Met-enkephalin. *N*-allyl-L-tyrosine (A) was synthesised from *N,O*-dibenzoyl-L-tyrosine methylester by reaction with sodium hydride and allyl bromide and purified by countercurrent distribution. Reaction of A with *tert*-butoxycarbonyl azide gave the *N*-protected derivative (B). D-Alanyl-glycyl-L-phenylalanyl-L-methionine (C) was prepared by stepwise condensation using *N*-hydroxysuccinimide esters. The crystalline tetra-peptide gave the expected amino acid analysis. B was converted to the *N*-hydroxysuccinimide ester and coupled to C. Following deprotection the homogeneity of *N*-allyl-[D-Ala]²-Met-enkephalin was ascertained by countercurrent distribution, the product showing this amino acid analysis (residues per mol): glycine, 1.00; alanine, 0.94; methionine, 1.00; *N*-allyl-tyrosine, 0.85 (C-value 0.09 of that of leucine; *N*-allyltyrosyl peptides are resistant to hydrolysis and A is subject to destruction); phenylalanine, 1.00.

the same study, the sodium sensitivity ratio of morphine was only 7 in contrast to the larger values of 38 obtained in these experiments and values of 33–40 consistently obtained in previous studies^{1,11,20}. The methodological difference which probably accounts for the discrepant values¹⁵ is the lack of incubation at 0 °C, since low temperature is essential for demonstrating sodium effects reproducibly²⁰.

The sodium shift value of Met-enkephalin⁹ suggests a predominantly agonist nature, but falls within the range of opiates which display partial antagonist activity in some test situations¹¹. A recent study²¹, indeed, suggests that Leu-enkephalin despite its three to fourfold reduced affinity for brain opiate receptors^{7,8}, maintains intraventricular self-administration behaviour more readily than Met-enkephalin because it is a 'purer' agonist. No antagonist activity is detectable for Met-enkephalin in the guinea pig ileum²², however.

In striking contrast to morphine and the opiate peptide agonists, *N*-allyl-[D-Ala]²-Met-enkephalin has a small (2.5) sodium response ratio which clearly suggests antagonist properties if sodium effects on peptides are as relevant to *in vivo* pharmacological activity as they are for opiate alkaloids. Intraventricular injection of the putative peptide antagonist, *N*-allyl-[D-Ala]²-Met-enkephalin, however, does not decrease the anti-nociceptive response obtained when [D-Ala]²-Met-enkephalin is injected alone (Table 1). In fact, the allyl peptide injected alone is a weak analgesic, suggesting the presence of weak agonist activity compatible with its rather low affinity for opiate receptors. The 'narcotic antagonists' (nalorphine, levallorphan and so on) also elicit analgesia in the tail-flick test and produce sodium ratios in the 1.5–3.0 range¹¹.

The various pharmacological assays for opiates are known to differentially emphasise their agonist or antagonist qualities. For example, chronic morphine administration causes a dramatic 'supersensitivity' to opiate antagonist activity so that much lower doses of antagonists are required to precipitate opiate withdrawal signs²⁴. We therefore examined the ability of *N*-allyl-[D-Ala]²-Met-enkephalin to produce withdrawal signs in

Table 1 Behavioural effects of *N*-allyl-[D-Ala]²-Met-enkephalin

Analgesic effects		Tail-flick latency
Drug		(s ± s.e.m.)
Vehicle		3.9 ± 0.16 (n = 7)
<i>N</i> -Allyl-[D-Ala] ²		7.4 ± 1.49 (n = 6)*
[D-Ala] ²		10.4 ± 1.23 (n = 7)*
[D-Ala] ² + <i>N</i> -allyl-[D-Ala] ²		13.5 ± 0.65 (n = 7)**
Withdrawal	Vehicle (n = 4)	<i>N</i> -Allyl-[D-Ala] ² (n = 7)
Wet-dog shakes	0.75 ± 0.75	3.86 ± 2.0
Chewing	0	1.71 ± 0.36

To test analgesic effects 27 rats were implanted with 22 ga cannulae guides, the tips of which were aimed for an area 2 mm above the third ventricle (AP 3.0, LAT 0.0, DV 2.5)²³. Two weeks after the operation the animals were divided into four groups and injected in the third ventricle with either 7 μ l water (vehicle), 35 μ g *N*-allyl-[D-Ala]² in 7 μ l water, 20 μ g D-Ala in 4 μ l water or 20 μ g D-Ala followed immediately by 35 μ g *N*-allyl-[D-Ala]². Analgesic effects were assessed 15 min after injection with the tail-flick task as previously described¹⁴ with the exception that the tail-flick response limit was increased to 15 s. In withdrawal tests 10 rats were implanted with intracerebral cannulae guides as above. One week later all animals were implanted subcutaneously with one 75 μ g morphine pellet. Forty-eight hours later they were implanted with two additional pellets and 48 h after the second pellet implantation the animals were divided into two groups and injected with either 35 μ g *N*-allyl-[D-Ala]² or 7 μ l water. Total number of wet-dog shakes and jumps were recorded for 30 min after injection. Ptosis, chewing, teeth chattering, eye twitching, or squeal on touch²⁵ were rated at 10-min intervals during the same observation period. Only wet-dog shakes and chewing were found to increase following *N*-allyl-[D-Ala]².

**P* < 0.05 for comparison of drug effects with vehicle control.

†*P* < 0.05 for comparison of D-Ala + *N*-allyl-[D-Ala]² with D-Ala alone.

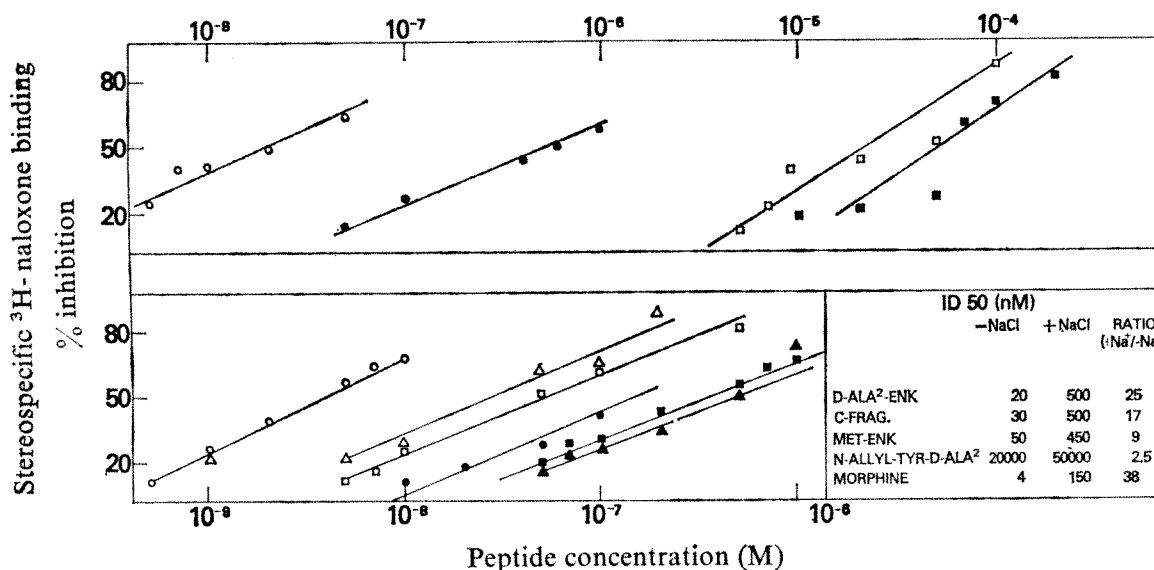


Fig. 2 Sodium sensitivity of the displacement of stereospecific ^3H -naloxone binding by several opiate peptides and morphine. Male Sprague-Dawley rats were killed by guillotine, their brain minus the cerebellum was rapidly removed and placed in 100 volumes of cold (4°C) 0.05 M Tris buffer (Trizma Preset pH crystals, pH 7.4 at 0°C) and homogenised with a Polytron set at number five for 15 s. The crude homogenate was centrifuged at $12,000g$ for 15 min, the supernatant discarded and the pellet reconstituted in 100 volumes of buffer. After repeating centrifugation and decanting, the pellet was resuspended in 10 ml of cold buffer by vortexing for 10 s and brief Polytron treatment. Membranes were prepared for use no later than 1 h after the final resuspension. The assay was incubated in duplicate in an ice water bath for 60 min in $10 \times 75\text{-mm}$ glass test tubes containing brain membranes (100 μl), peptides (abscissa), 50 μl ^3H -naloxone (2.4 nM, 20,000 c.p.m., 19.9 Ci mmol^{-1}), and 50 μl of 1 M sodium chloride (closed symbols) or buffer (open symbols), and sufficient Tris buffer required for a final volume of 500 μl . Membranes were added last. After 60 min each reaction mixture was briefly vortexed, then filtered under reduced pressure through Whatman GF/B filters and washed twice with 7 ml of ice-cold buffer in a filtration cycle of less than 10 s. Filters were transferred to vials and, after the addition of scintillation fluid (AquaSol, NEN) were counted in a Beckman LS-230 Scintillation counter at 45% efficiency. Control values of stereospecific ^3H -naloxone binding were calculated by subtracting nonspecific binding (c.p.m.) which occurred in the presence of 100 nM levallorphan. Control values from five experimental runs for total receptor binding and levallorphan binding were $1,625 \pm 72$ c.p.m. and 726 ± 172 c.p.m. respectively in the absence of sodium and $2,472 \pm 280$ c.p.m. and 429 ± 72 c.p.m. in the presence of sodium. ID_{50} and sodium ratio values, which varied less than 2-fold, were determined as the means from two experimental runs. All inhibition values between 15% and 85% for one experimental run are shown. In a: \bullet , \circ , [D-Ala]²-enkephalin; \square , \blacksquare , N-allyl-Tyr-[D-Ala]²-enkephalin. In b: \bullet , \circ , morphine; \blacktriangle , \triangle , C fragment (LPM 61-91); \blacksquare , \square , Met-enkephalin.

rats made dependent on morphine²⁵ in an attempt to demonstrate antagonist properties. Intraventricularly-injected morphine pellet-implanted rats indeed developed weak opiate withdrawal signs of 'wet-dog shakes' and chewing behaviour which were not present in the vehicle-injected controls (Table 1).

While further characterisation of the pharmacology of additional opiate peptides is required before a definite conclusion can be reached, these experiments show that the *in vitro* sodium effect provides information about the agonist-antagonist quality of opiate peptides when assessed in the very specific experimental conditions described here. The sodium effect probably occurs because receptors for endogenous as well as exogenous opiates exist as an inter-converting equilibrium between an agonist conformation and an antagonist conformation associated with a sodium binding site^{11,26,27}.

N-Allyl-[D-Ala]²-Met-enkephalin seems to be a weak agonist with mild antagonist character *in vivo* with a small sodium shift *in vitro* like that of opiate alkaloid partial antagonists. The clinically undesirable effects of chronic opiate administration have previously been demonstrated for [D-Ala]²-Met-enkephalinamide⁹, β -LPH (61-91) (ref. 28), and Met-enkephalin²⁶. Opiate peptides with partial antagonist quality might have diminished physical dependence and tolerance liability like the analgesics in the mixed agonist-antagonist benzomorphans group of opiates²⁹. In any case, these results suggest the feasibility of developing a 'pure' opiate peptide antagonist by *in vitro* analysis. Such an antagonist might more readily block the physiological actions of endogenous opiate peptide ligands; Lord *et al.* have already shown that the currently available alkaloid antagonists are surprisingly weak at blocking some actions of opiate peptide agonists³⁰.

We thank Dr Derek Smyth for the gift of the C fragment

of β -LPH used in these studies. Met-enkephalin and [D-Ala]²-Met-enkephalin were obtained from Dr Jaw-Ken Chang.

Note added in proof: Hahn *et al.*³¹ have found that N-allyl-Leu-enkephalin has antagonist properties in the guinea pig ileum.

CANDACE B. PERT
DONALD L. BOWIE
AGU PERT

Section on Biochemistry,
Adult Psychiatry Branch,
National Institute of Mental Health

JOHN L. MORELL
ERHARD GROSS

Section on Molecular Structure,
Reproduction Research Branch,
National Institute of Child Health and Human Development
Bethesda, Maryland 20014

Received 18 May; accepted 12 July 1977.

- Pert, C. B. & Snyder, S. H. *Molec. Pharmacol.* **10**, 868-879 (1974).
- Wilson, R., Rogers, M., Pert, C. B. & Snyder, S. H. *J. med. Chem.* **18**, 240-242 (1975).
- Pert, C. B., Snyder, S. H. & Portuguese, P. *J. med. Chem.* **19**, 1248-1250 (1976).
- Creese, J. & Snyder, S. H. *J. Pharmacol. exp. Ther.* **194**, 205-219 (1975).
- Kosterlitz, H. W., Leslie, F. M. & Waterfield, A. A. *J. Pharm. Pharmacol.* **27**, 73-78 (1975).
- Hughes, J. *et al.* *Nature* **258**, 577-579 (1975).
- Chang, J.-K., Fong, B. T. W., Pert, A. & Pert, C. B. *Life Sci.* **18**, 1473-1482 (1976).
- Horn, A. S. & Snyder, S. H. *Molec. Pharmacol.* **12**, 987-998 (1976).
- Pert, A. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 87-94 (Elsevier-North Holland, Amsterdam, 1976).
- Cox, B. M., Goldstein, A. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1821-1823 (1976).
- Pert, C. B., Snyder, S. H. & May, E. L. *J. Pharmacol. exp. Ther.* **196**, 316-322 (1976).
- Horn, A. S. & Rogers, J. R. *Nature* **260**, 795-797 (1976).
- Jacobsen, A. E., May, E. L. & Sargent, L. J. in *Medicinal Chemistry* **2**, 1327-1350 (Wiley, New York, 1970).
- Pert, C. B., Pert, A., Chang, J.-K. & Fong, B. T. W. *Science* **194**, 330-332 (1976).
- Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M. & Hulme, E. C. *Nature* **270**, 793-795 (1976).

- 16 Li, C. H. & Chung, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1145–1148 (1976).
 17 Lazarus, L. H., Ling, N. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2156–2159 (1976).
 18 Walker, J. M. *et al. Science* **196**, 85–87 (1977).
 19 Meglio, M., Hosobuchi, Y., Loh, H. H., Adams, J. E. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 774–776 (1977).
 20 Creese, I., Pasternak, G. W., Pert, C. B. & Snyder, S. H. *Life Sci.* **16**, 1837–1842 (1975).
 21 Beluzzi, J. D. & Stein, L. *Nature* **266**, 556–558 (1977).
 22 Morgan, B. A., Smith, C. F. C., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. *J. Pharm. Pharmacol.* **28**, 660–661 (1976).
 23 Pellegrino, L. J. & Cushman, A. J. *A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967).
 24 Way, E. L., Loh, H. H. & Shen, F.-H. *J. Pharmac. exp. Ther.* **167**, 1–8 (1969).
 25 Bläsing, J., Höllt, V., Herz, A. & Paschelke, G. *Psychopharmacologia* **46**, 41–51 (1976).
 26 Simon, E. J. & Groth, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2404–2407 (1975).
 27 Pert, C. B. in *Hormone Receptors* 2 (eds Birnbaumer, L. & O'Malley, B. W.) (Academic, New York, in the press).
 28 Wei, E. & Loh, H. *Science* **193**, 1262–1263 (1976).
 29 Eddy, N. B. & May, E. L. *Science* **181**, 407–414 (1973).
 30 Lord, J. A. H., Waterfield, A. A., Hughes, J. & Kosterlitz, H. W. *Nature* **297**, 494–499 (1977).
 31 Hahn, E., Fishman, J., Shiwaier, Y., Folders, T. F., Nagashima, H. & Duncalf, D. *Res. Commun. Chem. Path. Pharmac.* (in the press).

Subcellular localisation of leucine-enkephalin-hydrolysing activity in rat brain

THE naturally-occurring pentapeptides leucine- and methionine-enkephalin which exhibit agonist activity at the opiate receptor¹ are subject to rapid deactivation by tissue homogenates^{2,3}. The primary mode of degradation has been demonstrated to be cleavage of the Tyr-Gly amide bond^{4,5}. It is not surprising, therefore, that the opiate actions of the exogenously administered peptides are transient^{6–9} and the biological half life of the peptides is very short. We have previously studied degradation of the enkephalins in relatively crude preparations and we present here a study of the subcellular localisation of enkephalin-hydrolysing activity (EHA) in rat brain. We found a heterogeneous distribution of EHA in rat brain subfractions and evidence for enzyme heterogeneity. Opiate receptors are apparently functionally unrelated to the site of EHA in synaptic membranes.

Rat brain subcellular fractions were obtained from whole rat brain, excluding medulla and cerebellum, by the method of Whittaker¹⁰. Blood was obtained by cardiac puncture and

Table 1 Enkephalin-hydrolysing activity in rat plasma, CSF and unlysed brain subfractions

Tissue (fraction†)	Enzyme specific activity*	Total protein (mg)
Plasma	2.41 ± 0.31 (6)	39.2 ± 7.45 ml ⁻¹
CSF	2.00 ± 0.85 (10)	0.54 ± 0.26 ml ⁻¹
Brain soluble (S ₃)	30.54 ± 5.57 (5)	36.05 ± 5.41
Nuclear (P ₁)	7.51 ± 1.22 (5)	11.82 ± 1.57
Microsomal (P ₃)	3.29 ± 1.11 (5)	24.38 ± 5.63
Myelin (A)	0.96 ± 0.91 (5)	10.63 ± 3.07
Synaptosomal (B)	7.75 ± 1.47 (5)	15.80 ± 2.40
Mitochondrial (C)	4.84 ± 1.76 (5)	21.19 ± 5.71

Values are mean ± s.d.; number of determinations in parentheses. The assay mixture, containing [3,5-³H]-Tyr]-Leu⁵-enkephalin (1 μCi, final concentration 10⁻⁴ M), 0.5 M Tris-HCl buffer pH 7.4 (0.1 ml), and the preparation to be assayed (50–200 μg, protein), was diluted with distilled water to a final volume of 0.5 ml. The mixture was incubated at 37 °C for 15 min and an aliquot (0.2 ml) taken and added to ice cold methanol (0.1 ml). After separation of the denatured proteins by centrifugation, an aliquot (0.05 ml) of the supernatant was applied in a narrow band to the foot of a silica gel thin-layer plate (Kieselgel-GF₂₅₄, 5 × 20 cm, Merck) together with authentic samples of tyrosine and Leu⁵-enkephalin. After development using chloroform-methanol-acetic acid-water (45:30:6:9, v/v) as solvent, the markers were visualised with ninhydrin (1%, w/v in acetone). Zones corresponding to the substrate and product were scraped and the silica suspended in a mixture of water (2 ml) and PCS (5 ml, Amersham-Searle). Radioactivity was determined using a Packard 2450 scintillation counter. Protein concentrations were determined by the method of Lowry *et al.*¹¹.

*Enzyme specific activity expressed as nmol enkephalin hydrolysed per mg protein per min.

†Letters in parentheses refer to the classification of subcellular fractions as used by Whittaker¹⁰.

cerebrospinal fluid (CSF) by puncture of the cisternum magnum of rats. The EHA was assayed using [3,5-³H]-Tyr]-Leu⁵-enkephalin (Radiochemical Centre, Amersham) as substrate, by either determining the rate of formation of [3,5-³H]-tyrosine or the rate of disappearance of the substrate after thin-layer chromatographic separation of the two tritiated species.

The pH optimum for EHA in plasma, synaptosomes and brain high speed supernatant was pH 7.2. Significant EHA was found in all subcellular fractions (Table 1). Plasma, in contrast to CSF, was found to be a potent source of EHA. This is consistent with the results of studies reporting detection of enkephalin-like activity in CSF (refs 12, 13). The soluble fraction (S₃) of rat brain was also a good source of EHA. Of the particulate fractions examined synaptosomal (B) and nuclear (P₁) fractions showed highest enzyme specific activity. The even distribution of EHA in a crude membrane fraction (P₂) from various rat brain regions shown by Meek *et al.*⁵ would be expected from the considerable EHA associated with the mitochondrial fraction as this activity would contribute to a high background level of EHA of other than synaptosomal origin.

In view of the putative role of the enkephalins as inhibitory neurotransmitters or neuromodulators, it was of particular interest to explore the localisation of the synaptosomal enzyme. The synaptosomal fraction (B) was lysed by suspension in water for 30 min at 0–1 °C. The lysate was then fractionated on a discontinuous sucrose gradient as described by Whittaker¹⁰. The activity of the subfractions obtained is shown in Table 2. Some soluble enzyme was released which possessed similar activity to that obtained in the soluble fraction from whole brain. The synaptosomal

Table 2 Enkephalin-hydrolysing activity in rat brain lysed synaptosomal subfractions

Fraction (fraction†)	Enzyme specific activity*	Total protein (mg)
Soluble (O)	35.26 ± 12.0 (4)	2.49 ± 1.33
Vesicular (D, E)	0.91 ± 1.00 (4)	1.30 ± 0.56
Synaptosomal membranes (F, G, H)	2.62 ± 0.96 (4)	7.40 ± 4.93
Intra-terminal mitochondria (I)	3.26 ± 1.19 (4)	2.20 ± 1.70

Values are mean ± s.d.; number of determinations in parentheses. Assay methods as for Table 1.

*Expressed as nmol enkephalin hydrolysed per mg protein per min.

†Letters in parentheses refer to classification of Whittaker¹⁰.

mitochondrial fraction also possessed activity similar to that found in the mitochondrial fraction from whole brain. The EHA associated with the vesicular fraction was low whereas significant EHA was associated with the synaptosomal membrane fractions (F, G, H) and this latter activity may have a significant role in terminating the physiological action of the enkephalins.

Simantov *et al.*¹⁴ have shown that synaptosomes contain the highest levels of enkephalin activity and this study shows that substantial EHA is present in intact synaptosomes, so it follows that enkephalins must be protected from hydrolysis in some way, possibly by sequestration within vesicles. This would be consistent with the evidence presented here and analogous to the subcellular storage and inactivation of acetylcholine¹⁰.

It was of interest to examine the sensitivity of EHA in brain subcellular fractions to various reagents in an attempt to cast some light on the uniformity of enzyme activity present. The results are presented in Table 3. Rat brain EHA was uniformly inhibited by leucyl-β-naphthyl-

Table 3 Effect of various reagents on rat brain and plasma enkephalin-hydrolysing activity

Tissue (fraction)	Leucyl- β -naphthylamide	EDTA*	p-Chloromercuribenzoate
Plasma	41	14	0
Brain soluble (S ₃)	79	47	90
Synaptosomal (B)	92	1	90
Mitochondria (C)	62	15	93
Soluble (O)	82	0	19
Synaptic vesicles (D, E)	100	100	42
Synaptosomal membranes (F, G, H)	71	0	47
Intra-terminal mitochondria (I)	78	16	92

Values are % inhibition. Final inhibitor concentration was 0.5 mM.

* Tissue classification as in Tables 1 and 2.

*Adjusted to pH 7.4 with NaOH.

amide (0.5 mM). The patterns of inhibition observed with EDTA and p-chloromercuribenzoate did not run in parallel suggesting considerable enzyme heterogeneity in the fractions studied.

In view of the structural similarities demonstrated^{15,16} between the enkephalins and standard opiates it seemed possible that the latter could be competitive inhibitors of EHA. Of a series of compounds tested (buprenorphine, etorphine, fentanyl, ketocyclazocine, methadone, morphine, nalorphine, naloxone, pentazocine and pethidine) representing a wide range of structures and activities, only etorphine, fentanyl and methadone showed any inhibitory properties (about 50% at 0.66 mM, the highest concentration tested). In the light of the high affinity that the majority of these compounds demonstrate for the opiate receptor, it can be concluded that the pharmacological receptors and the sites of enzyme activity in synaptic membranes are functionally unrelated.

In summary, a heterogeneous distribution of EHA has been observed in rat brain subfractions. The demonstration of activity in the synaptic membrane fraction and the low level of activity found in the synaptic vesicle fraction are observations not inconsistent with the putative role of the enkephalins as neurotransmitters or neuromodulators.

A. C. LANE

M. J. RANCE

D. S. WALTER

Reckitt and Colman Pharmaceutical Division,
Dansom Lane,
Hull, UK

Received 20 June; accepted 18 July 1977.

- ¹ Hughes, J. *et al.* *Nature* **258**, 577-579 (1975).
- ² Hughes, J. *Brain Res.* **88**, 295-308 (1975).
- ³ Pert, C., Bowie, D. L., Fong, B. T. W. & Chang, J. K. *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 79-86 (North-Holland, Amsterdam, 1976).
- ⁴ Hambrook, J. M., Morgan, B. A., Rance, M. J. & Smith, C. F. C. *Nature* **262**, 782-783 (1976).
- ⁵ Meek, J. L., Yang, H. Y. T. & Costa, E. *Neuropharmacology* **16**, 151-154 (1977).
- ⁶ Bellinzi, J. B. *et al.* *Nature* **260**, 625-626 (1976).
- ⁷ Pert, C. B., Pert, A., Chang, J. K. & Fong, B. T. W. *Science* **194**, 330-332 (1976).
- ⁸ Goldstein, A. *Science* **193**, 1081-1086 (1976).
- ⁹ Chang, J. K., Fong, B. T. W., Pert, A. & Pert, C. *Life Sci.* **18**, 1473 (1976).
- ¹⁰ Whittaker, V. P. in *Handbook of Neurochemistry* (ed. Lajtha, A.) 327-364 (Plenum, London, 1969).
- ¹¹ Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265-275 (1951).
- ¹² Wahlstrom, A., Johansson, L. & Terenius, L. *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 49-56 (North-Holland, Amsterdam, 1976).
- ¹³ Hughes, J. in *Centrally Acting Peptides* (Macmillan, London, in press).
- ¹⁴ Simantov, R., Snowman, A. M. & Snyder, S. H. *Brain Res.* **107**, 650-657 (1976).
- ¹⁵ Bradbury, A. F., Smith, D. G. & Snell, C. R. *Nature* **260**, 165-166 (1976).
- ¹⁶ Horn, A. S. & Rodgers, J. R. *Nature* **260**, 795-797 (1976).

Unconventional serotonergic excitation in *Aplysia*

EXCITATORY potentials of long duration have been observed in vertebrate¹⁻³ and invertebrate^{4,5} neurones. In *Aplysia*, Gerschenfeld and Paupardin-Tritsch⁴ have found that 5-hydroxytryptamine (5-HT) can produce two kinds of long excitatory responses (A' and α) with different ionic mechanisms. The A' response was described as an increase in sodium conductance. The response became smaller as the cell was depolarised, and was extrapolated to a reversal potential of 0 mV. In contrast, the α response was attributed to a decrease in potassium conductance, since it had a reversal potential near -80 mV and became larger as the cell was depolarised. Krnjevic *et al.*³ and Weight and Votava¹ have hypothesised a similar mechanism for the long excitatory potentials which they observed in cat cortical neurones and frog sympathetic ganglia, respectively. Using the voltage clamp technique we observed a prolonged excitatory response to 5-HT, the characteristics of which are unlike either of those described previously. At hyperpolarised levels it resembles the A' response, becoming smaller with depolarisation, but at depolarised potentials the amplitude of the response dramatically increases.

Long excitatory responses with this characteristic could be recorded in cells of the left caudal quarter of the abdominal ganglion and in unidentified neurones of the buccal ganglia of *Aplysia californica*. The ganglion was pinned out and bathed in a solution of artificial seawater at pH 7.8. Cells were impaled with a glass capillary microelectrode filled with 1.5 M KCl. Membrane potential was clamped by the single microelectrode method of Wilson and Goldner⁶. An iontophoretic electrode containing a saturated solution of 5-HT was positioned extracellularly, and adequate bias current was applied to prevent leakage of the transmitter. To eject the 5-HT, standard pulses of positive current were passed through the electrode at a constant frequency of about once every 5 min. At this rate, desensitisation would not occur. It was ensured that the amplitude of the response was constant and that manipulation of membrane potential and the perfusion of seawater solutions would not alter the response. Since membrane potential was held constant by the voltage clamp, a depolarising response is expressed as inward current.

The membrane potential dependence of the long excitatory response is illustrated in Fig. 1a and b. As the cell is depolarised from -80 to -30 mV, the current response decreased in amplitude. Above -30 mV, the current response dramatically increased in size. In other cells this dramatic increase occurred at potentials as negative as -40 mV. Additionally, at depolarised levels, the current was prolonged and reached a maximum amplitude more slowly. The response seems to be comprised of two components: a linear component (1 in Fig. 1b) at hyperpolarised levels and a nonlinear component (2 in Fig. 1b) which begins to influence the response between -40 and -50 mV. This nonlinear component was observed in over 80% of the cells in which a prolonged excitatory serotonergic response was recorded (25 cells). In each case where component 2 was seen, it was as distinct and prominent as the sample shown in Fig. 1b.

Occasionally, the long response to 5-HT was accompanied by an excitatory response with a short time course (the A response of Gerschenfeld and Paupardin-Tritsch⁴). This fast response behaved in a totally conventional manner. As reported by Gerschenfeld and Paupardin-Tritsch⁴, the response became smaller with depolarisation and seemed to have a reversal potential near 0 mV. At potentials where the prolonged response was enlarged, the fast response in the same cell was extremely reduced in amplitude. This was

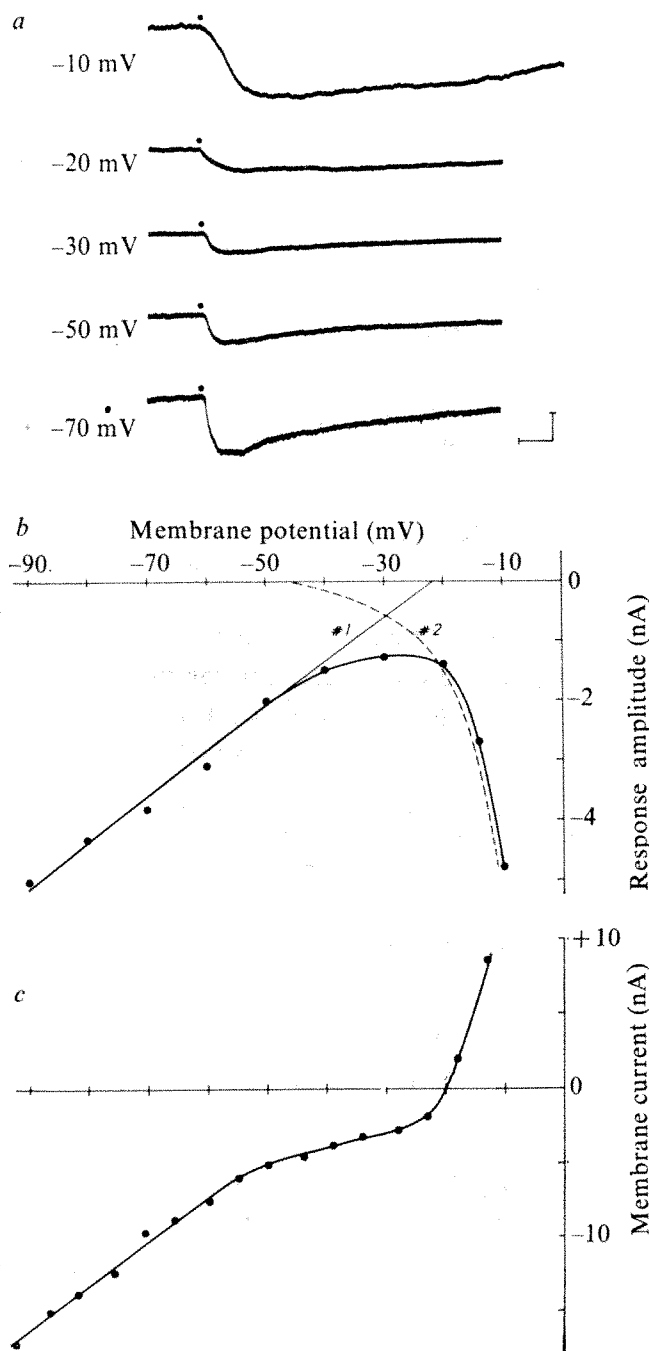


Fig. 1 *a*, Prolonged excitatory response to 5-HT. Cell membrane potential was voltage clamped and current was recorded. Ionophoretic pulse was applied at the dot. At -10 mV the response is prolonged and greatly enhanced. Calibration: 2 nA, 24 s. *b*, Plot of membrane potential against response amplitude for the same cell as in *a*. The response seems to be composed of two components: a linear component, labelled 1 and a non-linear component, labelled 2. *c*, Current-voltage relationship for the same cell as in *a* and *b*. Note the correspondence between the increase in the conductance of the cell and the enhanced response to 5-HT.

taken as evidence that the enhanced component 2 of the long response was not simply an artefact of the voltage-clamping system, for if it were, the short response would be equally enhanced.

A comparison of the current-voltage relationship of the cell (Fig. 1*c*) and the amplitude-potential relationship of the response to 5-HT (Fig. 1*b*) shows that the 5-HT response increased dramatically at the same potential where the membrane conductance increased. In the illustrated

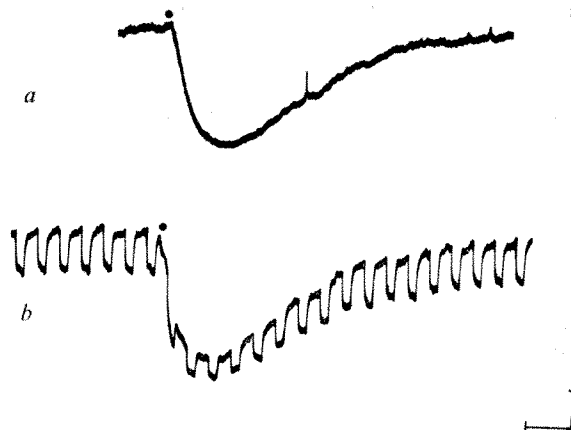
cell this point was approximately -20 mV (compare Fig. 1*b* and *c*). We considered the possibility that the anomalous response to 5-HT at depolarised levels (component 2) could be due to a reduction in the potentiated membrane conductance at these potentials. Figure 2 shows the results of conductance measurements before and during a response to 5-HT. While the potential of the cell was clamped to -12 mV, hyperpolarising command pulses were applied to the cell every 20 s. During the response to 5-HT the current change produced by these pulses was reduced, indicating an apparent conductance decrease. At membrane potentials more negative than -30 mV, this reduction in membrane conductance was not observed. In fact, there was either a small increase in conductance or, often, no measurable change at all in the membrane conductance of the cell during the response at -80 mV.

The apparent decrease in membrane conductance, at potentials above -30 mV could be due to a decrease in potassium or chloride conductance. Preliminary ion substitution experiments show that this is not the case. In both high (30 mM) and low (1 mM) extracellular potassium, the response was unaffected. Decreasing extracellular chloride concentration to 77 mM was also without effect on the excitatory response to 5-HT. Figure 3*a* shows a typical ion substitution experiment. This particular experiment illustrates that elevation of extracellular potassium does not alter the response.

The apparent decrease in conductance could also be due to a voltage-sensitive increase in inward sodium current. In Fig. 1*b*, the line labelled 2 can represent such a current. As the cell is depolarised, the inward current is enhanced. Summing this component of the response with the current produced by a voltage-independent conductance change (1 in Fig. 1*b*) would result in the observed response. Since component 2 would be reduced during a hyperpolarising command, there would be an apparent conductance decrease. Such a voltage-sensitive sodium or calcium conductance has been shown to underlie the regenerative slow wave potentials and negative resistance region of bursting cells⁷. This possibility is supported by ion substitution experiments. Replacement of extracellular sodium with sucrose consistently attenuated both components of the response, suggesting that the response is in some way dependent on sodium ions (Fig. 3*b*).

It is likely that the unconventional response shown here is actually the A' response of Gerschenfeld and Paupardin-Tritsch⁴, which was attributed to an increase in sodium conductance. Since their observations were made in an unclamped cell, rectification prohibited their recording at

Fig. 2 *a*, Long excitatory response to 5-HT, administered at the dot. Membrane potential was voltage clamped to -12 mV. *b*, Hyperpolarising voltage commands applied before and during an identical response to 5-HT. The current change produced by the voltage commands is reduced during the response. Calibration: 2 nA, 48 s.



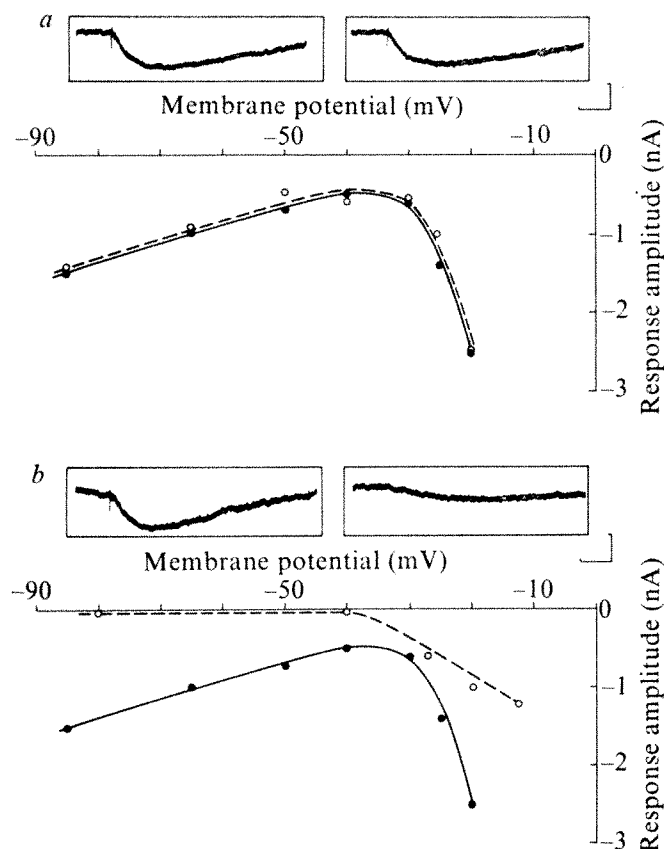


Fig. 3 Effect of ion substitutions on the long excitatory response to 5-HT. The responses shown in *a* and *b* were obtained from the same cell. *a*, Sample responses in normal (left) and 30 mM K^+ seawater (right) were recorded while the cell was voltage clamped to -20 mV. Calibration: 2 nA, 24 s. A plot of membrane potential against response amplitude illustrates that high potassium seawater (\circ) does not alter the responses obtained in normal seawater (\bullet). *b*, Sample responses at a membrane potential of -20 mV were greatly attenuated by Na^+ -free seawater, in which sucrose was added to maintain isotonicity. Calibration: 2 nA, 24 s. A plot of membrane potential against response amplitude illustrates that the Na^+ -free seawater (\circ) reduced the response at all membrane potentials. \bullet , Normal seawater.

very depolarised levels⁴. Fig. 1*b* shows that component 2 of the response would be missed unless potentials above -30 mV were sampled. Extrapolation of the curve consisting of only the points below -30 mV would indicate a reversal potential near -20 mV which is similar to that of conventional, sodium-dependent response^{4,6}. It is unlikely that the response we have observed is due to the stimulation of a combination of receptors which produce an increase in sodium conductance and a decrease in potassium conductance (A' and α , respectively). Our second component can first be seen to influence the current response near -40 mV and seems to have no effect on the response at more hyperpolarised potentials. Also the response is insensitive to changes in extracellular potassium concentration.

Our data show an unconventional long excitatory response to 5-HT. The response at hyperpolarised potentials behaves simply as an increase in sodium conductance, whereas at depolarised potentials the current is enhanced and accompanied by an apparent decrease in membrane conductance. This unusual component may be due to serotonergic activation of a voltage-sensitive sodium conductance, whereas conventional synaptic and iontophoretic responses result from voltage-independent conductance changes. The response we described represents a new type of neurotransmitter action: the induction of voltage-sensitive ionic channels.

This work was supported by a grant from the Veterans

Administration Hospital, Durham, North Carolina. T.P. received a stipend from the NIH. We thank Dr David Carpenter for reading the manuscript and T. Tulley for assistance.

T. C. PELLMAR
W. A. WILSON

Department of Physiology and Pharmacology,
Duke University Medical Center,
and Epilepsy Research Laboratory,
Veterans Administration Hospital,
Durham, North Carolina 27705

Received 22 April; accepted 5 July 1977.

- ¹ Weight, F. F. & Votava, J. *Science* **170**, 755–758 (1970).
- ² Libet, B., *J. Physiol., Lond.* **174**, 1–25 (1964).
- ³ Krnjevic, K., Pumain, R., & Renaud, L., *J. Physiol., Lond.* **215**, 247–268 (1971).
- ⁴ Gerschenfeld, H. M. & Paupardin-Tritsch, D. *J. Physiol., Lond.* **243**, 427–456 (1974).
- ⁵ Carew, T. & Kandel, E. R. *Science* **192**, 150–153 (1976); Yarowsky, P. J. & Carpenter, D. O. *Neurosci. Abstr.* **1**, 566 (1975).
- ⁶ Wilson, W. A. & Goldner, M. M. *J. Neurobiol.* **6**, 411–522 (1975).
- ⁷ Wilson, W. A. & Wachtel, H. *Science* **186**, 932–934 (1974).
- ⁸ Gerschenfeld, H. M., *Physiol. Rev.* **53**, 1–119 (1973).

Light-induced fluctuations in membrane current of single toad rod outer segments

VERTEBRATE photoreceptors respond to illumination with a reduction in the steady current of Na ions which in darkness flows inwards across the outer segment membrane; this results in hyperpolarisation of the cells¹. The light responses of the receptors can be studied by measuring extracellular voltage gradients² or by intracellular recording³, but these methods can only provide information averaged over many photoreceptors owing respectively to high extracellular conductivity and to the presence of electrical coupling between cells^{3–6}. This averaging and the presence of dark voltage noise in photoreceptors^{6,7} have prevented observation of the electrical effects of individual photoisomerisations. To try to record these elementary events and to localise the source of the dark noise, we have developed a method for recording the membrane current of a single rod outer segment. The technique is based on that used by Neher and Sakmann⁸ on muscle fibres.

Small pieces of retina were isolated from dark-adapted toads, *Bufo marinus*, and maintained in previously oxygenated toad Ringer⁹. On the stage of an inverted microscope fitted with an infrared image converter, a single rod outer segment was drawn under visual control into a close-fitting suction electrode containing Ringer (Fig. 1). The resistance of the suction pipette was initially 1–2 M Ω and rose to 4–10 M Ω with an outer segment in place. A current sensor between the pipette and a reference electrode in the bathing solution recorded the bulk of the membrane current flowing across the region of outer segment within the pipette. Recording resolution was limited by thermal noise in the leakage resistance of the space between the electrode wall and the outer segment, and to measure transient currents of the order of picoamps the bandwidth was normally restricted to 5–10 Hz.

Flashes of light evoked net outward photocurrents which were graded with stimulus intensity. As in previous extracellular² and intracellular^{3–5,9,10} measurements, the response amplitude saturated with increasing intensity in a hyperbolic manner. The half-saturating photon density was about 1 photon μm^{-2} for 'red' rods stimulated with 500 nm light, comparable with results obtained with intracellular voltage recording⁴, and the maximum photocurrent obtainable from a cell corresponded closely to complete suppression of its dark current, with values for different cells ranging up to 23 pA. Although intracellular voltage responses to bright

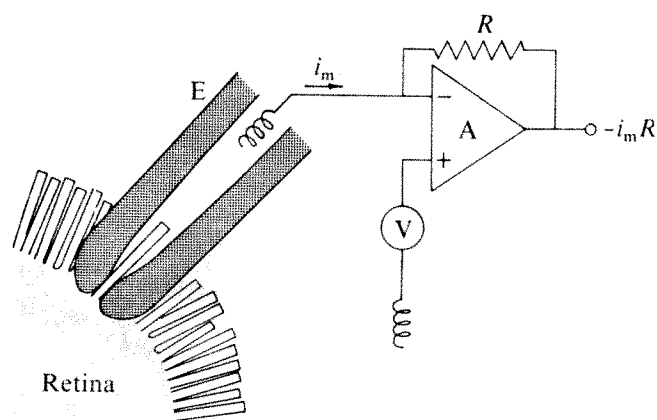


Fig. 1 Arrangement for recording membrane current of a rod outer segment. A suitably oriented outer segment was drawn by gentle suction into a close-fitting glass electrode, E, with fire-polished and vinyl-coated tip (Stoner-Mudge resin, Mobil). Membrane current, i_m , was recorded with amplifier A, a Function Modules model 380K, selected for low noise⁸. V, variable voltage used to compensate differences in electrode potentials and to measure electrode resistance. Amplifier output, $-i_m R$, was further amplified and recorded on a chart recorder and an FM tape recorder. Feedback resistance, R, 100 M Ω .

flashes display a prominent initial transient component^{4,9}, no such phase has been seen in the membrane current response from the outer segment. This may indicate that the voltage-sensitive channels generating the transient are not located in the outer segment. The dim flash responses were often considerably slower than the corresponding responses observed previously with internal electrodes⁴, with times to peak of 2–6 s. The reason for this is not clear, but it might result from our procedure of cutting the retina into small pieces.

Responses of a rod to a series of dim flashes of fixed intensity applied locally on its outer segment are shown in Fig. 2. There was considerable variation in response amplitude and four flashes seem to have failed to elicit a response. Measurement of the peak amplitude gave a mean of 2.0 pA and a variance of 2.2 pA², which, assuming a Poisson distribution, indicates an event size of 1.1 pA and

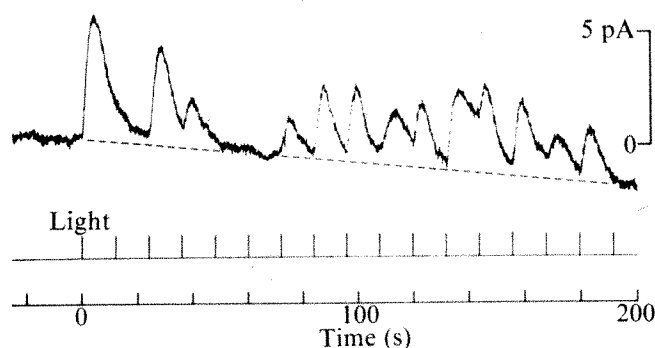


Fig. 2 Responses of a 'red' rod outer segment to brief flashes of blue-green light delivered at intervals of 12 s. Membrane current above, outward current upward, bandwidth 5 Hz; stimulus monitor below. Stimuli were local slits of light slightly larger than the 40 μm length of outer segment in the electrode. Intensity of the 500 nm stimulus at the preparation was estimated from readings of the unattenuated light made with a Tektronix digital irradiance meter and from *in situ* calibrations of the neutral density filters made with a photon counter (Princeton Applied Research, Quantum photometer). The flash width at half amplitude was 18 ms, and the estimated mean photon density was 7.9×10^{-2} photons per μm^2 per flash. The effective collecting area of the portion of the outer segment in the electrode was estimated as 12.5 μm^2 from measurements of cell dimensions, assuming an axial pigment density of 0.016 μm^{-1} and a quantum efficiency of isomerisation of 0.65.

a mean of 1.8 events per response. If a correction were made for nonlinear summation, the estimated event size would increase somewhat, and the mean number of events per flash would correspondingly decrease. The intensity of the light applied to the preparation was determined from photometer and photomultiplier readings as 0.079 photons μm^{-2} which was estimated to cause a mean of 0.99 photoisomerisations in the portion of outer segment within the suction pipette. This number of events is in reasonable agreement with the value of 1.8 estimated from the current fluctuations.

Figure 3 illustrates responses of the same rod to steady lights of increasing intensity. The lowest intensity (a) gave a response which rose with a considerable delay after the shutter opened and which fluctuated and then disappeared

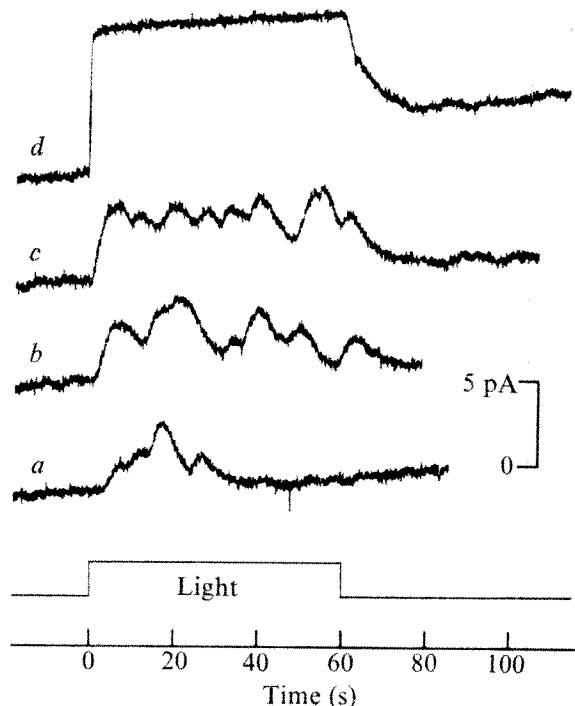


Fig. 3 Responses of the outer segment of Fig. 2 to steady lights of increasing intensity. Upper traces (a–d): membrane current, bandwidth 10 Hz; lower trace: stimulus monitor. For each response the intensity of 500 nm light was (photons $\mu\text{m}^{-2} \text{s}^{-1}$): a, 6.1×10^{-3} ; b, 2.0×10^{-2} ; c, 3.6×10^{-2} ; d, 30.

before the shutter closed. Two higher intensities (b, c) gave larger responses still marked by fluctuations, but a saturating light (d) gave a smooth response. The light for trace (a) had an intensity of 0.0061 photons $\mu\text{m}^{-2} \text{s}^{-1}$, which should on average have caused about 0.076 photoisomerisations per second in the recorded region of outer segment. On this basis the number of isomerisations expected in a 60-s stimulus would have been 4.6 ± 2.1 . Inspection of trace (a) shows four discrete deflections although it is possible that more events may have occurred. The absence of a response in the later part of the trace presumably resulted from the absence of any isomerisations during that period. In trace (b) the mean response during illumination was 2.5 pA and the variance was 0.7 pA². Ignoring nonlinear summation of elementary events and taking a shape factor¹¹ of 0.68 gives the amplitude of the elementary event as 0.42 pA, in rough agreement with that obtained from the flash run. The mean number of simultaneous events in trace (b) would then have been 6.0, which, with an integration time³ of 8 s (from the flash responses of Fig. 2), corresponds to 0.75 events per second, as compared with a rate of 0.25 photoisomerisations per second estimated from the light intensity and cellular dimensions.

Assuming that the driving potential on the light-

sensitive conductance is 40 mV (refs 1, 3, 10), the observed current event of about 1 pA would correspond to a photon-induced conductance decrease of 25 pS. This is comparable to the conductance of an acetylcholine channel^{11,12}, but several times larger than that of a sodium channel in nerve^{13,14}. An elementary current event of 1 pA together with the measured flash sensitivity of 700 μ V per photoisomerisation with diffuse light⁴ indicates that the resistance of an individual rod would be about 700 M Ω in the absence of coupling to other cells.

A noticeable feature of the presumed responses to single photons was their rounded shape. In contrast, the opening and closing of single channels in other preparations leads to rectangular-shaped changes in conductance^{8,15}. This may imply that a large number of channels are affected by each photoisomerisation or that a single channel fluctuates so rapidly between open and closed states that only the average behaviour is observed.

Responses to bright lights, such as that in Fig. 3d, occasionally showed suppression of the dark noise, similar to the effect seen with intracellular recordings^{6,7}. In one cell the variance measured in the band 0.2 to 5 Hz was 0.050 pA² in darkness and 0.017 pA² during a light which gave a saturating response of 13 pA. The difference power spectrum was broadly consistent with a Lorentzian of half power frequency 1.5 Hz. It is not yet clear whether such dark current noise arises from conductance fluctuations in the outer segment or from fluctuations in intracellular voltage arising from a noise source elsewhere in the cell.

This work was supported by grant EY-01543 from the National Eye Institute, USPHS.

Note added in proof: It has come to our attention that a similar method of recording from rod outer segments has been reported by Jagger¹⁶.

K.-W. YAU
T. D. LAMB
D. A. BAYLOR

Department of Neurobiology,
Stanford University School of Medicine,
Stanford, California 94305

Received 18 May; accepted 5 July 1977.

- 1 Tomita, T. *Q. Rev. Biophys.* **3**, 179–222 (1970).
- 2 Penn, R. D. & Hagins, W. A. *Biophys. J.* **12**, 1073–1094 (1972).
- 3 Baylor, D. A. & Hodgkin, A. L. *J. Physiol., Lond.* **234**, 163–198 (1973).
- 4 Fain, G. L., Gold, G. H. & Dowling, J. E. *Cold Spring Harb. Symp. quant. Biol.* **15**, 547–561 (1976).
- 5 Schwartz, E. A. *J. Physiol., Lond.* **257**, 379–406 (1976).
- 6 Lamb, T. D. & Simon, E. J. *J. Physiol., Lond.* **263**, 257–286 (1976).
- 7 Schwartz, E. A. *J. Physiol., Lond.* (in the press).
- 8 Neher, E. & Sakmann, B. *Nature* **260**, 799–802 (1976).
- 9 Brown, J. E. & Pinto, L. H. *J. Physiol., Lond.* **236**, 575–591 (1974).
- 10 Baylor, D. A. & Fuortes, M. G. F. *J. Physiol., Lond.* **207**, 77–92 (1970).
- 11 Katz, B. & Miledi, R. *J. Physiol., Lond.* **224**, 665–699 (1972).
- 12 Anderson, C. R. & Stevens, C. F. *J. Physiol., Lond.* **235**, 655–691 (1973).
- 13 Almers, W. & Levinson, S. R. *J. Physiol., Lond.* **247**, 483–509 (1975).
- 14 Conti, F., Hille, B., Neumcke, B., Nonner, W. & Stampfli, R. *J. Physiol., Lond.* **262**, 699–727 (1976).
- 15 Hladky, S. B. & Haydon, D. A. *Nature* **225**, 451–453 (1970).
- 16 Jagger, W. S. *Eur. J. Physiol.* **362** (suppl.), R47 (1976).

Transport mechanism operating between blood supply and osteocytes in long bones

WE show here that cyclic loading, as naturally occurring in long bones, produces a flow of liquids through canaliculi. The magnitude of such a stress-induced flow is so great that it significantly increases the efficiency of the transport mechanism operating between blood supply and osteocytes.

The present state of knowledge, according to the curricula in biology and medicine, describes the mechanism of supply of nutrients to Haversian bone cells (osteocytes) and disposal of the waste products from the cells by the process of diffusion^{1,2}. Diffusion is a very powerful mechanism in other areas of the body where, first, there is a short distance between the vessel and the

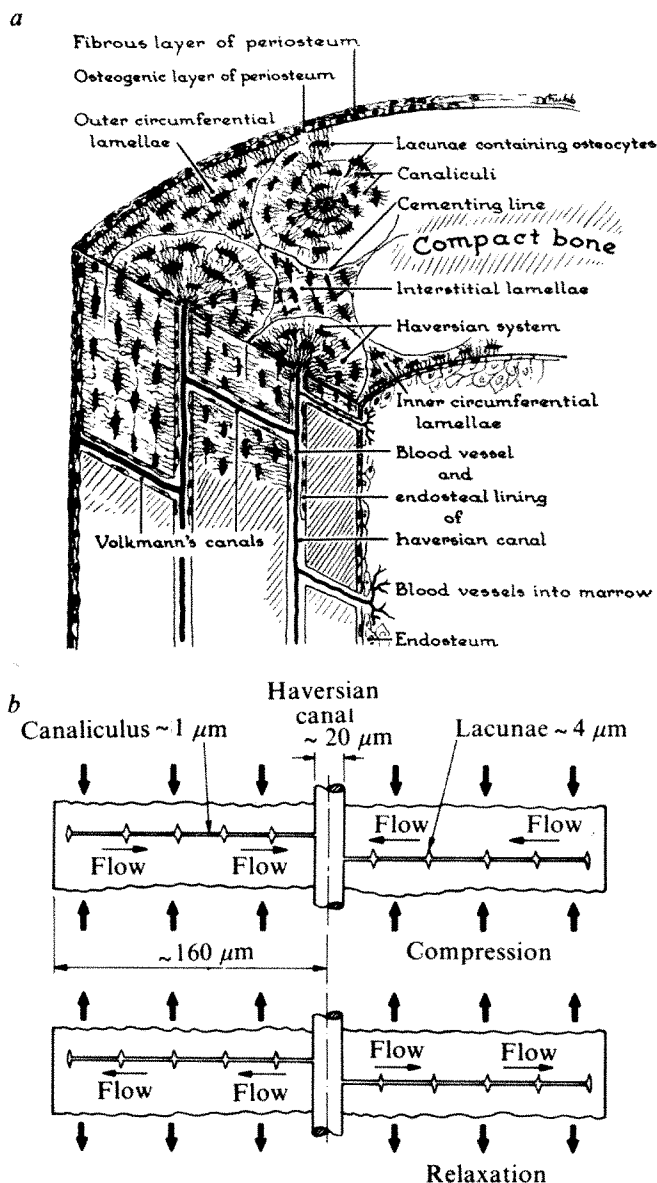
cell and, second, the cells are surrounded by fluid. The osteocytes of Haversian bone (Fig. 1a), however, are almost completely entombed in a calcified matrix except for the long and narrow canaliculi through which the nutrients and waste products must pass. Thus, diffusion is probably quite weak and it must be aided by another mechanism.

Assuming that bone is compressible and is loaded in compression, the volume of the canaliculus would decrease and thus pump the liquids out into the central vessel. After unloading, fresh nutrients will be drawn in from the Haversian canal into canaliculi (Fig. 1b). There is indeed much supporting evidence that cyclic loading of bone affects its mass; it grows thick and healthy. Immobilisation, bed rest or weightlessness, results in atrophy and in general loss of bone mass.

The proposed mechanism is examined here in greater detail. The lamellar structure of the Haversian system has been previously described and earlier studies established only that lamellae differ in the orientation of collagen fibres and perhaps in their mineral content. More observations were made recently by Vincent³, Ascenzi *et al.*⁴ and Boyde⁵. The pertinent information of these investigators may be summarised as follows.

Concentric cylinders of lamellae may differ from each other by their size, orientation of collagen fibres and ratio of mineral to

Fig. 1 a, Schematic representation of the microstructure of cortical bone¹. b, Simple model of stress-induced flow in the lacunar-canalicular system.



organic phases. Ascenzi *et al.*⁴ distinguished some of the lamellae as true lamellae, 5–7 μm wide, and some as interlamellar cementing zones, 1.5–2 μm wide. The latter displayed random orientation of collagen compared with the regular fibrous structures of lamellae.

Scanning electron micrograph of a fractured bovine bone treated with ethylene diamine, to remove the organic phase also revealed lamellar arrangements of phases in an osteon (Fig. 2). Similar observations were made on sectional specimens of bone treated with 95% hydrazine. It may reasonably be assumed that the empty spaces between the solid cylinders had a higher content of the organic phase, and any mineral phase present existed there in the classical form of discontinued crystallites which presumably dropped out on removal of the organic phase. Closer examination of the remaining mineral rings suggests a continuous polycrystalline structure (Fig. 3).

For the study of flow within the lacunar–canalicular network, organic material with suspended mineral was assumed to behave as a liquid, that is, capable of transmitting hydrostatic pressure. Thus, the Haversian system may be visualised as multiple concentric porous mineral cylinders separated by regions of liquid (Fig. 4). Such a structural arrangement can be easily analysed by a mathematical and physical model. It was also assumed that the pressure in the central cylinder would remain constant (equal to capillary blood pressure) and independent of flow through the canaliculi. The general concept and solution technique for axially loaded liquid-filled single and multiple concentric cylinders has been previously described in great detail (refs 6, 7).

After axial compressive loading of the model (Fig. 4), a hydrostatic pressure gradient is produced between the liquid regions. As flow occurs through the cylinders (canaliculi modelled as circular tubes), liquid on the inner side of the peak pressure moves radially inwards as in the simple model (Fig. 1b). Liquid on the outer side of the peak pressure, however, initially travels outwards and then, after $t = 0.0013$ s, inwards. Thus, in one-half of the loading cycle, the middle and outer areas of the model experience a two-way flow, whereas the inner areas only a one-way flow. This same pattern, opposite in direction, occurs after release of the load.

In both the simple and concentric cylinder models, it has been shown that cyclic stressing produces cyclic flows within the lacunar–canalicular network of the Haversian system. What remains to be shown is the order of magnitude of difference between the effect of the stress-induced flow and diffusion in transporting nutrients and waste products.

A set of calculations was performed for each liquid region to compare a common variable, the local displacement of a constant concentration, between a diffusion model and the stress-induced flow model for the period of 0.5 s, which approximates to one-half of a walking cycle, that is, a period of a normal loading of a long bone. The local displacement for the stress-induced flow model was calculated by assuming that the total volume change of that

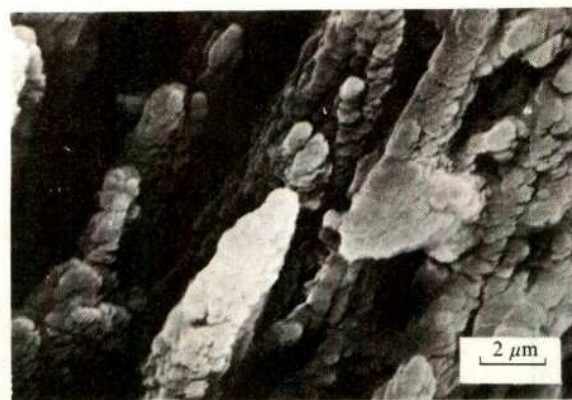


Fig. 3 Portion of a lamella of the osteon in Fig. 2 illustrating polycrystalline arrangement of the mineral phase.

region and its outer regions flowed through the canaliculi of that region.

For the diffusion model, a linear concentration gradient of the diffusing matter varying from 100% in the central liquid region to 10% in the outermost liquid region was assumed. One-dimensional diffusion in a semi-infinite medium was considered, that is

$$C = C_0 [1 - \text{erf}(x/2(DT)^{1/2})]$$

The estimate of the diffusion coefficient, $D = 10^8 \text{ cm}^2 \text{ s}^{-1}$, was based on the results of the *in vitro* bone diffusion experiments of Amprino⁸ and Lang *et al.*⁹. The local displacement for the diffusion model was the distance that the assumed local concentration moved in 0.5 s. The ratio of the local displacement of the stress model to that of the diffusion model as a function of radius is given in Table 1.

Table 1 Comparison of local displacement values for stress-induced flow and diffusion models

Liquid region	Radius (μm)	Ratio: induced flow/diffusion
2	16	3,700
3	23	1,140
4	30	490
5	37	230
6	44	110
7	51	46
8	58	12

Although no direct calculation can be made relating fluid movement and diffusion in the stress induced flow model, it is felt that diffusion would be aided significantly by increased mixing caused by the very large relative motion of liquids through both the rough-walled canaliculi and the lacunae.

In addition to this very basic comparison, an examination of the pattern of the flow in Fig. 4 indicates that the reversed directions of flow in canaliculi during compression and relaxation cycle would produce additional mixing of liquids, thus aiding further the mechanism of diffusion. It is also reasonable to assume considerable mixing to occur in each lacunae until diffused product would reach most outwardly located osteocytes.

The mechanism of the radial motion of molecules through the canaliculi and lacunae is completely unknown. The osmotic behaviour of cell membranes and processes surely plays an important part. An approximation of the canaliculus to a round tube is also inaccurate. An electron microscope examination of the wall of the canaliculus reveals uneven and porous surfaces¹⁰. Thus, there may be many other factors affecting diffusion processes between the blood vessels and bone cells. This investigation demonstrates the existence of one very powerful aiding mechanism

Fig. 2 Fractured surface of a bovine osteon deproteinised with ethylene diamine.



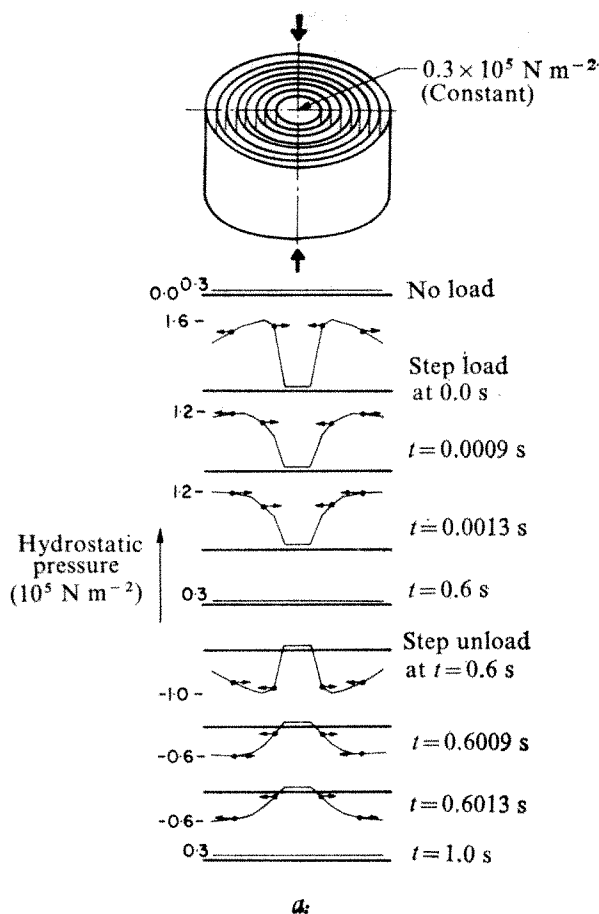


Fig. 4 Stress-induced flow in the lacunar-canalicular system modelled as a number of concentric cylinders separated by regions of liquid (detailed calculations in ref. 7).

which should not be neglected when considering transport of molecules through the canaliculi.

K. PIEKARSKI

University of Waterloo,
Department of Mechanical Engineering,
Waterloo, Ontario, Canada N2L 3G1

M. MUNRO

University of Cambridge,
Engineering Department,
Trumpington Road,
Cambridge, UK

Received 7 March; accepted 13 June 1977.

- ¹ Ham, A. W. *Histology* (Lippincott, Philadelphia, 1973).
- ² Warwick, R. & Williams, P. L. (eds). *Gray's Anatomy* (Longman, London, 1973).
- ³ Vincent, J. M. *Exp. Cell Res.* **12**, 422-424 (1957).
- ⁴ Ascani, A., Bonucci, E. & Bocciaelli, D. S. *J. Ultrastruct. Res.* **12**, 287-303 (1965).
- ⁵ Boyde, A. in *Biochemistry and Physiology of Bone* 1, (ed. Bourne, G. H.) 259-310 (Academic, New York and London, 1972).
- ⁶ Munro, M. & Piekarski, K. *Composites* **7**, No. 3, 195-199, (1976).
- ⁷ Munro, M. & Piekarski, K. *J. appl. Mech.* ASME Paper No. 77-APM-32, 1-4 (1977).
- ⁸ Amprino, R. *Experientia* **8**, 380-382 (1952).
- ⁹ Lang, S. B., Stipanich, N. & Soreni, E. A. in *Electrically Mediated Growth Mechanisms in Living Systems* 139-148 (N.Y. Acad. Sci., **238**, 1974).
- ¹⁰ Vose, G. P. *Anat. Record* **145**, 183-189 (1963).

Unstable protein mediated ultraviolet light resistance in *Anacystis nidulans*

CYANOBACTERIA are believed to have been precursors to eukaryotes during Precambrian evolution¹. It is also suggested that the oxygen evolved as a result of photosynthetic activity in these organisms, several of which are obligate photoautotrophs, was the major contributor towards the transition from anaerobic to aerobic atmosphere^{1,2}. It is, therefore, reasonable to expect cyanobacteria to have evolved in an environment with a relatively high flux of

solar ultraviolet light in the absence of ozone shield. This, presumably, could be possible with the evolution of efficient protective mechanisms or repair systems effective against damage by ultraviolet light. It is therefore not surprising that these organisms possess an extremely efficient photoreactivating repair system³. The presence of dark-repair in *Anacystis nidulans* has also been suggested by the isolation of mutants sensitive to ultraviolet light⁴. We present here physiological evidence for a dark-repair (or protective) system in this organism. A protein, unstable at least in the light, seems to be responsible for the resistance against lethal damage by ultraviolet light. This is observable in conditions of reduced photoreactivation achieved by a 24 h post-irradiation dark incubation, referred to here as 'dark-survival' following Asato, who showed that well within this period photoreactivability of damage by ultraviolet light is almost completely lost⁴.

Strain BD-1 *A. nidulans* was cultured and plated in synthetic medium, C-Mn, as described by Van Baalen⁵. The cells were grown in light (200 foot-candle (fc)) from tungsten lamps, at 35 °C in a shaker. Exponentially grown cells were used for all the experiments. The plates were incubated in the presence of 200 ± 10 fc of light from tungsten lamps and were maintained at 36 ± 1 °C in an incubator. After 5-6 d of growth the colony counts were taken.

Incubation of cells with chloramphenicol before exposure to ultraviolet light sharply reduced their dark-survival (Fig. 1). Almost the maximum reduction in the dark-survival at

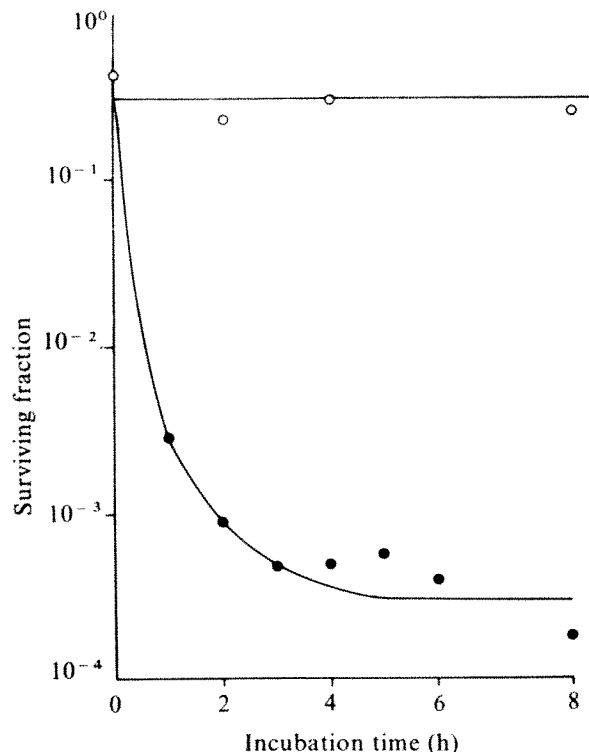


Fig. 1 Kinetics of survival by *A. nidulans* on exposure to ultraviolet light during incubation in light in the presence and absence of chloramphenicol. Exponential phase cultures of strain *A. nidulans* BD-1 at a concentration of 10⁷-10⁸ cells per ml without further washing were divided into two fractions. In one fraction chloramphenicol was added to a final concentration of 5 µg ml⁻¹ (●). The other fraction contained no chloramphenicol (○). Both the fractions were incubated at 36 ± 0.5 °C in a water bath shaker. Light from tungsten lamps was incident at an intensity of 200 fc. At indicated time after the addition of chloramphenicol samples were diluted in the growth medium at 35 °C and spread on synthetic agar plates prewarmed to the same temperature. Plates with cells were exposed to 165 J m⁻² of ultraviolet light (254 nm) from Philips TUV15W germicidal lamp at an intensity of 11 J m⁻² s⁻¹. The control and ultraviolet light exposed cells were kept in the dark for 24 h at 35 °C. After the dark-incubation all the plates were transferred to light (200 ± 10 fc) at 35 ± 1 °C. After incubation in light for 5-6 d the colony counts were taken.

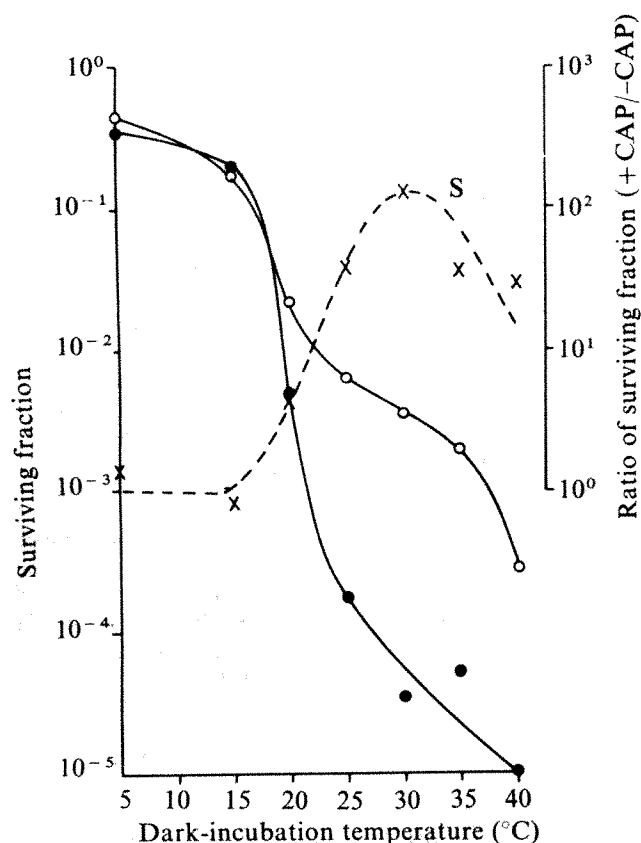


Fig. 2 Survival of *A. nidulans* on exposure to ultraviolet light when preincubated in visible light in the presence and absence of chloramphenicol (CAP), as function of post-irradiation dark-incubation temperature. Exponentially growing cells without further washing were incubated in light (200 fc) without (○) and with $5 \mu\text{g ml}^{-1}$ of chloramphenicol (●) for 3 h at $36 \pm 0.5^\circ\text{C}$ in water bath shaker. Control and treated cells were washed and resuspended in fresh growth medium at 35°C and 10 ml suspensions of each were immediately exposed to 330 J m^{-2} ultraviolet light. 1-ml aliquots of control and irradiated cells were transferred to thermally equilibrated tubes at indicated temperatures in the dark. After 24 h of dark-incubation at the respective temperatures the cells were diluted, plated and grown for colony counts as described in the legend to Fig. 1. The ratio of surviving fraction of untreated to chloramphenicol treated cells at the indicated temperatures is also shown (x).

35°C was achieved in about 3 h of chloramphenicol treatment. The extent of the dark-survival also depends on the post-irradiation temperature (Fig. 2). This dark-survival was significantly lower in the cells pretreated with chloramphenicol in the temperature range $20\text{--}40^\circ\text{C}$. The ratio of the surviving fractions of the untreated to the chloramphenicol treated cells was a function of the temperature of the dark-incubation, with a maximum at about 30°C . These results strongly suggest that some protein(s) normally present in the cell before the ultraviolet light exposure is involved in the dark-survival. The cells preincubated with chloramphenicol for 3 h or more are deficient in this protein suggesting its unstable nature. The higher survival at lower temperatures in Fig. 2 may be due to the arrest of (enzymatic) reactions leading to the loss of photoreversibility of the lesions which on subsequent incubation for growth in light may be removed by photoreactivation. When the chloramphenicol treated cells were exposed to photoreactivating light after ultraviolet light irradiation, their survival was comparable to untreated cells showing that the above protein(s) is not the photoreactivating enzyme. Incubation in the dark before ultraviolet light exposure is known to increase the dark-survival of this organism⁶. Chloramphenicol treated cells also showed a similar resistance when incubated in dark before ultraviolet light exposure. This unstable protein, therefore, may not have

a significant role in the development of the resistance of cells to ultraviolet light on preincubation in the dark.

The repair protein(s) described here is unstable in as much as the associated repair capacity is considerably reduced by the preirradiation treatment with chloramphenicol. In contrast, the same treatment does not measurably affect the photoreactivating capacity. A previous attempt to detect the presence of an unstable repair protein in yeast was unsuccessful⁷; in yeast pretreatment with nuclear specific protein synthesis inhibitor did not affect the liquid holding recovery⁷. In *Escherichia coli* an inducible repair protein synthesised after exposure of the cells to ultraviolet light has been detected⁸⁻¹⁰.

It is not clear whether this unstable protein has a role in unirradiated cells of *A. nidulans*. This organism may possess a constitutive or a visible light inducible repair pathway involving a protein which is independent of light for its action. Such a system could have a very important role in ensuring survival in an environment where photoreactivating system could not meet the challenge by a relatively high intensity of ultraviolet light. Regulation of repair processes by visible light has been suggested for eukaryotes^{11,12}. No specific mechanism is known.

I thank Dr S. K. Mahajan for critically reading the manuscript, and K. A. V. David, N. D. Shirke and G. Bhattacharjee for their cooperation.

S. K. BHATTACHARJEE

Biology and Agriculture Division,
Bhabha Atomic Research Centre,
Bombay 400 085, India

Received 5 April; accepted 7 July 1977.

- Schoff, J. W. *Biol. Rev.* **45**, 319-352 (1970).
- Margulis, L., Walder, J. C. G. & Rambler, M. *Nature* **264**, 620-624 (1976).
- Van Baalen, C. *Pl. Physiol.* **43**, 329-333 (1968).
- Asato, Y. *J. Bact.* **110**, 1058-1064 (1972).
- Van Baalen, C. *J. Phycol.* **3**, 154-157 (1967).
- Bhattacharjee, S. K. & David, K. A. V. *Nature* **265**, 183-184 (1977).
- Heude, M., Chanet, R. & Moustachi, E. *Mutat. Res.* **28**, 37-45 (1975).
- Witkin, E. M. *Genetics* **79**, 199-213 (1975).
- Sedgwick, S. G. *Nature* **255**, 349-350 (1975).
- McEntee, K., Hesse, J. E. & Epstein, W. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3979-3983 (1976).
- Chatterjee, S. & Bhattacharjee, S. K. *Nature* **259**, 676-677 (1976).
- Murphy, T. M. & Gordon, M. P. *Photochem. Photobiol.* **13**, 45-55 (1971).

Detection of casein messenger RNA in hormone-dependent mammary cancer by molecular hybridisation

THE growth and development of the normal mammary gland are regulated by the complex interaction of both peptide and steroid hormones¹. Whereas some breast cancers retain the hormone-dependent characteristics of normal mammary tissue^{2,3}, other tumours lose this responsiveness and grow autonomously. Following the pioneering work of Huggins⁴ on the hormonal dependence of both experimental and human breast cancer, investigators seeking a prognostic test have studied the relationship of specific oestrogen receptors to mammary cancer^{5,6} to the response of the tumour to endocrine ablation^{7,8}, and to the levels of other steroid⁹ and peptide hormone receptors^{10,11}. A superior marker for hormone responsiveness would, however, be a measurable product of hormone action rather than the initial binding interaction. In the mammary gland, casein synthesis has been used as a specific biochemical marker of differentiated function and hormone responsiveness. In the studies reported here, a specific complementary DNA copy (cDNA) of rat casein mRNA has been utilised to study the effects of hormones on the expression of differentiated function in chemical carcinogen-induced mammary adenocarcinomas. It is shown that casein mRNA as detected by cDNA may be a useful indicator of hormone dependence in experimental breast cancer. The cDNA probe was synthesised using as a template a 15S casein mRNA fraction

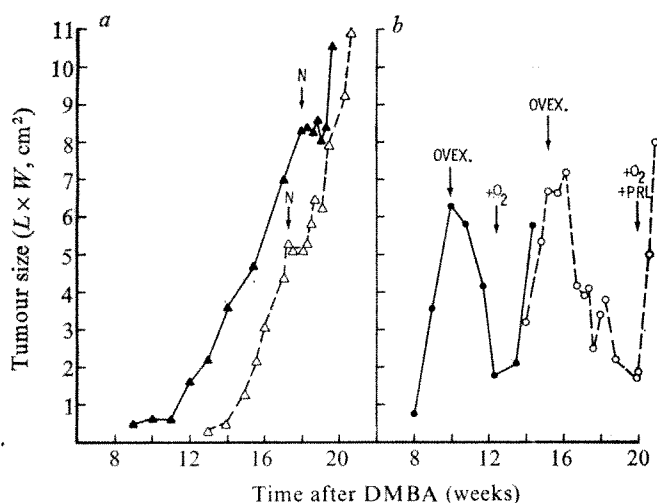


Fig. 1 Effect of hormonal treatment on the growth of DMBA-induced rat mammary carcinomas. Mammary adenocarcinomas were induced in 50-d-old female Sprague-Dawley rats by a single intubation of 20 mg DMBA. Tumours usually appeared 8–14 weeks after the administration of the carcinogen. *a*, Hormone dependence was assessed without ovariectomy by the single injection of an anti-oestrogen, nafoxidine 200 μ g s.c., given at the time indicated by the arrow. *b*, Hormone dependence of tumours was also determined by measuring the response to ovariectomy and the administration of hormones, either oestradiol benzoate (10 μ g every 3 d s.c. in sesame oil, ●), or oestradiol benzoate and prolactin (ovine, 35 IU mg⁻¹, (NIH-P-S12) 1 mg daily i.p., ○). Arrows indicate the time of ovariectomy (OVEX) followed by hormone administration.

purified 180-fold from a lactating RNA extract by chromatography on dT-cellulose and several precise sizing techniques^{12,13}. The template mRNA fraction represented greater than 90% casein mRNA, as estimated by specific immunoprecipitation of the total protein synthesised in the wheat germ assay, and by a careful analysis of its kinetics of hybridisation with the cDNA probe¹³. The specificity of the complementary DNA copy of the 15S casein mRNA has been demonstrated previously¹³. This cDNA probe

selectively hybridised to RNA from lactating tissue but not to rat liver poly(A)-containing RNA and the resulting hybrid displayed a high T_m characteristic of a well base-paired duplex. In addition, the rate of hybridisation of the casein-specific cDNA to various RNA preparations was directly related to the casein mRNA activity of these same preparations determined by a cell-free translation assay.

In these studies, 7,12-dimethylbenz(a)anthracene (DMBA)-induced rat mammary carcinomas were utilised as a model for hormone-dependent breast cancer^{3,4}. At least 80% of these tumours have been reported to respond to endocrine ablation and the levels of both oestradiol and prolactin receptors have been characterised previously¹¹. In addition, a limited number of autonomous mammary tumours induced in BALB/c mice have been screened for the presence of casein mRNA sequences. The amount of casein mRNA was quantified by analysing the rate of hybridisation of the complementary DNA with total RNA extracts of lactating and tumour tissue.

The characteristic growth response of the DMBA-induced rat mammary tumours to either ovariectomy and hormone administration or anti-oestrogen treatment are shown in Fig. 1. Ovariectomy resulted in a marked reduction in tumour size (Fig. 1*b*). Following either oestradiol administration or a combination of oestradiol and prolactin treatment, rapid growth was re-initiated. A single injection of nafoxidine, an oestrogen antagonist, also prevented the growth of hormone-dependent tumours for approximately 1 week without ovariectomy. (Fig. 1*a*). Following the return to the previous growth rate in the presence of the endogenous hormonal milieu, tumours were then removed and frozen before RNA extraction.

The hybridisation of the casein-specific cDNA to purified casein mRNA, RNA isolated from lactating tissue, and RNA isolated from some of the various hormone-dependent tumours is illustrated in Fig. 2. The 15S casein mRNA displayed a rate of hybridisation consistent with its high purity, and hybridised approximately 166 times faster than a total RNA preparation from 8-d lactating tissue. Both of these reactions occurred with the expected pseudo-first-order kinetics and proceeded to greater than 90% completion. Both the rates and extents of hybridisation of the

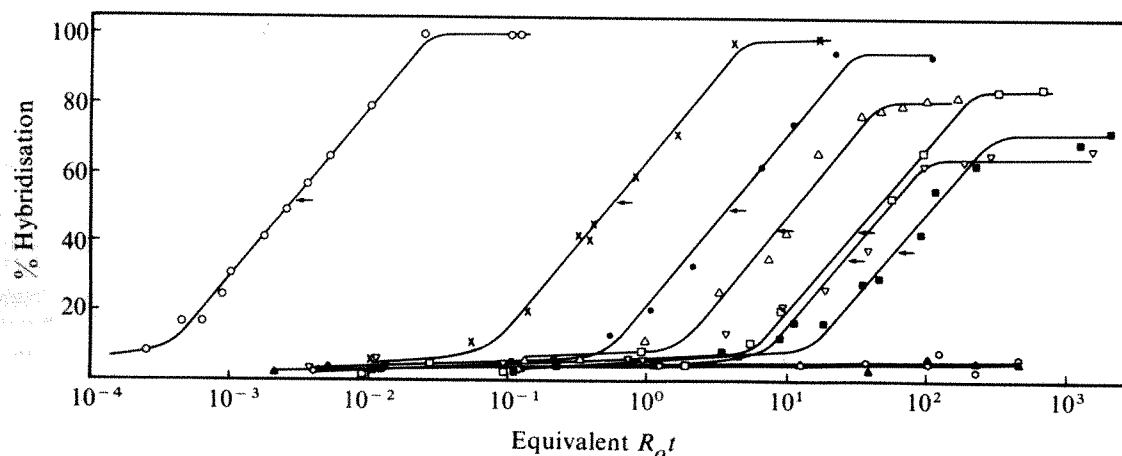


Fig. 2 Detection of casein mRNA sequences in hormone-dependent rat mammary carcinomas by molecular hybridisation. Tumours were excised, necrotic tissue removed and the remaining tissue rapidly frozen in liquid N₂. The isolation of total cellular RNA by the direct homogenisation of pulverised, frozen tumour tissue in a phenol-SDS solution at pH 8.0 has been described previously¹². Before hybridisation all RNA extracts were treated with Proteinase K (25 μ g ml⁻¹ at 37 °C for 10 min) followed by a final phenol-CHCl₃ extraction at pH 8.0. RNA excess hybridisations were carried out in 0.6 M NaCl, 0.01 M HEPES, pH 7.0, 0.002 M Na₂EDTA at 68 °C, and the extent of hybridisation was determined by S₁ nuclease as described previously¹³. The data are expressed as the percentage hybridisation compared with the log equivalent R_0t , the concentration of RNA (mol l⁻¹) times the time (s). The rates of hybridisation have been adjusted to the standard conditions of 0.18 M Na⁺ at 62 °C. The amount of DNA in the total nucleic acid extracts was determined by a standard diphenylamine assay and the rate of hybridisation adjusted accordingly. DNA did not contribute to the extent of hybridisation observed in any of the tumours at the R_0t values assayed¹³. The following typical hybridisation curves are presented in this figure. Hybridisation of ³H-cDNA with purified 15S rat casein mRNA (○), RNA isolated from 8-d lactating mammary tissue (×), RNA isolated from DMBA-induced tumours treated with oestradiol + prolactin following ovariectomy (●, △, ▽); treated with oestradiol alone following ovariectomy (□, ▲); and from tumours growing in a rat previously treated with nafoxidine (○, ▽).

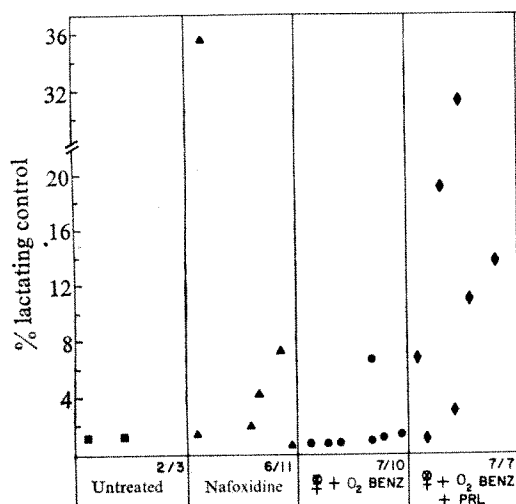


Fig. 3 Quantification of casein mRNA levels in hormone-dependent DMBA-induced rat mammary carcinomas. A total of 31 DMBA-induced tumours were assayed for the presence of casein mRNA by molecular hybridisation as described in Fig. 2. The tumours were divided into four groups based on the treatment received (see Fig. 1). The data are expressed as a percentage of an 8-d lactating RNA preparation, which was run as a control during each group of hybridisations. The lactating RNA contained approximately 0.52% of the total cellular RNA as casein mRNA, equivalent to approximately 80,000 molecules of casein mRNA per alveolar cell¹³. The number of positive tumours containing casein mRNA divided by the total number of tumours assayed in each treatment group is given as a fraction beneath each group.

tumour RNA preparations were less than those observed for the RNA preparation from lactating tissue. The maximum hybridisation observed at saturation or achieved by a R_{0t} (the concentration of RNA, mol l^{-1} , times the time, s) value of 1,000 for the casein mRNA containing tumour RNA extracts ranged from 25–100%. Any RNA extracts which displayed less than 20% hybridisation by a R_{0t} value of 1,000 were considered not to contain significant amounts of casein mRNA.

The data obtained from these hybridisation experiments are summarised in Fig. 3. Significant hybridisation was observed for 22 out of 31 (approximately 70%) of the RNA preparations isolated from the hormone-dependent tumours. The levels of casein mRNA found in the hormone-dependent tumour RNA preparations ranged from 0–36%

of those found in the 8-d lactating RNA preparation, which had been estimated previously to contain as many as 80,000 molecules of casein mRNA per alveolar cell¹³. The highest levels of casein mRNA were observed in tumours of ovariectomised rats treated with both oestradiol and prolactin and the lowest in tumours growing under endogenous hormonal stimulation. The amount of casein mRNA present in tumours of animals treated with both oestradiol and prolactin was also greater than the level found in animals treated with oestradiol alone. This result is consistent with the role of prolactin in the induction of casein synthesis and casein mRNA in normal mammary tissue¹⁴. A wide range was observed, however, in casein mRNA sequence levels in the different primary DMBA-induced tumours even within a given treatment group. A similar variability in the levels of prolactin and oestradiol receptors has also been reported in these DMBA-induced tumours¹¹. This variability is most likely explained by the heterogeneity of cell types that exists within these tumours. Some cells may contain prolactin receptors and display the normal hormonal response, whereas others may either lack prolactin-binding sites or display an altered response. Two such distinct populations of cells have actually been identified in DMBA-induced mammary tumours by autoradiographic localisation of prolactin receptors using ¹²⁵I-prolactin¹⁵.

Whereas the majority of the DMBA-induced mammary adenocarcinomas in Sprague-Dawley rats are dependent on hormones for growth, those which appear in BALB/c mice are characteristically hormone-independent. The molecular basis for this autonomy and the difference in response of related species to the same chemical carcinogen are not well understood. Several transplantable mouse mammary tumour lines were also screened for the presence of casein mRNA sequences. Because of the previously reported sequence divergence of mouse and rat casein mRNAs¹³, a homologous cDNA probe was synthesised using a purified 15S mouse casein mRNA fraction as the template for avian myeloblastosis virus RNA-directed DNA polymerase. This cDNA probe displayed similar hybridisation kinetics with the purified mouse 15S casein mRNA and a total RNA preparation obtained from lactating mice as previously observed for the rat cDNA and mRNA fractions (Fig. 4). In addition, casein mRNA sequences were detected in RNA extracts obtained from 14-d pregnant mouse mammary tissue at levels less than one-fifth those observed during lactation, illustrating the sensitivity of the hybridisation assay. In none of the three autonomous mouse mammary tumour RNA samples tested, however, was any significant hybridisation detected. This assay is capable of detecting

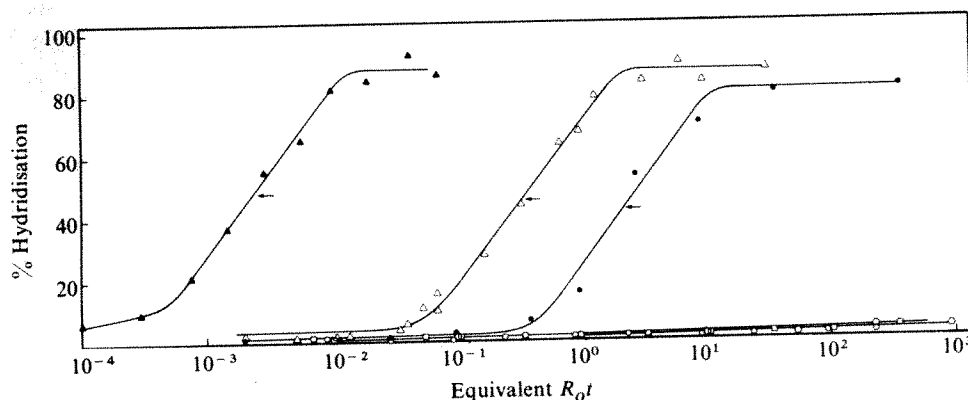


Fig. 4 Measurement of casein mRNA sequences in hormone-dependent mouse mammary tumours. The mouse mammary tumours used in these studies were transplantable lines originally induced by DMBA administration to BALB/c mice. These tumours grew equally well in intact female or ovariectomised animals. Hybridisation of mouse ³H-cDNA with purified mouse 15S casein mRNA (▲), RNA isolated from a 10-d lactating mouse (Δ), RNA isolated from a 14-d pregnant mouse (●) and RNA isolated from three different transplantable lines of mouse mammary carcinomas induced with DMBA (○, ◇, □).

casein mRNA sequences at levels of less than at least 0.1% of the normal lactating gland. Thus, these three tumours seem to represent variants, which have lost the hormonal responsiveness characteristic of the normal mammary gland and the accompanying expression of differentiated function.

The absence of casein mRNA sequences in the hormone-dependent mouse mammary carcinomas is consistent with the autonomous growth of these tumours. The failure to synthesise casein mRNA in response to endogenous hormones may reflect the absence or low levels of oestradiol and prolactin receptors in these cells¹¹. A more thorough examination of other tumour variants, especially receptor-positive cells which are unable to synthesise casein, may help elucidate the mechanisms regulating casein synthesis. A larger selection of both hormone-independent and dependent rat and mouse mammary tumours should be screened before a definitive correlation between the presence of casein mRNA sequences and hormone independence of mammary tumours can be ascertained. The detection of casein mRNA in hormone-dependent rat mammary carcinomas in this study, however, is consistent with the previously reported expression of normal, differentiated function in both experimental^{16,17} and human breast cancer¹⁸⁻²⁰. In addition, prolactin-inducible casein mRNA activity has also been detected recently in experimental breast cancer using a cell-free translation assay²¹. In conclusion, the data presented in this communication suggest that casein mRNA may be a useful molecular marker for determining hormone dependence, in addition to the presence of steroid and peptide hormone receptors.

We thank Mr David Cooke, Ms Carol Waugh and Ms Gail Brady for assistance. Drs William McGuire and Daniel Medina kindly provided some of the DMBA-induced rat and mouse mammary carcinomas, and some of the nafoxidine-treated animals were provided through the courtesy of Ms Cheryl Watson and Dr James Clark.

JEFFREY M. ROSEN
SUSAN H. SOCHER

Department of Cell Biology,
Baylor College of Medicine,
Houston, Texas 77030

Received 19 April; accepted 16 June 1977.

- ¹ Topper, Y. J. *Rec. Progr. Hormone Res.* **26**, 287-308 (1970).
- ² Smithline, F., Sherman L. & Kolodny, H. D. *New Engl. J. Med.* **292**, 784-792 (1975).
- ³ McGuire, W. L., Chamness, G. C., Costlow, M. E. & Shepherd, R. E. *Metabolism* **23**, 75-100 (1974).
- ⁴ Huggins, C. & Bergenstal, D. M. *Cancer Res.* **12**, 134-141 (1952).
- ⁵ King, R. J. B., Cowan, D. M. & Irman, D. R. *J. Endocrin.* **32**, 83-90 (1965).
- ⁶ McGuire, W. L. & Julian, J. A. *Cancer Res.* **31**, 1440-1445 (1971).
- ⁷ Jensen, E. V. *Cancer Res.* **35**, 3362-3364 (1975).
- ⁸ McGuire, W. L., Carbone, P. P. & Vollmer, E. P. *Estrogen Receptors in Human Breast Cancer* (Raven, New York, 1975).
- ⁹ Horwitz, K. B., McGuire, W. L., Pearson, O. H. & Segaloff, A. *Science* **189**, 726-728 (1975).
- ¹⁰ Turkington, R. W. *Cancer Res.* **34**, 758-763 (1974).
- ¹¹ DeSombre, E. R., Kledzik, G., Marshall, S. & Meites, J. *Cancer Res.* **36**, 354-358 (1976).
- ¹² Rosen, J. M. *Biochemistry* **15**, 5263-5271 (1976).
- ¹³ Rosen, J. M. & Barker, S. W. *Biochemistry* **15**, 5272-5279 (1976).
- ¹⁴ Houdebine, L. M. & Gaye, P. *Molec. cell. Endocrin.* **3**, 37-55 (1975).
- ¹⁵ Costlow, M. E. & McGuire, W. L. *J. natn. Cancer Inst.* (in the press).
- ¹⁶ Archer, F. L. *J. natn. Cancer Inst.* **42**, 347-362 (1969).
- ¹⁷ Hilf, R. *Science* **155**, 826-827 (1967).
- ¹⁸ Young, S., Pang, L. S. C. & Goldsmith, I. *J. clin. Path.* **27**, 94-102 (1974).
- ¹⁹ Bussolati, G., Pich, A. & Alfani, V. *Virchows Arch. Anat. Hist.* **365**, 15-21 (1975).
- ²⁰ Ross, H. N. & McGrath, C. M. *Science* **190**, 673-675 (1975).
- ²¹ Nardacci, N. J. & McGuire, W. L. *Cancer Res.* **37**, 1186-1190 (1977).

A theory for genetic regulation by chromosome folding

This theory to explain gene expression in higher organisms is based on the spatial re-arrangement of genes by directed, specific folding of the chromosome. Tissue-specific differences in genome structure exist¹ and profound modulation of genome structure can occur during differentiation². It is postulated that specific proteins recognise specific sequences in double-stranded repetitive DNA and that, as a consequence, the structural genes encompassed by these repetitive sequences become accessible

to the transcribing proteins. Transcription and translation then result in synthesis of proteins characteristic of the particular differentiated state. The interactions of metals and other ions with repetitive DNA (ref. 3) may be important in this recognition.

The model is further described in the following example where S_1 , S_2 and S_3 are structural genes specifying proteins P_1 , P_2 and P_3 and R_1 , R_2 and R_3 are repetitive sequences. Suppose that a sequence of DNA is

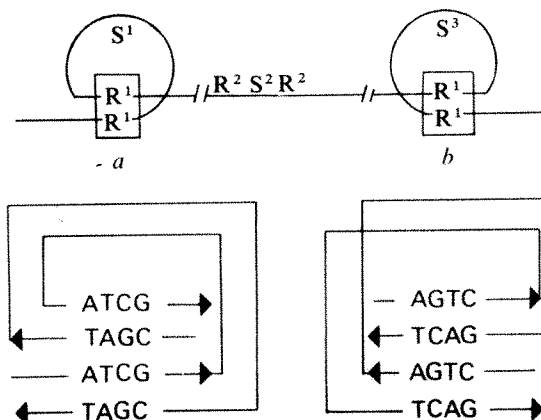
$$R_1 S_1 R_1 \dots R_2 S_2 R_2 \dots R_3 S_3 R_1$$

and that P_3 is a tissue-specific recognition protein that initiates and stabilises the association of double-stranded repetitive sequences R_1 with each other. These R_1 sequences need not be identical, but if they are identical they must be orientated in opposite directions (Fig. 1). This results in a localised distortion and/or unwinding of the DNA double helices (perhaps through the formation of a localised four-stranded structure) which is transmitted to the encompassed DNA, thus loosening the 'nucleosome' structure and making the DNA available for transcription. Thus both S_1 and S_3 , but not S_2 , are transcribed and translated giving proteins P_1 and P_3 characteristic of the particular tissue. The presence of protein P_3 will maintain the particular differentiated state. Such an arrangement of repetitive and unique DNA is consistent with the data (but not the analysis) of Davidson *et al.*⁴

Since most proteins are synthesised in more than one tissue, it is necessary to postulate that a structural gene may have more than one associated repetitive sequence. This will result in a continuum of repetition frequency classes, the frequency of each is dependent on the number of different cell types in which its associated structural gene is expressed. For example, $R_1 R_3 S_1 R_1 R_3 \dots R_2 R_3 S_2 R_2 R_3 \dots R_1 S_3 R_1$. Thus, in different tissues the genes expressed are: (a) S_1 and S_2 ; (b) S_2 ; (c) S_1 and S_3 . Such an arrangement of repetitive and unique sequences is suggested by Cech and Hearst⁵. Where several sequential genes are coordinately expressed in a given tissue, the group will be encompassed by the appropriate repetitive elements.

Development of the differentiated state can also be explained. For example, suppose cell a differentiates to give cell b which gives rise to the mature cell c. The genome of cell a is stabilised in its specific conformation by protein X and produces proteins A, B and C. At cell division X is broken down and nuclear reprogramming occurs⁶. Protein C thus has access to the genome and stabilises it in the conformation characteristic of cell b, which produces proteins D, E and F. At the next reprogramming, protein F has access to the genome and stabilises it in the conformation characteristic of cell c which produces proteins F, G and H. The continuing presence of F ensures the maintenance of the differentiated state.

Fig. 1 Interaction of tissue-specific recognition protein P_3 stabilises the association of double-repetitive sequences R_1 with each other. a, Identical R_1 sequences; b, non-identical R_1 sequences. As a result, structural genes S_1 and S_3 , but not S_2 , are made available for transcription.



Such transcription active regions may be equivalent to the interbands of dipteran polytene chromosomes⁷ and the 'loops' observed in lampbrush chromosomes⁸. Such structures are dynamic in that they show developmental and tissue-specific differences in size and configuration⁹.

I thank Dr K. G. H. Dyke for criticism of the manuscript.

STEVEN VAUGHAN

Wadham College,
Oxford, UK

Received 27 January; accepted 7 July 1977.

¹ Baserga, R. *et al.* *Structure and Function of Chromatin*, Ciba Symposium (New Series) 28, 269–279 (Elsevier/North Holland, Amsterdam, 1975).

² Friend, C. *et al.* *Exptl Cell. Res.* 99, 301–309 (1976).

³ Sissoëff, I. *et al.* *Progr. Biophys. molec. Biol.* 31, 165–199 (1976).

⁴ Davidson, E. H. *et al.* *Cell* 4, 217–238 (1975).

⁵ Cech, T. R. & Hearst, J. E. *J. molec. Biol.* 100, 227–256 (1976).

⁶ Gurdon, J. B. & Woodland, H. R. *Curr. Top. devl Biol.* 5, 39–70 (1970).

⁷ Crick, F. *Nature* 234, 25–27 (1971).

⁸ Scheer, U. *et al.* *J. Cell Sci.* 22, 503–520 (1976).

⁹ Lima-de Faria, A. *Hereditas* 81, 249–284 (1975).

Water binding by antifreeze glycoproteins from Antarctic fish

Low molecular weight glycoproteins isolated from serum of Antarctic fish have been shown¹ to be responsible for freezing point depression which permits survival at temperatures down to -1.9°C . The principal active antifreeze glycoprotein (AFGP) of several Antarctic species consists of a repeating tripeptide Ala-Thr-Ala with a disaccharide galactosyl-*N*-acetylgalactosamine linked to the threonine residue^{2–4}. The AFGP is more effective in freezing-point depression on a weight basis than NaCl and several hundredfold more effective than ordinary proteins². Melting point, however, is virtually unaffected by the AFGP⁵. We have investigated the possible role of protein-bound water in the unusual antifreeze activity of the AFGP. Ordinary proteins such as bovine serum albumin, haemoglobin, and lysozyme show hydration levels of about 0.4 g H₂O per g protein⁶. This water does not freeze at temperatures down to -35°C , but is sufficiently mobile to produce a measurable proton magnetic resonance signal. Quantitative determination of the amount of this 'bound' water is therefore possible in frozen protein solutions⁷. We have used this technique to examine water binding by the AFGP over a range of temperatures below the freezing point of bulk water.

Antifreeze glycoproteins 1–6 (ref. 2) were isolated from *Dissostichus mawsoni* (Norman) at McMurdo Sound Station, Antarctica and purified by DE-22 cellulose ion-exchange chromatography. The product was dialysed exhaustively against distilled water and lyophilised. Samples for magnetic resonance were prepared in 0.01 M KCl at concentrations of 25–100 mg ml⁻¹ determined by weight and ultraviolet absorbancy. Solutions of standard globular proteins, bovine serum albumin (Schwarz-Mann, crystalline, 100%) and human oxyhaemoglobin (gift from R. Banerjee) were prepared similarly. Nuclear magnetic resonance (NMR) spectra were recorded on a Perkin-Elmer R12 spectrometer operating at 60 MHz. No accumulation procedures were necessary. The samples in commercial NMR tubes were cooled from room temperature in 5° steps to -5°C . At this temperature solvent alone froze and the proton resonance signal disappeared completely. In the case of the protein solutions which supercooled, bulk water freezing was induced by lowering the instrument temperature control to -20°C until the first indication of loss of signal was observed (about 2 min). The control was returned to -5°C and signals were followed until equilibrium (constant peak area and line width) was achieved. The time dependency of the signal response was followed with each subsequent change of temperature in 5° increments to -40°C (cooling curve) and in the return to the melting temperature of the solution (warming curve). Peak area stabilised in about 15 min, line width

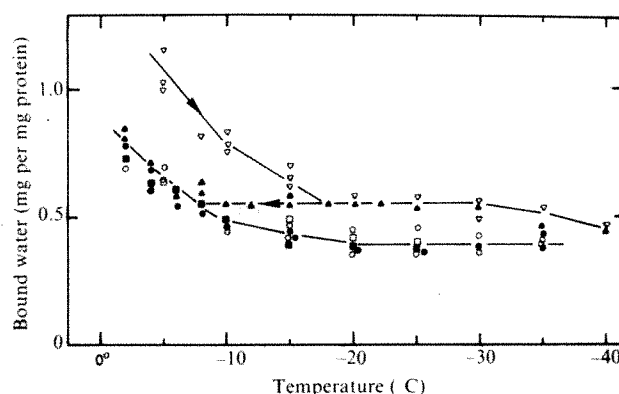


Fig. 1 Amounts of 'bound' (unfrozen) water in protein solutions at temperatures below the freezing point of bulk water. Open symbols represent data taken during cooling from -5°C ; closed symbols are data collected during warming. ∇ , Δ , AFGP; \circ , \bullet , bovine serum albumin; \square , \blacksquare , human haemoglobin.

in about 30 min, after a temperature change. The reported data were obtained after equilibration for 60 min; in these conditions line widths for cooling and warming coincided except in the case of the AFGP above -10°C . Line width values represent the width at half-height of the absorption curves. Areas were calculated as the product of line width and height and were converted to absolute values for hydration by comparison with a standard water sample (4.5 M LiCl, 0.01 M MnCl₂)⁷.

Figure 1 presents hydration results for the three proteins studied during cooling from -5 to -35 or -40°C and subsequent warming to -2°C . Amounts of unfrozen water were directly proportional to protein concentration in the range studied. In the case of the two globular proteins identical results were obtained during cooling and warming. At temperatures of -20 to -35°C the hydration averaged 0.40 g per g protein, in agreement with previous studies^{8–9}. In the AFGP, hydration at the low temperatures is 0.55 g per g protein; above -15°C hysteresis in the temperature dependency is observed. During cooling, hydration values as high as 1.1 g per g protein are observed, whereas with warming values of about 0.6 g per g are found, as in the two protein standards.

Line width data show the expected exponential increase with decreasing temperature (Fig. 2). Arrhenius activation energies are 4.0, 6.5 and 7.7 kcal mol⁻¹ for the AFGP, haemoglobin and serum albumin, respectively. The results for the two standard proteins are thus in good agreement with previous values for activation enthalpies of protein-bound water: 7.3 kcal mol⁻¹ for haemoglobin (from dielectric relaxation)¹⁰ and 5.0 kcal mol⁻¹ for BSA (from proton resonance in frozen solutions)⁸. The result obtained for the antifreeze glycoprotein falls within the range observed for polysaccharide hydration, 3.6–4.8 kcal mol⁻¹ (ref. 11). Estimated activation entropies^{8,9} in this study are -9 e.u. for AFGP, $+4$ e.u. for haemoglobin, and $+9$ e.u. for bovine serum albumin. Comparison of results for the three proteins suggests that the higher level of hydration in the AFGP is associated with somewhat weaker binding, presumably fewer hydrogen bonds, and a less structured environment.

Relaxation behaviour in the frozen AFGP solutions during cooling was suggestive of a two-phase system with slow exchange¹². Thus, at -5°C a sharp resonance of line width about 100 Hz seemed to be superimposed on a broad band of width 600 Hz (Fig. 3), merging at lower temperatures. Both were distinguishable from bulk water which, before its freezing by means of the cold pulse, showed a line width less than 10 Hz. The additional component of unfrozen water in the AFGP apparently represents a category intermediate between Type I (bulk

water) and Type II (bound water)⁹. In quantitative terms the 100 mg ml⁻¹ AFGP solution at -5 °C contains about 90% Type I water, 5% Type II water, and 5% additional unfrozen water. Type III, irrotationally bound water⁹, is not measurable by this technique, but is anticipated to be less than 0.1%.

The level of Type II water in the AFGP seems to be consistent with its chemical composition. On the basis of hydration values of 1.5 H₂O/alanine residue and 2 H₂O/threonine residue¹³, hydration of 0.37 g per g protein would be predicted for the copolymer (Ala-Ala-Thr)_n. Since the polypeptide accounts for 39% of AFGP weight², it would therefore contribute 0.15 g H₂O per g AFGP to the total hydration. Hydration of the sugar portion of the molecule would have to be 0.66 g per g carbohydrate to account for the remaining AFGP hydration. This figure is similar to the value of 0.59 g per g reported for Agarose¹¹. The magnitude of the line width for the Type II water in the AFGP is consistent with an unstructured conformation of the molecule, as shown by circular dichroism² and confirmed for the samples used in this study. Signal broadening has been observed in urea-denatured bovine serum albumin⁹ and in random-coil polypeptides⁹.

Of particular interest is the possible significance of either the Type II water or the additional unfrozen water in the AFGP relative to freezing-point depression. AFGP occurs as several molecular forms in fish plasma at a total concentration of about 25 mg ml⁻¹; the active forms studied here account for up to 10 mg ml⁻¹ (ref. 2). Thus, the amount of water bound by the AFGP in plasma represents only 1% of total water. Nevertheless, the similarity in temperature-dependent hysteresis of the resonance data and that of the freezing-melting behaviour of the AFGP¹⁴ suggests a possible role for this water in the antifreeze activity. Studies of partition of the AFGP in frozen solutions indicate that the AFGP molecules are themselves incorporated into ice during freezing¹⁵,

Fig. 2 Arrhenius plots of line width data for unfrozen water in protein solutions. ▽, ▲, AFGP; ○, ●, bovine serum albumin; □, ■, human haemoglobin.

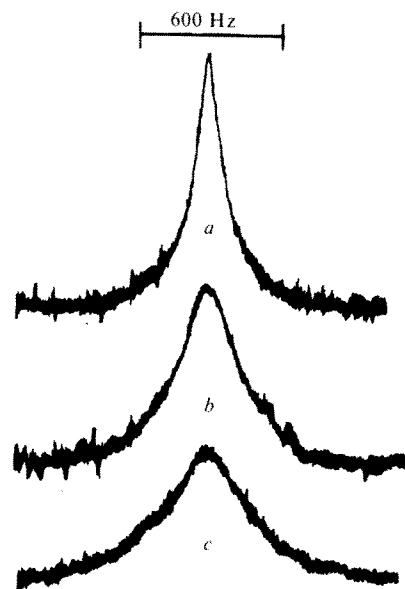
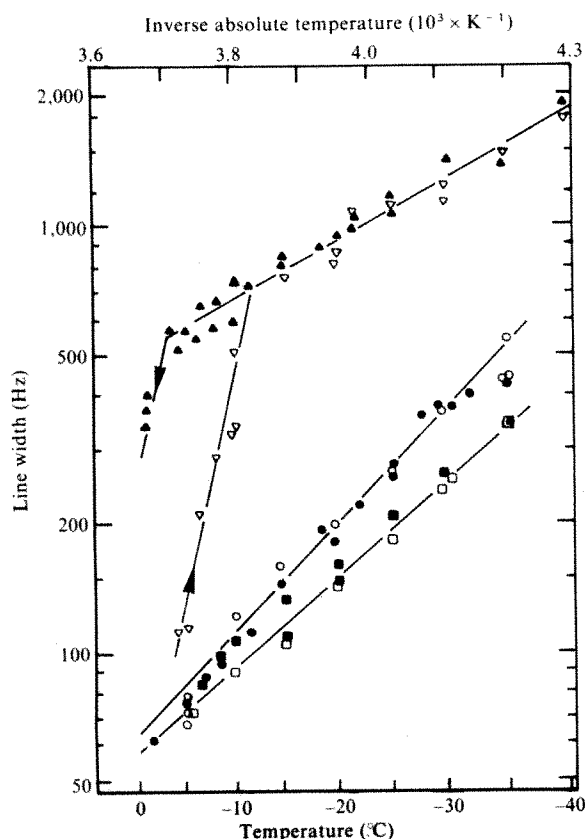


Fig. 3 Proton resonance spectra of unfrozen water in solutions of the antifreeze glycoprotein at -5 °C (a), -8 °C (b) and -10 °C (c).

although they do not affect other freezing characteristics such as salt compartmentalisation¹⁶. It has been suggested that AFGP may prevent ice propagation by direct binding to ice surfaces, thus increasing surface free energy or simply coating the ice with a barrier of unfreezable water¹⁷. Normal protein-bound Type II water is apparently not effective in freezing-point depression, as evidenced by the absence of this activity in ordinary proteins². The explanation for AFGP activity, however, may lie in the elevated levels of water that is resistant to freezing, and in the extended conformation of the molecule which serves to optimise surface contacts with ice crystals. Comparative studies of water binding in model polysaccharides and in related fish glycoproteins which lack antifreeze activity should shed further light on the activity of this unusual protein.

This work was supported by a grant from the American Cancer Society (to A. E. V. H.) and NSF grant OPP74-07917 (to A. L. DeVries).

A. E. V. HASCHEMEYER*
W. GUSCHLBAUER

Service de Biochimie,
Centre d'Etudes Nucleaires de Saclay,
B. P. No. 2, F-91190-Gif-sur-Yvette, France

ARTHUR L. DEVRIES
Department of Physiology and Biophysics,
University of Illinois,
Urbana, Illinois 61801

Received 23 March; accepted 14 July 1977.

*Permanent address: Department of Biological Sciences, Hunter College, City University of New York, 695 Park Avenue, New York 14208.

- DeVries, A. L. & Wohlschlag, D. E. *Science* **163**, 1073-1075 (1969).
- DeVries, A. L., Komatsu, S. K. & Feeney, R. E. *J. biol. Chem.* **245**, 2901-2908 (1970).
- DeVries, A. L., Komatsu, S. K. & Feeney, R. E. *J. biol. Chem.* **245**, 2909-2913 (1970).
- DeVries, A. L., Vandenheede, J. & Feeney, R. E. *J. biol. Chem.* **246**, 305-308 (1971).
- DeVries, A. L. *Science* **172**, 1152-1155 (1971).
- Kuntz, I. D. & Kauzmann, W. *Adv. Protein Chem.* **28**, 239-345 (1974).
- Kuntz, I. D., Brassfield, T. S., Law, G. D. & Purcell, G. V. *Science* **163**, 1329-1331 (1969).
- Kuntz, I. D. & Brassfield, T. S. *Archs Biochem. Biophys.* **142**, 660-664 (1971).
- Cooke, R. & Kuntz, I. D. *Ann. Rev. Biophys. Bioengng* **3**, 95-126 (1974).
- Pennock, B. E. & Schwan, H. P. *J. phys. Chem.* **73**, 2600-2600 (1969).
- Derbyshire, W. & Duff, I. D. *Faraday Disc.* **57**, 243-254 (1976).
- Lynch, L. J. & Webster, D. S. *J. Polymer Sci. Symp.* No. 49, 43-63 (1975).
- Kuntz, I. D. *J. Am. chem. Soc.* **93**, 514-516 (1971).
- Lin, Y., Duman, J. G. & DeVries, A. L. *Biochem. biophys. Res. Commun.* **46**, 87-92 (1972).
- Duman, J. G. & DeVries, A. L. *Cryobiology* **9**, 469-472 (1972).
- Lin, Y., Raymond, J. A., Duman, J. G. & DeVries, A. L. *Cryobiology* **13**, 334-340 (1976).
- DeVries, A. L. in *Biochemical and Biophysical Perspectives in Marine Biology* (eds Malins, D. C. & Sargent, J. R.) 289-330 (Academic, London, 1974).

reviews

The problematique

Eric Ashby

The Human Quality. By Aurelio Peccei. Pp. 214. (Pergamon: Oxford and New York, 1977.) Paperback, £3.25; \$6. Hardback, £6.50; \$12.

To write about this book as a reviewer is easy, but as a critic is difficult. I shall try to do both.

The early part of the book is an artless life-story of a modest, sincere, and courageous man. Aurelio Peccei was a freedom-fighter in the Resistance in Italy, imprisoned at one time by the Nazis. He then became a highly successful industrialist. It is ironic, in the light of his present activities, that his industrial success was to provide large numbers of mass-produced automobiles which polluted Europe and—through Peccei's special efforts—Latin America.

But Peccei's conscience survived this material success and he turned in 1957 to something with a dash of altruism added to industrial acumen: an engineering and economic consultancy which was to do work in developing countries on a non-profit-making basis. It was not a Pauline conversion exactly, for Peccei still maintained his connections with the manufacture of automobiles, but he had come to the view that multinational companies have a responsibility to society above that of making profits for their shareholders. By the 1960s, his family being now grown up and his level of affluence being adequate, Peccei found himself free to reflect on human destiny.

The rest of the book describes Peccei's reflections and the practical steps he has taken to disseminate them. The most significant of these steps was the formation of the Club of Rome, which is an informal group of people who are concerned with what they call the *problematique*. The *problematique* is (Peccei writes) the "intractable tangle of difficulties" which constitute the present "predicament of mankind."

That there are intractable difficulties facing industrial societies and the developing nations no-one questions for a moment. But that Peccei and the Club of Rome are the first people to have realised this, is nonsense. The Club (writes Peccei, observing that "we know incredibly little about our

changed conditions") is "the first to rebel against this well nigh suicidal ignorance." This claim to priority is just one of a string of unacceptable assertions in the book. Has the Club of Rome not heard of H. G. Wells, Aldous Huxley, George Orwell, Lewis Mumford, Bertrand de Jouvenal? Were they not aware of the *problematique*?

Peccei offers a somewhat querulous defence of the best-known project of the Club of Rome, which was to sponsor the book *Limits to Growth*. The warning in this book, that continued exponential growth would lead to collapse, was (Peccei writes) "denounced as nothing more than a heretical and misinformed libel." This is a grossly inaccurate way to describe the sober and careful criticism which *Limits to Growth* received. Peccei does nothing to refute these criticisms; indeed he does not even mention what the criticisms are: not a word about the reasoned indictments from the University of Sussex or the World Bank. These critics were not (as Peccei suggests) defending the "sacred cow of growth" nor were they under the impression (as Peccei suggests) that the Club of Rome advocated zero growth. The critics were simply exposing the spurious assumptions which had been fed into MIT's computer. In a later chapter, but without acknowledging the debt which the Club of Rome owes to these critics, Peccei concedes some of their criticisms. "Perhaps," he writes, "science and technology are capable of placing at mankind's disposal the immense resources required. . . ."

It is over 50 years since Raymond Pearl published data on the sigmoid curves of population growth and discussed some of their consequences. The Club of Rome has performed a useful service in stimulating popular interest in the climacteric of modern society: the transition from accelerating growth rate with positive feedback to decelerating growth rate with negative feedback; and I am willing to acknowledge that the issue had to be made sensational in order to capture public attention. But the basic strategic error which the Club has made (and which Peccei makes even more egregiously) is to deflect people from paying attention to thousands of mini-

solutions to mini-problems (which is the way society is most likely to survive this transition) by dangling before them one Comprehensive Maxi-Solution.

Assertions about the maxi-solution occupy most of the space in Peccei's book. They are offered in the tedious platitudes which are the despair of people who are seriously engaged in the minute particulars of trying to improve the condition of society. "[S]ociety must see to it that *all* the benefits which the system can provide—including goods and services—are actually put at the disposal of *all*." Mankind "must undergo" a process of "total re-education." It is the use of the word "must" which riles me. Even if you accept the premise—that "must" implies "ought"—there are two consequential questions which follow the use of the word "must"; one is: "How?" The other is: "Imposed by whom?" Neither Peccei nor the Club of Rome offer any answers to these two questions. Without answers, these intoxicating declarations simply save people the trouble of considering far more important problems, such as how to see to it that *some* of the benefits of the system can be put at the disposal of *some* people, and that in place of the airy policy of total re-education for *everyone* (New York stockbrokers, Italian politicians, Indian peasants, Ghanaian cocoa farmers, Moscow policemen!), there is encouragement for thousands of people who are trying to do a little re-education for a few.

So I reject Peccei's whole approach to the predicament of mankind on the simple criterion that these vague and sweeping clichés which he offers as the recipe for achieving social justice are as impotent to solve the *problematique* as similar vague generalisations would be for the production and sale of automobiles. But—and here I am in complete agreement with Peccei—the *problematique* (much as I dislike the word) does stand for something real which confronts governments all over the world. What can take the place of Peccei's sincere but futile gestures to mankind at large? I think there are modest and much less colourful alternatives. It would be more effective if diagnoses of the predicament of mankind were to separate

clearly what *is* (that is, to get the facts straight) from what *ought to be* (which is legitimate ethically provided you say *why* it ought to be); and never to proceed to "*must*", for on that way lies compulsion, dictatorship, the rule of Leviathan. A wise assumption would be that social attitudes cannot be changed except incrementally, and that there are "tolerance limits", so to speak, to the magnitude of a change which people will contemplate and be prepared to accept. Any proposal which lies outside these tolerance limits is rejected; it creates resentment, sales resistance, is (in modern jargon) counter-productive.

And, finally, all attempts to define goals for mankind are likely to be mirages, vanishing as one approaches

them. There is another way to move toward goals: that is not to demand a grandiloquent metamorphosis of mankind, but instead to encourage little groups of people all over the place to make wise choices over small changes in society. For it is one lesson of history (and it would do the Club of Rome no harm to read a little history) that ends are continually being re-defined by the means being used in groping toward those ends; and it is another lesson of history that *men and women* do respond to messages which lie within their range of understanding, but *mankind* is deaf to all messages. □

Eric Ashby is at present Walgreen Professor of Human Understanding at the University of Michigan.

GC-MS system

Fundamentals of Integrated GC-MS. Part III: The Integrated GC-MS Analytical System. By B. J. Gudwinowicz, M. J. Gudwinowicz and H. F. Martin. Pp. vii+603. (Dekker: New York and Basel, 1977.) SFr.190.

THIS third part of the text on analytical chemistry was written in mid-1976 and surveys knowledge about the integrated gas chromatography-mass spectrometry (GC-MS) system up to that date.

The first chapter deals with basic vacuum technology and the interface between the GC and MS, seeks to provide enough of a mathematical treatment of gas flow characteristics to permit an understanding of the problems associated with interfaces to be appreciated. All of the usual enrichment devices are discussed in detail and their characteristics compared. The authors have made excellent and very full use of tables, graphs and drawings of apparatus as well as including in the text many references to original work. The chapter contains 181 pages with 18 tables, 88 figures, 263 mathematical equations and 221 references.

The middle chapter deals with the methods which are available to optimise the operational parameters of each separate part of the integrated GC-MS analytical system. The authors consider each operational parameter in turn and deal with the optimisation of carrier gas flow rates, injection systems, the thermostability of liquid phases, chromatography columns of various types, inlet and effluent splitters, separator enrichment factors and characteristics, source temperature and pressure, ionising potentials, sensitivity, scan rates, dynamic resolution, ratio recording, and the use of peak matching techniques to measure the

accurate mass of fragment ions during GC-MS scans. This long chapter is fully illustrated and well referenced, containing 202 pages with 29 tables, 96 figures and 484 references.

The final chapter deals with the role of a mass spectrometer as a GC detector and the data processing of the mass spectral results. The role of the ion monitor in producing a total ion monitor (TIM) graph is discussed, and multiple ion monitoring and single ion monitoring techniques are reviewed in detail. Several examples of the use of mass fragmentography techniques for the analysis of biological samples are described and the sensitivity of detection and quantification using single ion monitoring are discussed.

The history of the application of data processing techniques to mass spectrometers is reviewed and the use of these systems for the calculation of the accurate masses and the molecular formulae of fragment ions, and their display in terms of element maps, is described. Repetitive cyclical scanning GC-MS-COMP systems are described where the mass and abundance information is stored in disc systems ready for regeneration in the form of individual mass spectra, outputs from library search routines or mass chromatograms. The final chapter contains 140 pages with 15 tables, 68 figures and 171 references.

The authors have provided a book which is a mine of information for those involved in, or about to embark on this field. It is prepared to a high standard and is well indexed. It is a 'must' for every GC-MS laboratory but it is priced (190Fr=£46) well beyond the resources of most individuals.

James A. Ballantine

James A. Ballantine is Senior Lecturer and operator of a GC-MS system in the Chemistry Department of the University College of Swansea, UK.

Statistical mechanics revised

Statistical Mechanics. Second edition. By J. E. Mayer and M. G. Meyer. Pp. 491. (Wiley: Sussex, New York, Sydney, Tokyo, and Mexico City, 1977.) £17.50; \$29.

A CLASSIC BOOK revised by its author 37 years later for a second edition has to be approached with the respect one would normally reserve for, say, a fine old whisky. This respect is indeed deserved, for this book has retained its clarity, relevance and coverage. It has nevertheless lost some of the quality of the first edition: the small print—an author's whisperings into a reader's ear—has gone. So has the author index; so have the problems and the glossary. The effect is that the material, though necessarily altered, covers the same number of pages as the first edition. I bought my 1948 copy, however, for one-seventh of the present price.

Turning to the contents, substantial parts of the book are unchanged; for example, the early chapters and also the treatment of electric and magnetic fields. The discussion of black-body radiation is postponed to the quantum theoretical part, as it was in the first edition, and so comes rather late (p368) for so important a topic. On the other hand, new sections on computer experiments (p320), on the coupling of nuclear spins to electrons and on spin echo, up-date the book in the author's lively style. Some unexpected references are left, for example (p38), one to Gibson's Amsterdam thesis of 1933, which readers are unlikely to want to look up to check on the viscosity values for hydrogen. A "constant entropy paradox" is named, stated, and resolved, but the simple lesson that coarse-graining is to some extent responsible for entropy increase is not very explicitly drawn. There is not much on stability or negative temperatures and on p106 the author might have noted that heat capacities *can* be negative (in gravitational systems).

This book, written by two great experts in the field, and now revised with extreme care by one of them, remains a good introduction, is a source of much useful information, and should enjoy continued popularity.

Peter Landsberg

Peter Landsberg is Professor of Applied Mathematics at the University of Southampton, UK.

Heterocyclic compounds

Stereochemistry of Heterocyclic Compounds. Part 1: Nitrogen Heterocycles. By W. L. F. Armarego. Pp. 443. Wiley: London, New York and Sydney, 1977. £27; \$45.

DR ARMAREGO'S aim is to survey the entire stereochemistry of heterocyclic compounds, excluding most natural products, in two volumes. He has chosen to do this by referring freely to specialised reviews and other secondary sources, thereby avoiding duplication of effort and leaving himself free to give quite detailed discussion to the remainder of the subject, with emphasis on recent work. This approach clearly adds to the value of the book to the specialist but runs the risk of bittiness and lack of balance for the more general reader. Fortunately, Dr Armarego's style and wide-ranging interest in his subject save the book from these dangers, and large parts can be read with enjoyment and a sense of continuity.

This volume is concerned with compounds with nitrogen as the only heteroatoms in the rings and is divided into a short introductory chapter and

three very long chapters with compounds classified by ring size: chapter 2 covers three-, four-, and five-membered rings, chapter 3 six-membered rings, and chapter 4 the larger rings. Each chapter is subdivided into increasingly complex classes of compounds in a way that makes it generally easy to locate information without working through the detailed table of contents.

A book of this type must be up to date, reliable and uniform in its coverage of the original literature, clear in its diagrams, and concordant with currently accepted theory in its interpretations of the properties of the compounds described. In most respects Dr Armarego's book meets these requirements well. The references are very full for the period from 1965 to about the middle of 1975. A check on the references in chapter 3 showed that the average number each year for 1965 onwards is about six times as high as for 1964 and earlier. The abrupt change in the frequency of references suggests the possibility that Dr Armarego's book may have gaps in respect to the earlier literature but I have not found any that are important and not indirectly covered by the references to secondary sources. Occasionally there is a lack of balance: for example, Kipping's work on 2,3,5,6-

tetramethylpiperazine in the 1930s is described in some detail when a reference to Harris and Sheppard's conclusive NMR study in 1955 would have been sufficient. In contrast, the diazabicyclo[3.3.1]nonanes are dismissed very briefly. This section contains a rare but bad example of chemical misunderstanding because the 'normal' amide carbonyl group in 3-isopropyl-2-oxo-1,3-diazabicyclo[3.3.1]nonane is regarded as a violation of Bredt's Rule and is inappropriately contrasted with the ketonic carbonyl group in 2-oxo-1-azabicyclo[2.2.2]octane. The formulae are usually clear and well drawn, particularly when this is most important as with bridged and caged polycyclic systems, but there are, unfortunately, some very distorted drawings of fused six-membered rings (notably, formulae 260 and 296 in chapter 3) that result from the uncritical use of stencils.

Dr Armarego has been very successful in realising his aim. This book will be bought by many chemists with interests in stereochemistry or heterocyclic chemistry, as well as by all chemical libraries. **M. J. T. Robinson**

M. J. T. Robinson is a University Lecturer in Organic Chemistry at the University of Oxford and a Fellow of Magdalen College, Oxford, UK.

Weak interactions

Weak Interactions. By David Bailin. Pp. ix+406. (Chatto and Windus: London, 1977.) Paperback £9.

THE subject of weak interactions has developed very rapidly in the past decade. As a result there is a fairly wide and growing gap between good old textbooks, such as the well known book by Okun, and the moving frontiers. Since there are no signs that the motion of the frontier is slowing down, it is wise to educate graduate students in particle physics in the field of weak interactions. David Bailin was aware of this real problem and this book is his answer to it.

The book starts with a lengthy introduction to the Dirac equation and the construction of invariant amplitudes for fermions, and then discusses in detail leptonic, semileptonic and non-leptonic weak interactions as well as CP violation and unified gauge theories.

The new knowledge gained in the past decade is indeed covered by Bailin's book, which is useful as a guide to what is known and as an introduction to the literature. Its main strength lies in the material which is covered and the detailed elaboration

of computational details. I could find very few misprints and no errors of calculation.

The emphasis on calculations is also the source of the main weakness of the book: it is lacking in pedagogy. There is much more material than could be covered in a one-year course. The author could, however, have avoided stressing hard computation rather than easy insight. Many topics are discussed in a way which, though correct, are likely to be difficult for beginners. For example, SU_3 and the Cabibbo angle are introduced in an abstract fashion starting with the structure constants f_{ijk} of the algebra. It is much more easy to start with a quark model and introduce the Cabibbo rotation at the level of the quark currents and only talk about SU_3 afterwards. This way is also easier to generalise to a larger number of quarks—a contemporary problem.

I recommend Bailin's book to all those who want to become familiar with modern developments in theoretical technology for weak interactions. The publishers must anticipate a huge increase in graduate student grants, judging from the price of this book.

G. Karl

G. Karl is Professor of Physics at the University of Guelph, Ontario, and is at present on sabbatical leave at the SRC Rutherford Laboratory, Chilton, UK.

**tins
will
change
your
reading
habits**

from July, 1978

**ELSEVIER/NORTH HOLLAND
BIOMEDICAL PRESS**

Circle No. 14 on Reader Enquiry Card.

Vector analysis

Vector Fields: Vector Analysis Developed Through Its Applications to Engineering and Physics. By J. A. Shercliff. Pp. 329. (Cambridge University: Cambridge and London, 1977.) Hardback £13.50, paperback £4.25.

THIS book is stimulating and occasionally irritating; but few stimulants are without their side-effects. It is aimed at physicists and engineers at the equivalent of British second-year undergraduate level. Teachers of engineering may be happier with it than teachers of physics, although many physics students might find that it taught them more than would a more conventional approach. The approach is unconventional, to this physicist at least, because it fuses the entire content of a classical electromagnetic theory course and of a hydrodynamics course in order to illustrate the tools and methods of vector analysis.

The introductory chapters certainly would not replace proper courses on basic electricity and magnetism or on vectors. The summary is brief and might be rather obscure to, for instance, a student whose knowledge of vectors was based on 'modern mathematics'. The argument is pictorial and intuitive, with some stress placed on a rather woolly property which all authentic vectors are supposed to have; 'cosine quality'. Someone who has played with scalar products, work integrals and components of forces will grasp 'cosine quality' straight away, just as someone who knows Fleming's left-hand motor rule will understand how magnetism is 'electricity looked at sideways'. The good thing about this cavalier treatment is that it should give the student a feeling for the eventual simplicity of material which he may have found heavy-going in the first year.

The next chapter is on line and surface integrals, assuming some knowledge of partial differentiation and of Taylor's theorem. Ampere's law, the Biot-Savart law and various aspects of current electromagnetism are followed by volume integrals, with illustrations from simple plasma theory. Then comes 'A crisis in electromagnetic theory', a rather wordy treatment of the arguments leading to the concept of displacement current. The stage now seems to be set for the derivation of Maxwell's equations. But no; this consummation is delayed for another five chapters. Divergence and gradient must be introduced; then Gauss' theorem, Laplace's equation, a variety of applications to hydrostatics, fluid flow and heat flow, Poisson's and Helmholtz' equations with examples including the

criticality condition in a nuclear reactor. The treatment of waves and of the superposition principle is surprisingly sketchy after the detail given to many other topics.

Curl comes next, with a great deal of illustrative detail in hydrodynamics, leading to Stokes' theorem. Then, at last, we are ready for Maxwell's equations, followed by discussions of the skin effect, electromagnetic waves and the Poynting flux. The final chapter is on 'Integrals in fluid mechanics', beginning with derivations of Kelvin's and Helmholtz' theorems and applying them to such problems as the jet from an orifice, flow over an infinite airfoil and the lift and drag properties of an aircraft wing.

The book is illustrated with a large number of clear, simple line drawings.

Search for life

Chemical Evolution of the Giant Planets. Edited by Cyril Ponnamperuma. Pp. 240. (Academic: New York and London, 1976.) \$11.50; £8.15.

THIS book contains the invited lectures presented at the first of a series of colloquia to be organised by the Laboratory of Chemical Evolution at the University of Maryland, aimed at bringing together scientists for formal and informal discussion on topics pertinent to chemical evolution. The meeting, held in October 1974, was stimulated by the availability of data from NASA's Pioneer 10 and 11 and attended by astronomers, chemists, biologists and engineers, involved with, or interested in, the outcome of those missions. The overall theme of the discussion was intended to consider whether conditions prevail in the atmospheres of the giant planets which are conducive to chemical synthesis, or able to support life of a terrestrial nature. The title of the volume may be to some extent a misnomer since many of the authors did not restrict themselves to the location suggested. The outer planets in general, Mercury, Venus, Mars, meteorites, comets and the satellites of Jupiter and Saturn, particularly Titan, are freely discussed.

The book consists of sixteen papers by twenty authors; its content may be broken down into several general subject areas. T. Gehrels, D. M. Hunten and T. Owen provide essential background information concerning atmospheric composition, structure and chemical composition. W. F. Libby, J. S. Lewis and C. Ponnamperuma cover various aspects of chemical synthesis in the Miller-Urey vein. The biological

There are more than two hundred problems on a great variety of applications, distributed in clumps in mid-chapter as well as at the ends of chapters. Answers are provided to all the problems.

Although most departments will not wish to telescope courses together in the way Shercliff has done, the book should be generally useful as an additional text. Weak students will benefit from the wealth of applications for each abstract idea. Stronger students may be led on to read and understand material which a more formal textbook would leave out.

David J. Miller

David J. Miller is Lecturer in the Department of Physics and Astronomy at University College, London, UK.

papers (R. D. MacElroy, D. J. Kushner, R. L. Dimmick and M. R. Chatigny, R. S. Hanson and N. H. Horowitz) centre on the adaptability of microorganisms and their ability to survive in harsh environments having a variety of temperatures, pH, salt content, and even in aerosols. The energy requirements of a biosphere in terms of light input are considered separately by B. Kok and R. Radmer. Two essentially technique papers by E. E. Russel and M. G. Tomasko, and R. A. Hanel cover spin-scan imaging and infrared spectroscopy, respectively. NASA philosophy towards further planetary exploration is put forward by D. H. Herman and S. J. Grivas. Because of funding constraints rather than through any fault of the authors, the treatment is of a very general nature. A more important philosophical question raised by R. S. Young and R. D. MacElroy, concerns policy with respect to the possible irreversible contamination of any planetary surface or atmosphere, by organisms or molecules, carried on unsterilised or uncleaned space probes.

All in all, I feel this is a very useful book. Not, perhaps, of great value to the specialist chemist or biologist, but eminently readable by anyone with fringe knowledge of a variety of subjects and interested in studies on the origin of life. It provides many appropriate references and has a full index. As the object of the colloquium was to provide a forum for discussion, I wish an edited version of the question and answer sessions could have been included at the end of each chapter.

C. T. Pillinger

C. T. Pillinger, of the Department of Mineralogy and Petrology, University of Cambridge, UK, is a principal investigator in NASA's lunar sample analysis program.

obituary

Benjamin Lee

PROFESSOR BENJAMIN W. LEE died in an automobile accident on 16 June 1977. Director of Theoretical Physics at Fermi National Accelerator Laboratory and Professor of Physics at the University of Chicago Enrico Fermi Institute, Ben Lee was at the age of 42 deeply engaged in scientific activity at the forefront of high energy physics.

Born in Seoul, Korea, Ben Lee emigrated to the United States in 1956 and obtained citizenship in 1968. He studied at Miami University (Oxford, Ohio), the University of Pittsburgh and the University of Pennsylvania, where he was awarded a doctorate in 1960. His earliest work dealt primarily with dispersion relations and related techniques in the analysis of strong interaction phenomena, but he also had an early interest in Lagrangian symmetries and spontaneous symmetry breakdown, which ultimately became the focal points of his research.

By 1964 Ben Lee was a leading contributor to the study of the transformation properties of weak interactions with respect to strong interaction symmetries, and the intimately related question of the full symmetry properties of the strong interactions themselves. Through such studies the relevance of a spontaneously broken chiral symmetry gradually became apparent, and Lee contributed significantly to the construction and study of Lagrangian field theories which realise this property. His work demonstrating the renormalisability of these theories provided an encouraging model for later developments which led in 1971 to the establishment of renormalisability of spontaneously broken gauge theories. Following this breakthrough, Lee's work on the formal demonstration of renormalisability of gauge theories and on the elaboration of calculation techniques was of major importance in the further development of this field which has taken a dominant position in our present thinking.

As soon as the relevance of gauge theories for weak interactions began to appear highly probable, Ben Lee turned to the study of their practical implications, both for the newly discovered neutral current interactions and for the previously established properties of charged current interactions.

The use of new theoretical developments allowed a confrontation with experiment which placed severe restrictions on the possible choice of gauge models. It became increasingly clear that a viable model should incorporate charmed particles which had been postulated before achievements in renormalisation made such an inference compelling. Lee devoted considerable study to the expected properties of these particles and the possibilities for their experimental detection.

Along with the experimental discovery of charm, there has been a continuous experimental and theoretical evolution in the field of both weak and strong gauge theories, in which Ben Lee continued to participate fully. His rare combination of a familiarity with formal mathematical techniques and a close touch with experimenters and experimental results kept him at the forefront of many different aspects of these developments. At the same time he continued to reflect on the outstanding problems related to his earlier work on weak interaction symmetries and was quick to apply new developments to the clarification of old problems. He encouraged precision experiments designed to resolve long-standing questions as well as pioneering experimentation on the new phenomena.

Ben Lee's decision to remain permanently as head of the Fermilab theory group was not an easy one for him. He felt that at a university he would be freer to pursue fundamental theoretical questions. Yet while continuing to participate in the fast pace of theoretical research, he took extremely seriously the responsibilities of his position at Fermilab. On the one hand he attracted theoretical physicists of the highest quality as visitors to the laboratory and was in the process of putting together a small but strong nucleus of resident theorists. On the other hand he was deeply involved in laboratory policy concerning its present and future experimental programme, a job which he undertook with singular conscientiousness, endeavouring to learn the fine points of experimental techniques.

Before joining the Fermilab staff in 1973, Benjamin Lee was Professor of Physics at the State University of New York at Stony Brook. In addition to his responsibilities at Fermilab he served on the scientific policy com-

mittees for several major laboratories. He was the recipient of a number of prestigious scientific fellowships and had held appointments at many institutions in the United States and abroad. Perhaps his most meaningful experience in recent years was a visit to his native Korea where he was struck by the developing prospects for scientific research and found himself "working six days a week on upgrading graduate education in basic sciences" at the Seoul National University.

Ben Lee shared a close family life with his wife Marianne and teen-aged son and daughter at their home in Glen Ellyn, Illinois. Whenever possible they combined business with pleasure on trips to Aspen in Colorado, Europe and the Far East. An immeasurable personal tragedy for his family, Benjamin Lee's untimely death is a deep loss for the entire high energy physics community.

Mary K. Gaillard

Fred Hardy

PROFESSOR FRED HARDY, CBE, who died at St Augustine, Trinidad, in May at the age of 88 was the doyen of British tropical soil scientists. During his professional life the study of the soil developed from a subject that had little impact on practical agriculture to a discipline that has made major contributions to agricultural development, and Professor Hardy played a notable role in this transformation for tropical soil studies.

Almost his whole adult life was spent in the West Indies and Central America. His first appointment after leaving Cambridge University, was as lecturer in natural and agricultural sciences at Harrison College, Barbados, in 1911. He returned to England in 1917 towards the end of the First World War, then after the war spent a year at the School of Agriculture at Cambridge and then went back in 1920 to the West Indies as soil chemist with the Imperial Department of Agriculture, which was centred at St Augustine. This later became the Imperial College of Tropical Agriculture, and he became its first Professor of Chemistry and Soil Science. After his retirement in 1956 he served as Professor of Soil Science in the Inter-American Institute of Agricultural Sciences at Turrialba, Costa

Rica, for nine years before finally retiring to Trinidad.

Hardy's research career began after his return to the West Indies in 1920. He began by being interested in problems of soil water and the effect of water on some soil properties; then in the 1930s he became interested in the characteristics and genesis of tropical soils and laterites; and finally in the 1940s and 1950s he developed his well known studies on the soil factors that affect the fertility of tropical soils and their suitability for specific crops. In addition he was one of the early British soil scientists who realised the need for appropriate soil surveys for agricultural development, and I believe the first in the colonial Empire to institute a systematic soil survey, and he succeeded obtaining funds over a period of years for a survey of most of the British West Indies.

Professor Hardy had, however, a far greater influence on British colonial agriculture than would be judged from his research work, for almost every recruit to the Colonial Agricultural Service since the mid-1920s spent a year in Trinidad and most would study soils under him. His enthusiasm, his great friendliness and approachability, and his gift for picking out the most important points in any discussion, all left their mark; and his appreciation of the relation of the soil and the crop to the natural landscape must have influenced the ecological approach to agricultural development shown by many outstanding colonial agricultural officers.

E. W. Russell

William Parker

THE sudden and untimely death of William Parker, Professor of Chemistry at the University of Stirling, at his home in Dunblane on May 9 at the age of 45, has left the world-wide chemistry community with an immense feeling of loss.

Born in Glasgow in 1932, Willie Parker was a distinguished pupil of Whitehill Secondary School after which he went to the University of Glasgow where he took a first class honours degree in Chemistry in 1953. He started his PhD studies in Glasgow with Professor R. A. Raphael and completed them at the Queen's University of Belfast when Raphael moved there to his first professorial post. In 1956 Willie joined Professor G. Stork's group at Columbia University and then returned to the University of Glasgow where he rapidly moved up the academic ladder, culminating in his appointment to a senior lectureship in 1966. Promotion to readership saw him back in Ireland in 1968 at the

New University of Ulster where he spent two happy years helping to build up the new chemistry department there with characteristic drive and enthusiasm under the leadership of Professor M. Grundon. Armed with this valuable experience he returned to Scotland in 1970 to take up the first chair in organic chemistry at the University of Stirling.

Natural products, particularly the sesquiterpenes, were Willie's first love and in this area he published many important papers relating to their synthesis, biogenesis and rearrangements. In particular, he and his co-workers unravelled many of the problems associated with the molecular acrobatics of caryophyllene and humulene and their derivatives. Although he maintained this interest throughout his research career, a sabbatical year with Professor J. Berson at the University of Wisconsin in 1965 served to promote and nurture his growing interest in organic reaction mechanisms with particular emphasis on the solvolytic behaviour and preferred conformation of a wide range of bridged bicyclic compounds. Of particular note were his contributions to the understanding of the reactivity of bicyclo[3,3,1]nonane, bicyclo[3,3,2]decane and bicyclo[3,3,3]undecane (manxane) derivatives. At the other end of the spectrum he also had a keen research interest in the chemical factors influencing germination and dormancy in wild oat seeds. Shortly before his death, his group, together with collaborators in other disciplines, had made significant advances in this area.

Over the past eighteen years I knew him in many different rôles; as a teacher, as a research supervisor, as a colleague, as an opponent on the squash court and, above all, as a friend. In all these aspects his boundless enthusiasm, inexhaustible drive, unadulterated Glasgow humour, and extrovert personality made a tremendous impact on me as they did on so many other people. If ever there was a teacher who practised the subtle art of instilling a sense of dedication and professionalism in his students there was no greater practitioner than Willie Parker. Under his guidance and tuition organic chemistry came across as an exciting and challenging field of study.

Over the years many students, of whom I was one, continued their studies with Willie as their PhD supervisor. He shared in our triumphs and disappointments at the bench, our laughter and our tears. All of us greatly benefited from that experience and I have no doubt that his guiding principles and aspirations had a profound influence on us.

His rumbustious character came

through vividly in all the many facets of his work at the University. Willie was by no means an isolationist in an ivory tower; he enjoyed all his teaching commitments whether in the first year undergraduate laboratory with his sleeves rolled up or at the Monday evening postgraduate seminars. He approached his administrative duties both in the chemistry department and in the many university committees on which he served with a refreshing, down-to-earth attitude.

More than anything else he wanted to and did put the Chemistry Department of Stirling University on the map. I believe that he achieved this not only by his own personal reputation in the world of organic chemistry and by his service on national committees such as the Chemistry Panel of the Science Research Council, but also by his active encouragement of other members of staff to apply for grants and to seek and establish links with the chemical industry. Above all he had the unique ability to muster and channel our individual energies towards the common cause of creating a resourceful and stimulating department. His door was always open to those seeking advice and counsel. It is not surprising that he achieved so much during his relatively short trip to India last year while acting as project co-ordinator for a UNDP/UNESCO programme 'Special Assistance to Selected Science Departments in Indian Universities.' The United Nations could not have picked a better man for such a stamina-sapping mission.

Willie's tough professionalism really knew no boundaries; it overflowed onto many other areas including the sports field. His light blue tracksuit is a legend at Stirling University. Even beyond the campus his zest and vigour were manifest. At various times he was chairman of the local gardening club, a member of the male voice choir, and a coach to one of the Cub football teams. Just as Willie knew how to advise and encourage young academics, so too did he help the nine-year-old footballer. From his own student days as a keen footballer he remembered the importance of a new strip and the morale-boosting sing-song in the car returning from a 26-nil defeat.

All his many friends, both in the UK and abroad, have shared in one unique privilege—that of knowing the truly remarkable character of Willie Parker. For all of us, we are the beneficiaries of that enriching and rewarding experience, the memory of which will live on to compensate in part for our tragic loss which we share with his wife and three children.

James S. Roberts

nature

8 September 1977

When science turns up the unpalatable

THE Presidential Address of Sir Andrew Huxley to the British Association for the Advancement of Science, meeting in Aston, has been hyperbolically described in the press as "the first shot in what could become one of the most crucial and vitriolic scientific battles of the century". It certainly is an unusual, even courageous address.

His line of arguing is this. He asserts that "Science as a whole—the scientific approach to questions of all kinds—has come increasingly under attack . . . it is repeatedly suggested that the speed of scientific discovery should be slowed, and that scientists ought to suppress discoveries that seem capable of being used to the detriment of humanity". In a lengthy aside, he then acknowledges that scientists themselves often rely too much on grand unifying principles which inhibit their collection of evidence. But in a broader context too the collection of scientific evidence may be inhibited if it seems likely to touch on some particularly sensitive nerve in ordinary human affairs. Resistance, he claims, may be based on "Authority" or on the fear of the consequences of accepting the conclusion. Not for nothing does a Huxley hark back to the 1860s and the debate on this very matter occasioned by the publication of *Origin of Species*.

He asks if any topic stirs similar emotions these days. And he sees many features in common between the evolution debate of the last century and the current debate on the extent to which human ability is inherited, although he points out that the analogy would be better had Darwin gone public twenty years earlier when his case would have been weaker, but certainly not hopeless. The resistance these days to research in the field of the heritability of human ability comes from a feeling that our ethics may be undermined because the existence of substantial inherited differences would lead to unjust treatment of the less-well endowed, and that discoveries of below average ability would damage self-respect.

More sinister than anything in the evolution controversy, says Sir Andrew, is that there are actually scientists who regard the assumption of equal inherited ability as "something which does not require experimental evidence to establish it and which it is positively wicked to question because the conclusion might disagree

with their social and political preconceptions". Thus when someone (William Shockley) had the "courage to suggest systematic and scientific investigation" he was repeatedly turned down by the National Academy of Science. Sir Andrew accepts that at the time Shockley was advocating eugenic measures unacceptable to public opinion, but thinks the main reason for refusals was fear that it would be represented as a "commitment to an illiberal point of view unfavourable to American Negroes". He feels that such behaviour is an unjustifiable obstacle to human enquiry and impedes or distorts the advance of science.

Freedom for scientists to investigate whatever they want may be a valid rallying point in some circumstances and under some tyrannies. But scientists are already under some external constraints on their freedom, for ethical and financial reasons, and it is clearly not possible simply to say that everything that is observable and measurable ought, in the name of science, to be observed and measured. The question then revolves around the motives for doing research into heritability. There are, of course, two extremes—those who believe that the world should know that the less well-endowed are going to stay that way and those who believe that the whole thing is both scientifically unsound and an affront to certain people, and should be stopped forthwith.

Is there middle ground? If there is, and many intelligent and thoughtful geneticists believe there is, it needs staking out with immense care. Those who propose that research be stopped have some very human motives behind them, and it is no answer simply to wave freedom of scientific enquiry in their faces. Questions are bound to be asked about motives for doing more research, especially as there is little doubt that inheritance does play at least some and maybe a very significant part in the acquisition of ability. Some who want to do more only want to do so to rub in assumed inferiorities. What Sir Andrew should have done is take his argument further and set forth reasons why more research at this time, with its close proximity to sensitive issues, is desirable. Such a case can probably be made out but it would need a rather more spirited explanation of why we are better off with the knowledge than without it. □

La Soufrière, volcanology and forecasting

Last year Haroun Tazieff, of the Laboratoire de Volcanologie, Centre de Faibles Radioactivités, Gif-sur-Yvette, France, had to leave his post as director of the Service Volcanologique at the Institute of Physics of the Globe over the eruption of La Soufrière. Below, his account of the problems

IN THE past six years at least four erroneous volcanological diagnoses, based either on wrong interpretations of actual facts or on deliberately false data, have induced governmental authorities in four countries to take unnecessary and expensive measures. Together they show that volcanologists may sometimes be confronted with quite serious socio-economic consequences of their own forecasts.

● The first case was in 1970 in Italy's Neapolitan area. A submarine eruption was claimed to have burst out offshore of the city of Pozzuoli, approximately 2 km west of the well-known Solfatara fumarolic field. After the mass-media told of possible explosions, ash-falls and sea-waves, the Army and the *carabinieri* (police) helped the frightened population to evacuate the city. The claimed symptoms were an upheaval of the ground in the whole area, which had been pushed up about one metre in a "very short while"; extremely shallow earthquakes, located 1 to 2 km offshore; a conspicuous temperature-rise of the Solfatara's fumaroles; and the outflow of hot fluids on the bay's floor, inferred from dead boiled fish fishermen took in their nets.

The last three items were subsequently proved totally false by a team of six scientists led by myself, and I disclosed the fact at a press conference. On the other hand, the upheaval, the so-called brady-seism of Pozzuoli, had not been faster than usually noticed over the centuries during which up-and-down level variations of that peculiar area had been classically observed. Eventually it was stated that deliberately wrong data had been produced by the scientist in charge of the geophysical institute of Naples. This was connected to a construction operation in which high-rank people were involved. The evacuation of the inhabitants was proved to be expensive and useless.

● The second case happened during the 1972 eruption of St Vincent's Soufrière in the West Indies. Notwithstanding the clearly expressed and reassuring opinion of Dr J. Tomblin, the volcanologist in charge, an alarming cable from a scientist some thousand kilometres away was taken into consideration. This 'televolcanologist' had deduced his frightening conclusions from earth-satellite imagery and the whole population was consequently moved away. Here too it proved both expensive and useless.

● The third case happened in 1973 during the last days of the Heimaey eruption. Persuaded by a somewhat inexperienced foreign volcanologist, Icelandic authorities agreed to use fire-boats to sprinkle water on a tongue of the thick lava flows which over several weeks had progressed at a distressing speed towards the harbour entrance. No arguments could prevent the exercise: not even the evidence that the Atlantic ocean itself, with all its water, had not been able to stop the main part of the flows which had crawled over the sea-floor for two months.

● The fourth error occurred in 1976 during the eruption of La Soufrière in Guadelope in the West Indies. Here too, in spite of the firm statement of the volcanologist in charge that no danger lay ahead, the authorities followed the alarmist opinion expressed first by a petrographer and then

by a geochemist, both of whom lacked experience of eruptive phenomena. Consequently 73,600 people were evacuated on 16 August and were kept away from their homes and jobs for 3½ months. It proved very expensive, dramatic for the population, and totally useless.

A volcanologist is actually as responsible for his diagnoses as is a physician—even more so, because of the number of people involved and because the costs are usually far bigger. This implies that some sort of deontological code, similar to the medical one, should exist for practising volcanologists, and that illegal practice should be prohibited in this field just as it is for medicine. Whatever their field, scientists cannot remain detached from the eventual effects of their work on the everyday life of other people.

The easiest course for the authorities is naturally to choose, amongst various scientific counsels, the most pessimistic if not necessarily the best one. Their responsibility is then shielded against the worst, and if the catastrophe eventually does not occur the only reproaches they suffer are minor ones that are swiftly forgotten. To express a volcanological forecast is always an awkward business. Like civil administrators, many a volcanologist will naturally tend to be pessimistic rather than optimistic, if only to avoid exposing people to some danger he would have disregarded; he would want to avoid being accused if his own optimistic forecast proved unfounded. Though understandable enough, such an attitude is not in accord with the deontology code; a deliberately pessimistic physician is not necessarily considered a good medical doctor.

But some points seem clear. First of all, no volcanologist should state any opinion if his knowledge of eruptive phenomena is below a minimum level. Secondly, the consultant volcanologist should express his scientific opinion without altering it in either an optimistic or pessimistic way. An honest volcanologist, whatever his experience, all too frequently will not be able to describe much more than his incapacity to tell what will follow next. Nevertheless, a valuable forecast is sometimes possible and our own experience shows examples of it.

It actually happened in 1976 at the Guadelope Soufrière, when a rather unusually flat and valid conclusion seemed evident to any experienced volcanologist: absolutely no risk existed that the volcanic event which the whole Caribbean population most feared—*nuées ardentes* (glowing avalanches) of the Mt Pelée type—could happen at all in the near future. I delivered this view, while several other scientists claimed the opposite. But it was obvious that the eruptive events, which were started during the autumn of 1975 by a volcano-seismic crisis, had then passed to phreatic kinds of outbursts in which no fresh magma at all was involved, and would not develop into *nuées ardentes*. The French government nevertheless chose not to listen to this plain argument, asked geologists unskilled in volcanology and proceeded to evacuate the entire population of 73,600 people. The whole issue developed in phases.

● The seismic activity of the volcano started in October 1975 when the seismographic array of the Soufrière Observatory began recording swarms of microearthquakes. For several months, these swarms increased in both number and intensity; the first felt earthquake occurred in late March 1976. At this point several scientists expressed anxiety, more because of the high number of recorded microearthquakes than because of their actual energy. None amongst them had any experience in eruptive phenomena.

The opposite opinion was based upon the two following facts. First, far more frightening volcano-seismic crises had previously been observed in the Caribbean, and many more

still in Japan, where the most earnest attempts in the world have been made in volcanological forecasting. At the Omuro volcano in 1930, for example, 4,880 shocks located between 2–7 km below the surface were recorded in less than three months; at the Hakone volcano in 1959–60, the foci of the recorded earthquakes were located 0.8–5 km below the surface, but on the basis of the complete lack of any B-type earthquake shallower than 0.8, Minamaki predicted that no volcanic eruption would happen and he was right. In the Carribean, two volcano-seismic crises occurred at Montserrat island in 1897–98 and 1933–36, and are well known to volcanologists; neither of these well-studied crises finished with volcanic eruption. It therefore made sense not to be unduly alarmed by the 1976 seismic events of the Soufrière.

Secondly, the focal depths of the earthquakes during the Soufrière crisis were located at 2–6 km, depths quite similar to those which had characterised the Hakone and the Omuro crises. This meant that many months, or more probably years, should be necessary for the Soufrière viscous magma to reach the surface, for any magma is supposed to be far slower than its own lavas, and all the Soufrière lavas had obviously been comparatively viscous and slow. Experience has shown that most frequently the average speed of lava flows of the same kind as those of the Soufrière varies between less than 1 and about 100 cm h⁻¹. From a spot located below 6 km down, the corresponding feeding magma should therefore require several years to reach the surface. If any factors had been overlooked, or if the magma velocity would drastically increase, several months seemed a minimum for it actually to become eruptive. Consequently one could quite confidently speak of a period of several weeks, at least, before the start of any magmatic event.

To the objection that a body of molten (and therefore not seismic) magma could be stored *above* the uppermost foci, that is, between the surface and the 2 km depth—meaning that a hypothetical reservoir was located within the 1,400 m thick volcanic heap itself—the answer was that both the temperatures measured close by in the geothermal field of Bouillante (70 °C near the surface and 240 °C 350 m down) and the gas composition of the Soufrière fumaroles (in vol % : CO₂, 90–93%; H₂S, 0.6–1%; CH₄, 0.5%; H₂, 0.5–1.2%) proved such a hypothesis to be quite improbable.

● On 8 July 1976 the eruption started its second, actually eruptive, phase when so-called ‘ash’ outbursts were superimposed on the almost continuously increasing volcano-seismic activity. This second phase lasted eight months, during which 20 phreatic eruptions occurred, the last one on 1 March 1977. Each outburst lasted only a few minutes (less than 20) and expelled steam and water droplets mixed with some volcanic gases laden with ejecta. These ejecta, ‘ashes’, sands and blocks, were exclusively composed of old rock material, with no trace of any fresh magmatic material. The first of these phreatic eruptions started noiselessly and propelled a dark mushroom hundreds of metres above the top of the mountain. This plume was then driven south-westwards by the wind. From the volcano windwards, the sky became totally dark, and a thick rain of small lapilli and dust started, which lasted for about 20 minutes. Several thousand people fled as soon as the darkness cleared up.

The scientists called by the authorities were divided. Some of them felt afraid, firstly because they feared that these ‘ash explosions’ could lead either to *nuées ardentes* or to mud-flows, or both. The opposite opinion was that these risks did not exist because this eruption obviously belonged to the phreatic kind: gas analyses, measured steam temperatures, as well as examination of ejecta showed that no fresh hot magma or lava were involved and that, consequently, no *nuées ardentes* could be expected. On the other hand, as in phreatic eruptions, the

very first outburst usually is the strongest one, and as the 8 July one had generated only a small mud-flow, it was to be supposed that further lava hazards would be minor. In spite of an apparent tendency to prefer pessimistic diagnosis to reassuring ones, the government authorities were consequently prevented from evacuating people.

The second phreatic outburst occurred on 24 July. Asked by the *prefet* and having obtained the data from and opinion of my collaborators, now permanently monitoring the fluid and solid exhalation of the volcano, I concluded that there was no more danger ahead than two weeks before.

● The third outburst happened on 9 August. I was in the Ecuadorian Andes involved in a volcanological expedition with two of my collaborators, but our three colleagues were still on La Soufrière, mainly to monitor the chemical and physical evolution of the gas phase, which may be considered as a good indicator of the eruptive evolution itself. These skilled volcanologists declared that no threatening change was to be feared in the eruptive events. This was because of the following facts: first, the gas composition had not changed. The proportion of CO to CO₂ was about 10⁻³–10⁻⁴, and of H₂S to SO₂ was about 10; in addition, the focal depth of the earthquake had not moved, no trace of any magmatic activity was detectable anywhere, either in the erupting area or on the lower slopes of the volcano, and old rocky material *exclusively* was to be found around the eruptive vents.

Disregarding these volcanologists’ opinions, the civil authorities nevertheless called from France two geologists without any experience in the field of eruptive activity. The first decided to have the whole population immediately evacuated; the second decided to enforce the precautions and maintain them for several months on.

Their diagnoses were based, first, on the assumption that the seismo-volcanic crisis—up to 1,257 shocks, half a dozen of which had been slightly felt, in one day—had reached such a climax that nothing but a catastrophe could proceed, and secondly on the claimed presence of fresh volcanic glass in the erupted ‘ashes’, the meaning of which was the imminence of *nuées ardentes*. Professor C. Allègre, on 5 September 1976, produced an official report in which he stated that 50–60% of fresh volcanic glass was present in the erupted ash. He was later compelled to admit that actually no trace of fresh material had ever been detected.

According to *Le Guern* (in press) the twenty outbursts which occurred between the first (8 July 1976) and the last one (1 March 1977), as well as the more or less continuous emission observed at the active vents throughout the same period, have poured out a total output of the order of 6–10 × 10⁶ tons of steam and 1.5–2 × 10⁶ tons of ‘ashes’ and blocks. According to the same author, a surface less than 1 km long and 300 m wide received a total ‘ash’ fall 300 m thick for the whole nine-months-long event. The closest town, Matouba, some 3.5 km west (windwards) from the craters, got a total ‘ash’ fall of 5–15 mm according to the spots. The closest city, St-Claude, 4.5 km west-south-west from the erupting vents, got a total 2–5 mm thickness of ‘ash’. The longest mud-flow, which resulted from the first rain eastwards over less than 3 km.

These figures show how modest was the scale of this eruption. The above mentioned criteria and their interpretation explain how our so-called ‘optimistic’ diagnosis had been attained. The main lesson of this unfortunate experience is perhaps to confirm the absolute necessity for a good volcanological interpretation of all the available, geological, geophysical, chemical and phenomenological data before expressing any forecast. Volcanologists, just as medical doctors, should be responsible, skilled, experienced, different specialists closely co-operating with each other. And they should keep as cool as a cucumber. □

Voyage to the outer planets

Both NASA's Voyager spacecraft are heading for the outer planets. **Stuart Sharrock** describes the journey they will make

WHEN the outer planets were last aligned so that a spacecraft could visit each in turn on a single voyage, Thomas Jefferson was President of the United States. Understandably none took advantage of that opportunity. But in the 1980's, the opportunity to fly past each outer planet will occur again and if it is not seized then it will be lost for several generations.

NASA's original plan to take advantage of the 1980's alignment consisted of sending two spacecraft on a visit to every planet from Jupiter to Pluto. Nicknamed the 'Grand Tour', that project soon fell victim to budget cuts and the current Voyager mission was developed as something of a consolation prize. The Voyager mission, however, is itself ambitious enough. Its two spacecraft, launched on 20 August and 5 September, are intended to scrutinise Jupiter, Saturn and their moons, with an option for one to continue to Uranus and Neptune.

A mission to the outer planets is a radically new adventure. The inner planets, Mercury, Venus, Earth and Mars are rocky worlds which have been intensively studied. They seem to bear little resemblance to the gaseous outer planets that dominate the solar system. But very little is known about Jupiter and Saturn and next to nothing about Uranus and Neptune. Only two spacecraft have ever reported from the region beyond Mars; Pioneers 10 and 11 flew past Jupiter and returned intriguing but inconclusive data. Pioneer 11 is expected to reach Saturn in 1979.

The two Voyager spacecraft will reach Jupiter in 1979. More massive than all the other planets put together, Jupiter is an enormous ball of gas at the centre of a miniature solar system of its own. Four of its thirteen known moons are as large as some planets and Voyager will take a close look at five of them, concentrating on Io with its associated strong concentration of charged particles which appear to modulate the powerful radio bursts from Jupiter itself.

By visiting Jupiter, Voyager will be able to halve the journey time to Saturn. It will take advantage of the increase in velocity provided by Jupiter's gravitational field and should make the flight to Saturn in just over three years instead of the normal six. This 'gravitational assist' technique may be used again at Saturn to sling one of the spacecraft to a rendezvous with distant Uranus in 1986.

The spectacular rings around Saturn will be only one object for investigation. Like Jupiter, Saturn emits more energy than it receives and it has an extensive system of moons. Voyager will visit seven of the ten moons and will take particular interest in Titan, a moon larger than the planet Mercury and with an atmosphere. Titan is thought to be a far better place than Mars to search for evidence of extra-terrestrial life; the theory that life on Earth developed from a primitive reducing atmosphere implies early conditions on Earth similar to those which now exist on Titan.

Jupiter, Saturn and Titan are the prime objectives of the mission. If the first spacecraft, Voyager 1—which takes a faster route to Jupiter and was therefore the second to be launched—fails to complete its investigation of Titan, Voyager 2 will be redirected to do the job again. Only if Voyager 1 is successful will Voyager 2 fly directly towards Uranus and eventually, after a 13-year flight, to Neptune.

Special design features

Even if the mission does not extend beyond Saturn, its lifetime will be long and the distance travelled vast compared to previous missions. To cope with these conditions, the Voyager spacecraft require special design features. The familiar arrays of solar panels are missing as the energy received from the Sun as far away as Jupiter is only 4% of that at the Earth; at Saturn it is down to 1% and at Neptune 0.1%. Instead, radioisotope thermoelectric generators, which should last the journey to Neptune,

supply power to the spacecraft. The power requirements are great because signals have to be sent across such large distances. The spacecraft will be the first to communicate via the high frequency X-band channel, allowing high data rates which are essential at various stages of the mission.

The 11 instruments on board include television cameras, infrared and ultra-violet spectrometers, charged particle detectors, magnetometer and plasma wave detectors and, for the first time on an interplanetary probe, radio wave detectors. All experiments have to operate at liquid nitrogen temperatures and because of the length and complexity of the mission almost everything that could fail has a back-up system—including the two spacecraft themselves which are identical.

With such a complex mission the chance of something going wrong is high, but Voyager has already had more than its fair share of troubles. Very near 20 August, the launch date of the first spacecraft (designated Voyager 2), computer problems were discovered. A rapid switch of the two spacecraft meant that Voyager 2 could be launched on time and that Voyager 1 which was due for launch on 1 September would be delayed by a few days. But the first launch was rough. Computer failure occurred again, there were apparent problems with gyroscopic stabilisation and the boom containing the television cameras did not deploy fully. There were even speculations that the spacecraft had been struck by its own discarded rocket engine. The problems were overcome but inevitably the mission has already been compared with Mariner 10 which had to be nursed continually during its 17 month trip to Venus. The outlook for Voyager 1, though, is brighter. According to NASA it went up on schedule and with no hitches on 5 September. □

Voyager mission highlights

Date	Event	Range (km)	Resolution (km)	Spacecraft
20 August, 1977	Launch			Voyager 2
5 September, 1977	Launch			Voyager 1
15 December, 1978	Start Jupiter imaging	80×10^6	1.5×10^3	Voyager 1
5 March, 1979	Closest approach Jupiter	278×10^3	6	
5 March, 1979	Io	22×10^3	0.5	
April, 1979	Conclude Jupiter imaging			
20 April, 1979	Start Jupiter imaging	75×10^6	1.5×10^3	Voyager 2
10 July 1979	Closest approach Jupiter	643×10^3	13	
August, 1979	Conclude Jupiter imaging			
August, 1980	Start Saturn imaging	100×10^6	2×10^3	Voyager 1
11 November, 1980	Titan	4×10^3	0.5	
12 November, 1980	Closest approach Saturn	138×10^3	3	
January, 1981	Conclude Saturn imaging			
June, 1981	Start Saturn imaging	100×10^6	2×10^3	Voyager 2 (assuming Voyager 1 successful at Titan)
27 August, 1981	Closest approach Saturn	102×10^3	2	
27 August, 1981	Edge Saturn rings	38×10^3	0.8	
October, 1981	Conclude Saturn imaging			
January, 1986	Uranus encounter			
September, 1989	Neptune encounter			

USA

Action on antibiotics

Colin Norman reports from Washington on the Food and Drug Administration's (FDA) recent action to ban the use of some antibiotics in animal feeds

AFTER more than a decade of controversy and several years of vacillation, the Food and Drug Administration (FDA) last week took the first formal step towards removing antibiotics from animal feeds, a step long urged by some scientists but bitterly resisted by the agricultural and pharmaceutical industries. The FDA issued a regulation which, provided it survives an expected slew of legal challenges, would prohibit the addition of small amounts of penicillin to premixed feeds for pigs and poultry. A similar regulation banning tetracyclines will follow soon, FDA announced.

Antibiotics have been added to animal feeds in the United States for 25 years, following a discovery that they help promote growth and hence shorten the road to the slaughterhouse. The practice has become so common that, according to figures collected by FDA, some 48.6% of all antibiotics produced in 1975 were added to animal feeds. In the mid-1960's, however, some scientists began to warn that such widespread use of antibiotics may be contributing to the growing problem of antibiotic resistance among microbes which cause human and animal diseases—resistance which is seriously compromising therapeutic uses of some drugs.

One concern is that bacteria which cause disease in animals will become resistant to antibiotics, thus making the disease more difficult to treat. Another concern is that the resistant bacteria may be transferred from animals to humans either directly or through meat, milk or eggs. An added dimension to the concern is the fact, which is now widely established, that antibiotic resistance can be passed from one strain of bacteria to another—for example, an animal bacterium which becomes resistant to an antibiotic may pass its resistance to a human pathogen through a chance encounter. (Antibiotic resistance is largely determined by so-called R-plasmids, tiny rings of DNA which sit inside a bacterial cell and are reproduced each time the bacterium divides).

Those concerns led the British government in 1968 to establish a special committee, under the chairmanship of Professor Sir Michael Swann,

to review the problem. The Swann Committee concluded "the administration of antibiotics to farm livestock, particularly at the sub-therapeutic levels, poses certain hazards to human and animal health", and it recommended a ban on the routine addition to animal feeds of antibiotics used to treat human and animal diseases. The British government swiftly adopted the recommendation, and other European governments have since followed suit.

Following publication of the Swann Report, FDA established a task force of its own to look into the matter, and in February 1972, the task force recommended that restrictions similar to those adopted in Britain should be imposed in the United States. But the task force put an important proviso on that recommendation: the ban should be delayed for up to two years to allow the drug industry time to try to prove its claim that the use of antibiotics in animal feeds does not lead to human health hazards. The deadline slipped by, but FDA took no formal action and non-therapeutic drug use on the farm has continued to increase. According to FDA estimates, 1.2 million pounds of antibiotics were added to animal feeds in 1960, 7.3 million pounds in 1970, and an average of 7.7 million pounds each year between 1971–75.

The first public indication that FDA hadn't completely forgotten about the problem came last April when Donald Kennedy, the new FDA commissioner, announced that FDA was finally prepared to act. "Although we can point to no specific instance in which human disease is more difficult to treat because drug resistance has arisen from an animal source, it is likely that such problems have gone unnoticed", Kennedy said. "The benefit of using these drugs routinely as over the counter products to help animals grow faster or in prophylactic programs does not outweigh the potential risk posed to people", he added. Consequently, Kennedy announced that FDA would seek to eliminate use of penicillin and severely restrict use of tetracyclines in animal feeds. (It should be noted that some antibiotics most critically needed for therapeutic use in man and animals, such as chloramphenicol, semi-synthetic penicillin, gentamycin, and kanamycin, have never been allowed in animal feeds.)

Last week FDA formally published a regulation barring use of penicillin in animal feeds, (it is used chiefly for pigs and poultry), and a similar ban



Donald Kennedy: new commissioner

on tetracyclines is expected in the next few weeks. Other antibiotics are under consideration, but FDA officials say they do not expect to take action on them in the near future.

The FDA decision is sure to be controversial, and the agriculture and pharmaceutical industries are expected to challenge it in court. One reason is the economic impact. FDA reckons that eliminating penicillin alone will cost some \$12 million in lost sales and increase feed costs: the impact of eliminating tetracyclines is expected to be even higher.

The industry is expected to argue on several different grounds. First, there is no demonstrated case in which antibiotic resistance derived from lacing animal feeds has led to untreatable bacterial infection in humans. Second, that resistance to penicillin and tetracyclines is already so widespread that banning their use in animal feeds will make little difference. And third, the chief reason why antibiotic resistance has become such a serious medical problem is that antibiotics have been overused and inappropriately prescribed by the medical profession.

The industry has a right to appeal the FDA decision by filing a complaint and requesting a public hearing, a process which could take up to a year and use of the drugs in animal feeds would be permitted until the appeal is exhausted. After that, the industry could go to court in an attempt to overturn the ban.

Even if the FDA action is ultimately upheld, the use of antibiotics in animal feeds will not be ruled out entirely. The pharmaceutical industry has produced a number of antibiotics which are not used to treat human disease and which may therefore be safer replacements for penicillin and tetracyclines. According to FDA officials, those antibiotics are not in widespread use because they are more expensive than penicillin and tetracyclines. □

USSR

Discrediting the dissidents

Vera Rich reports on efforts within the Soviet Union to play down the strength of dissident opinion

THIS autumn, the Soviet Union will be celebrating the sixtieth anniversary of the October Revolution. Unlike previous anniversaries, this one will take place against a background of dissident opinion which, to the obvious embarrassment of the official concept of Marxist-Leninism as a 'scientific' basis for society, includes among the dissidents a number of scientists.

Accordingly, it would seem that there is a last-minute campaign to reduce the impact of the dissident movement on the outside world before the celebrations commence, by means of media propaganda.

A recent article in the prestigious *Literaturnaya Gazeta* attacked the Oxford conference on physical chemistry and hydrodynamics which was held in honour of the sixtieth birthday of Corresponding Academician Veniamin G. Levich. Totally ignoring the scientific work of the meeting (for which see *Nature* 268, 298-299, 28 July, 1977), the article set out to prove that the organisers, Sir Derek Barton and Professor Brian Spalding, had been little more than figure-heads for organisers

"far removed from science". It cited the fact that a few of the Soviet scientists invited were already dead. Professor Spalding said that he had found the names of the Soviet scientists in a 1976 edition of the *World of Learning* and added that a few clerical errors were inevitable in organising a large conference.

Commenting on the article's other main charge, that Professor Levich had long ceased to work in science, Professor Spalding pointed out that this was a particularly wicked accusation, since it was the Soviet authorities' harassment that hindered Levich from continuing his scientific work. Spalding further noted that the author of the article seems to have violated the secret archives of the Academy of Sciences, concerning Levich's failure to be elected full Academician. Since the Academy elections are still by secret ballot, this seems ominous.

Somewhat similar in tone were the numerous broadcasts (largely in western languages) which preceded the Honolulu Congress of the World Psychiatric Association (WPA). The message was simple: Soviet psychiatry is a purely therapeutic activity, and all suggestions of the psychiatric repression of dissidents are simply propaganda. A number of western psychiatrists were cited

as having concurred in the diagnoses given to alleged dissidents. However, Dr Avtandil Papiashviliar, a psychiatrist who recently emigrated to the West, said in London that such claims were "a straightforward lie". He further maintained that during the WPA Symposium in Tbilisi, in 1974, the KGB had arranged for a genuine mental patient to break into the meeting, claiming to be persecuted by the police, in the hope that the visiting delegates would first protest, and then have to admit that the man was indeed in need of psychiatric attention. In the event, the Honolulu Congress passed (albeit narrowly) a motion censuring Soviet psychiatric repression of dissidents and the Soviet delegation decided not to secede from the WPA.

Honolulu can rightly be claimed as a success for all those concerned with the human rights movement in the Soviet Union. No such success, however, can yet be claimed for the recent Anatolii Shcharanskii hearing in the Swedish House of Parliament. Shcharanskii, a young mathematician who was a founder member of the Moscow 'Helsinki Monitoring Group', was arrested last March accused of spying for the CIA. The Stockholm hearing, before Hårje Stenberg, Master of the Swedish Appeal Court, not only presented circumstantial evidence of Shcharanskii's innocence, but also pressed for his human right of a fair trial. □

NEW ZEALAND

●The latest budget from the New Zealand government gives a substantial fillip to the country's Department of Scientific and Industrial Research. Although the country is going through difficult economic times expenditure on science-related activity is to rise by 16% to \$NZ 35 million for the financial year 1977-78 (£1=\$NZ 1.78). That increase should keep pace with inflation and provide for some real increase as well but staff figures in the Department continue to be held at just over 2,000.

The budget allocation breaks down into \$NZ 14 million on agricultural work; \$NZ 3.15 million on energy; \$NZ 3.41 million on manufacturing; \$NZ 6.81 million on the natural environment; and the balance on miscellaneous activities. The department is making a special effort on energy research in efforts to discover long-term alternatives to New Zealand's reliance on overseas sources of fuel.

Meanwhile university research personnel gained some benefit from the budget, having gone through a very

lean time. Last year the Universities Research Fund was denied funds by the government in a savage attack on spending. This budget allocates the Fund \$NZ 750,000.

●A master file of over 14,000 earthquakes in earthquake-prone New Zealand has been established. The file has been placed on computer tape and runs from the first recorded earthquake in AD 1460 to the most recent shocks of 1975. The file will greatly assist the task of scientists working out the geographic patterns of earthquakes and researchers in the construction industry.

●A Royal Commission on Nuclear Power is sitting in New Zealand in an effort to discover the real needs of the country before any step is taken to set up nuclear power stations. The Labour opposition has been vigorously opposed to nuclear power but the Conservative government is waiting on the Commission. The Minister of Energy Resources, Mr George

Gair, says he believes the country has at least five years in hand before any decision need be taken.

●The government has set up a working party to examine the need for legislation to control genetic engineering experiments which are going on in New Zealand. So far, the few scientists working in the field have agreed to abide by the Williams Committee guidelines from the United Kingdom, but now the government believes further study is needed.

●Just to produce and supply electricity to New Zealand's three million people is calculated to cost about \$100 for every person. In an effort to recoup the costs the government continues to allow energy prices to rise. But in the search for alternative sources the government will control a major on-shore drilling programme, take over an off-shore mining company and the Natural Gas Corporation which controls the Maui gas field.

Bruce Wallace

IN BRIEF

Instant data

By 1979 experimental data should be travelling between four major European high energy physics laboratories almost as fast as it can be processed. Under a project called STELLA (Satellite Transmission Experiment Linking Laboratories), which will involve the UK's Rutherford Laboratory, DESY in Germany, Saclay in France and CERN in Geneva, the data will be transmitted via the Orbital Testing Satellite, the European Space Agency's communications satellite scheduled for launch on 8 September.

The project has recently won approval from the Council of Ministers of the European Economic Community for funding worth SF 1.2 million, which will be spent on building a small satellite earth station and associated computer at CERN. It should demonstrate to industry and Europe's posts and telecommunications organisations, the value of using the new design of

European satellite for transmitting data almost instantaneously at very low error rates.

Spacelab selection

Last week, 12 European countries put forward 53 candidates for selection as Europe's first astronaut. Whoever is chosen will travel with one American on the first mission of the European Space Agency's (ESA) Spacelab, a reusable space laboratory which will be put into earth orbit in 1980 by NASA's Space Shuttle.

By the end of this year the 53 candidates will have been whittled down to six who will undergo further tests by NASA and ESA. Before the middle of next year, three will have been chosen for intensive training for the first Spacelab mission. A few months before the launch, one will be finally selected to travel in Spacelab while the other two work on ground-based activities.

The UK has put forward five candi-

dates, the maximum number any member country of ESA was allowed to submit. They are William Grut from Surrey University, Geoffrey Firmin from Associated Nuclear Services Ltd, Arthur Ince from East Birmingham Hospital, Keith Mason from the Mul-lard Space Science Laboratory and M. J. Rycroft from Southampton University.

Whilst Europe has been selecting candidates for Spacelab, NASA has been working out how much to charge customers for flights on the Space Shuttle. The cheapest fare, at \$10,000, is likely to be for payloads involving research, weighing less than 200 pounds and with a volume less than five cubic feet. The maximum fare will be around \$21 million for full use of the Shuttle by non-US government customers. NASA already has 40 payloads for 1980, the first year of the Shuttle's operation. Spacelab is due to go up on Space Shuttle flight 11 for a fare somewhere in the middle of the price range.

AGRICULTURAL productivity throughout the world is increasingly dependent on the liberal use of chemical fertilisers, particularly those containing nitrogen. This is true of the sophisticated farming systems in Europe and North America, and where the 'green revolution' is successful in developing countries. A substantial amount of energy and oil goes to produce these fertilisers, and therefore many people are doubtful whether their continued and increasing use will be possible in an energy-hungry world in the twenty first century.

As I showed on 25 August, oil-hungry tractors actually use less energy than horses on the farm. Chemical fertilisers may, on balance, increase the amount of energy available to the farmer and to the population he serves. Returning to our Kalahari bushman, so idolised by some ecologists for his allegedly-economical use of energy; he certainly extracts many more calories than he puts into the system, but he only obtains enough food from a square mile or more of territory to feed himself. The modern arable farmer feeds several people on the produce of a single acre. He puts more into the system, but he takes a great deal more out. The difference between his energy expenditure, in cultivation, spraying and harvesting, and that stored in his crops, is several hundred times that of the bushman's total productivity per unit area.

In the UK we use less than a million

tons of oil to produce over a million tons of nitrogenous fertiliser. Only a part of this is used on cereal crops which now yield annually some fifteen million tons of grain and ten

Food for energy



KENNETH MELLANBY

or more million tons of straw. Without the fertiliser the yields would be reduced by a factor of two or even three. The energy wasted by burning some three million tons of unwanted wheat straw could, in theory, power all our tractors and the factories making our manures.

Unfortunately we cannot, at present, use the straw in this way, but we should surely try. To turn straw, readily available on the surface of the

ground, into useable fuel should be a simple problem for our research workers to solve. A fraction of the money used for more esoteric agricultural research would be well invested to this end.

Although arable farming uses energy efficiently, further improvement is desirable, if only to reduce costs. In Britain we waste a substantial amount of organic manure which would save synthetic chemicals and might improve soil structure, but which, when fuels are cheap, is expensive to use. As fuel prices rise, this waste will be avoided. Cultivation may be further reduced when weeds are controlled by modern herbicides. Though it will probably be a long time before research to make it possible for cereals to fix atmospheric nitrogen gives positive results, we now make too little use of the ability of leguminous crops to perform this function. World phosphate supplies are limited, but fungal mycorrhizae may be increasingly used to mobilise insoluble compounds in the soil. All the indications are that in future agriculture will use less and not more energy, without any loss in productivity.

Thus though we may face a global energy shortage in the next twenty five or thirty years, so that the extravagant use of cars and aeroplanes may have to be restricted, and we may have to stop overheating our houses and offices, we should, with a rational system of priorities, have plenty of energy to produce our food.

correspondence

Alaskan earthquake

SIR,—The item on Alaskan earthquakes by P. J. Smith (7 July, page 12) is misleading—the point at issue is not the frequency of seismic events in Northern Alaska, but their magnitude.

The section of the pipeline of which he writes was required to be designed to withstand an earthquake of magnitude 5.5 without spillage; the federal requirements were only drawn up after extensive analysis by a variety of groups not depending solely on seismic data. Obviously these people appreciated that the absence of recorded activity was more apparent than real. I can see no reason to assert, as Smith does, that a report on the frequency of seismic events in the magnitude range 1 to 4 for a section designed to resist magnitude 5.5 shocks reveals an example of oversimplification through ignorance.

I might add that many crude oil pipelines around the world were built before good seismic data became available (for instance in Iran early in the century) and there is no record of any of them ever having ruptured as a result of an earthquake.

Yours faithfully,

F. G. LARMINIE

British Petroleum, London, UK

China and scientific unions

SIR,—In your editorial (28 July, page 283), you accuse The People's Republic of China of "trying to import" politics into the international scientific unions. You ignore the fact that politics must inevitably play a role in the unions as they are at present constituted. For example, how can a union, composed of member countries and financed by their governments, fail to consider the question of what is meant by a country?

In discussing Chinese representation in the unions one should remember that Taiwan has never been claimed to be other than a province of China by the authorities either in Peking or Taipei. Thus recognising Taiwan as an independent country would place the international unions in the strange position of giving Taiwan a status not claimed for it by any international authority including the United Nations and UNESCO, or even by its own administration. Although this is a course of action that was taken by

several of the unions (eg the International Astronomical Union in 1958), such a decision withdrew the right of the Academia Sinica in Nanking to represent a part of China which it had previously represented and in my view thereby often violated the statutes of the union.

Such actions took place during the fifties when a successful campaign was instigated by the US State Department to secure the membership of China in the unions for representatives of the Academia Sinica in Taipei. Often the Taiwanese applied and were admitted to membership of unions for which any relevant activity in Taiwan was miniscule or non-existent. The acquiescence of ICSU and its unions in this attempt to isolate the Chinese from the international scientific community is responsible for the bitterness with which many Chinese scientists still view ICSU and the insistence with which they demand that the previous decisions be reversed.

Yours faithfully,

GEORGE K. MILEY

Leiden, Netherlands

No arms link

SIR,—In your 'in brief' column (18 August, page 582) you state that "African National Congress officials are reported as saying that 15 German research institutions, including the Max Planck Institute for Nuclear Physics at Heidelberg, have contributed to South Africa's arms programme". We would like to state that the Max Planck Institute for Nuclear Physics is devoted only to basic research in the physics of nuclear structure and nuclear reactions. This research is completely unrelated to arms production.

Yours faithfully,

U. SCHMIDT-ROHR

*Max Planck Institute for
Nuclear Physics,
Heidelberg, West Germany*

Sunflower: a misnomer?

SIR,—It is commonly believed that the movement of the sunflower (*Helianthus annuus*) is a typically heliotropic movement and that with a certain lag, heads follow the path of the sun across the skies in the wake of its daily east-west movement. Observations on a field of

sunflowers growing in the essentially cloudless Mediterranean summer climate at the Ma'abaroth kibbutz settlement in the Sharon plain in Israel reveal that during the night the extent of sunflower head movement is more rapid than during the day.

At dusk all heads do indeed face west, but by 2 am, before any trace of light on the horizon, all heads had reverted to an east facing position. Calculated average velocity of movement during daylight is $\pm 13^\circ$ per h as against $\pm 26^\circ$ per h in the dark.

Furthermore, when complete rotation towards the east had occurred, by 2 am the heads were held at $\sim 40^\circ$ – 45° above the horizontal and upon the appearance of the sun heads 'nodded' eastwards to the extent of a further 15° , as if slightly bowing to greet the rising sun, and only subsequently started their daily journey westwards.

These observations suggest an endogenous 'spring' mechanism, apparently wound up by the sun and released in the dark or light period. The fact that the night movement is twice as rapid as that of the day seems to support the concept that the 'spring' release occurs in the dark and the winding in the light since unhindered mechanical spring release is presumably more rapid than winding, which demands active energy input. If, however, in the future the converse proves to be true, botanists may possibly reach the conclusion that the designation 'sunflower' (of similar etymology in most languages) may be a misnomer and in its stead 'nightflower' may be suggested.

Yours faithfully,

YA'ACOV Y. LESHEM

*Bar-Ilan University,
Ramat-Gan, Israel*

'The Iron Sun'

SIR,—Roman Znajak, in his review of my book *The Iron Sun* (30 June, page 867) suggested, no doubt unintentionally, that I had misquoted Einstein. In fact, I had made a proof-reading error. The passage in Einstein's *The Meaning of Relativity* to which I had wanted to draw attention is on page 26 of the 1973 Chapman and Hall Science Paperback edition.

Yours faithfully,

ADRIAN BERRY

London W8

news and views

Predators that switch

from Robert M. May

PREDATION is an important factor in enhancing the diversity of plant and animal communities. One classic example of the way predation can promote coexistence among competitors that would otherwise not persist together is Paine's (*Am. Nat.* **100**, 65; 1966) study of what happened when the dominant predator (a starfish, *Pisaster*) was systematically removed from an intertidal community of marine invertebrates: in less than 2 years, the community collapsed from 15 to 8 species. A variety of other broadly similar examples, from communities on the land and in the sea, have been drawn together by Connell (in *Ecology and Evolution of Communities*, (eds Cody & Diamond) Harvard University Press, 1975).

Suppose we have two or more competing species, one of which would exclude the other in the absence of predation. One pervasive mechanism whereby a predator can enable these competing prey species to coexist is by directing a disproportionately large amount of its attention to the prey species that happens to be most abundant at any one time. This 'switching', to concentrate attacks largely on the most common prey species, may be a simple consequence of the predator's searching behaviour; for example, many predators form a 'search image', which is likely to be set by the locally most abundant prey species. Such patterns of 'switching predation' are common among vertebrate predators (Holling *Can. Ent.* **91**, 293; 1959; Murdoch *Ecol. Monogr.* **39**, 335; 1969), and also among many invertebrate ones (Murdoch & Oaten *Adv. ecol. Res.* **9**, 1; 1975; Hassell Lawton & Beddington *J. Anim. Ecol.* **46**, 249; 1977).

There is no doubt that predation with switching exerts a generally stabilising effect on the dynamics of a community. Elementary accounts of the phenomenon are, however, liable

to two common misapprehensions, which several recent papers have helped to clarify.

The first misunderstanding concerns the extent to which switching predators contribute to the overall stability within the single trophic level of the competing prey species. Too often, simple accounts portray switching as a kind of Universal Stabiliser. In fact, as first emphasised by Steele (*The Structure of Marine Ecosystems*, Harvard University Press, 1974), although switching in a 1-predator/ n -prey system (in which one species of predator preys on n species of prey) will tend to stabilise the competitive interactions, it can result in overall instability if each individual 1-predator/1-prey system is by itself unstable. Steele lucidly combines qualitative reasoning, analytic results and a computer simulation to show that in this case the internal dynamics within the prey trophic level will settle to some stable state (some stable proportion of each prey species), but nonetheless the overall prey/predator system remains subject to the basic instability manifested by the individual prey/predator interactions. Steele's discussion is for overlapping generations, with differential equations; another illuminating study is by Comins and Hassell (*J. theor. Biol.* **62**, 93; 1977), who consider a 1-predator/2-prey system with non-overlapping generations obeying difference equations. In the model discussed by these authors, it can be seen explicitly that predator switching enlarges the domain of parameter space wherein the two prey species can coexist, yet it has no influence on the boundaries of the domain of parameter space wherein the overall prey/predator system is stable. These themes are succinctly summarised by Murdoch (*Am. Nat.* **111**, 383; 1977).

The second misunderstanding concerns the extent to which generalised predation, without any switching, can help competing species to coexist. In very simple mathematical models

(where all prey have the same intrinsic growth rate) a perfectly generalised predator, which attacks all prey species in impartial fashion, makes no difference to competitive coexistence among the prey; the internal dynamics within the trophic level of the prey is unaffected by predation of this evenhanded kind (Van Valen *J. theor. Biol.* **44**, 19; 1974; May in *Some Mathematical Problems in Biology* **6** (ed. Cowan) Amer. Math. Soc., Providence, Rhode Island, 1974). But a small amount of switching behaviour on the part of the predator alters this situation, and can make possible a significantly greater degree of 'niche overlap' among the competing prey species than would otherwise be possible (Roughgarden & Feldman *Ecology* **56**, 489, 1976; Janson, in manuscript, 1975; Murdoch and Oaten, *op. cit.*). Roughgarden and Feldman's study is a particularly clear and rigorous account of the way predator switching can permit increased niche overlap among the prey; yet at least one recent publication quotes it as affirming the broader and unqualified conclusion that "predation increases niche overlap."

Of course, predation can modify the basic conditions under which competition takes place, even if there is no switching. Thus generalised predation may promote coexistence if, among the prey, the less able competitors have higher intrinsic growth rates. Specific examples arise in pastures subject to 'predation' by grazing. Here species diversity can be increased because plants that would be shaded out, or otherwise outcompeted, are given a chance. Harper's (in *Diversity and Stability in Ecological Systems*, US Natn. Bur. Standards, Springfield, Virginia, 1969) review of such situations includes deliberate and accidental (following myxomatosis) experiments where rabbits were withdrawn, and controlled experiments in pastures grazed by sheep. Paine's example is of this general kind.

The above discussion (despite its

pedantic nit-picking) is directed mainly at affirming that predation in general, and particularly predation with switching, can make it easier for prey species to coexist, and thus lead to communities that are more diverse at the species level. If we shift our perspective to focus on the coexistence of different genotypes within a single prey species, analogous considerations arise. By concentrating its attacks on the most common genotype, a switching predator can enable the rarer genotype to enjoy higher fitness; this amounts to 'frequency-dependent natural selection', and is a powerful mechanism whereby genetic polymorphism can be stably maintained. This case has been argued by Clarke (*Sci. Am.* **233** (2), 50; 1975), who emphasises that predator switching is only one of a variety of biologically plausible ways in which frequency-dependent natural selection may be realised. □

Two-faced neurones

from Key Dismukes

THE classic concept of neuronal operation—dendritic summation of synaptic inputs, generation of all-or-none action potentials, propagation along axons, and release of neurotransmitter from terminal boutons—increasingly seems to be an oversimplification. For several years evidence has been accumulating for an array of information processing operations that go beyond the framework of traditional neuronal doctrine (Schmitt, Dev & Smith *Science* **193**, 114; 1976). In the retina, for example, the dendritic projections of axonless local neurones interlace with the synaptic connections of other cells communicating bidirectionally without the use of action potentials.

Recent biochemical studies suggest that the traditional view of the neurone as transmitting information in only one direction will have to be modified. The dopaminergic cells of the substantia nigra have provided the most striking evidence, recently reviewed by L. Iversen (at the Neurosciences Research Program Study Program at Boulder, Colorado in July (to appear in *The Neurosciences: Fourth Study Program* (eds Schmitt & Worden) MIT Press), in whose laboratory much of the work has been done.

Cytochemical studies in several laboratories have indicated the presence of dopamine and the enzymes of its bio-

synthetic pathway not only in cell bodies of the substantia nigra, but also in dendritic fields. This surprising discovery raised the suspicion that dopamine might be released from dendrites, in addition to its normal secretion from axon terminals. To investigate this possibility, Iversen's group studied the release of dopamine from nigral tissue *in vitro* by applying the superfusion chamber techniques that have been widely used to elucidate mechanisms of neurotransmitter secretion. In contrast to the usual conditions, the tissue in these experiments contained no axon terminals bearing the transmitter being studied (the dopaminergic axons of the substantia nigra terminate remotely, in the forebrain). Thus, if dopamine release occurred, it would have to be from the dendrites and/or the cell bodies.

Dopamine was in fact found to be released from the substantia nigra, in a fashion closely paralleling that seen in the neostriatum, which does contain dopaminergic nerve terminals (Geffen *et al.* *Nature* **260**, 258; 1976). Depolarising tissue fragments by raising the external concentration of K^+ produced a substantial efflux of dopamine. This efflux was blocked by removing Ca^{2+} from the superfusion medium, or by Mg^{2+} concentration. Ca^{2+} flow is known to be a central mechanism in neurotransmitter release; thus dopamine efflux from substantia nigra satisfies one of the major criteria for distinguishing the specific secretion of a transmitter molecule from nonspecific leakage through membranes.

Most of these experiments were performed by labelling endogenous dopamine pools with 3H -dopamine and following the appearance of radioactivity released into the superfusion fluid. This was possible because the substantia nigra possesses a high-affinity uptake mechanism for dopamine. This is an interesting fact in itself, because these specialised uptake systems are generally associated with nerve terminals, and are thought to provide a means of inactivating transmitter molecules released into the synaptic cleft. Verification of the results obtained with 3H -dopamine *in vitro* was obtained by using a highly sensitive radioenzymatic assay to demonstrate the minute efflux of endogenous dopamine released by depolarisation. Release of dopamine from substantia nigra has recently been directly demonstrated *in vivo*. Nieoullon *et al.* used push-pull canuli to superfuse the substantia nigra with 3H -tyrosine and to monitor the efflux of 3H -dopamine synthesised intracellularly (*Nature* **266**, 375; 1977). As in *in vitro* experiments, elevated K^+ concentration stimulated the release of 3H -dopamine.

Does dopamine released from the substantia nigra come from dendrites or from cell bodies? That question was answered by making use of the segregated topography of this nucleus. Dopaminergic cell bodies are confined to a central *zona compacta*, whereas dendritic processes invade both that zone and the surrounding *zona reticulata*. 3H -dopamine was taken up and released by depolarisation in both regions indicating that dendrites are indeed capable of secreting neurotransmitter.

What are the targets of dopamine released from the substantia nigra? Evidence suggests that one possibility is the GABA-containing axons from the striatum which terminate in the substantia nigra. Dopamine was recently reported to stimulate the overflow of 3H -GABA from nigral tissue *in vitro* (Reubi, Iversen & Jessell *Nature* **268**, 654; 1977). This stimulation was blocked by antagonists of dopamine, or by lowering Ca^{2+} concentration, indicating that the effect was specific.

Iversen also reported that modulation of GABA release from striato-nigral terminals is also suggested by lesion studies. Neural responses to dopamine seem generally to be mediated by cyclic AMP, whose formation is controlled by adenylate cyclase linked to dopamine receptors. Lesions of striato-nigral pathways containing GABA were found to cause disappearance of dopamine-sensitive adenylate cyclase in the substantia nigra. In contrast, destruction of substance P pathways or of dopaminergic cells themselves did not reduce activity of this adenylate cyclase.

Aghajanian and Bunney have shown that microiontophoretic application of dopamine to the substantia nigra *in vivo* inhibits the firing of dopaminergic cells (Neunyn-Schmeidebergs *Archs Pharmac.* **297**, 1; 1977). This finding, coupled with the GABA studies, suggests a functional role for dendritic release of dopamine. GABA should inhibit the firing of dopaminergic cells in the substantia nigra. Dendritic release of dopamine, by stimulating GABA release from pre-synaptic terminals, could provide a local feedback mechanism to modulate the activity of dopaminergic neurones.

Morphological features suggest that dopamine may be released from dendrites by a different mechanism from that in axon terminals. Iversen reported that electron micrograph studies give no suggestion that dopamine-bearing dendrites contain synaptic vesicles. (This raises a puzzling question about the function of Ca^{2+} , whose role in secretion is believed to involve triggering extrusion of vesicle-bound

transmitter). Nor is there any indication that dopaminergic dendritic elements form synaptic connections with other cells. However, several authors have suggested, on the basis of morphological evidence, that amine neurotransmitters in the brain can be released nonsynaptically (Chan-Palay *Cerebellar Dentate Nucleus* 418-425, Springer Verlag, Berlin, 1977, and references therein). This mode of secretion could be aimed at more distributed targets, and might represent a form of communication intermediate between the exclusive addressing of synapses and the broadcasting of hormones.

Giant molecular clouds

from M. G. Edmunds

A workshop on Giant Molecular Clouds in the Galaxy was held at Gregynog Hall, Newtown, Wales from 8-13 August 1977. It was organised by University College, Cardiff.

THE recent rapid growth in knowledge of the distribution of the carbon monoxide (CO) molecule in the Galaxy by observation of its line radiation at millimetre wavelengths has led to the discovery of a new and very significant component of galactic structure; the Giant Molecular Cloud. The importance of CO is as an indicator of the otherwise virtually unobservable hydrogen molecule H_2 . Where CO forms, it is expected that H_2 will also form, and since hydrogen is by far the most abundant element in the Galaxy, the CO maps can trace the overall density of the interstellar medium. The result of surveys such as those outlined by P. M. Solomon (Stonybrook, New York) suggest that there exist very large 10-80 pc clouds of molecules with masses which are typically 10^5 solar masses and may even reach as high as 10^7 solar masses. Of particular importance have been new maps in ^{13}CO radiation which do not suffer from the severe optical depth uncertainties of ^{12}CO but confirm the large masses of the clouds. The densities are high in these clouds, on average about 700 molecules per cubic centimetre, compared with a value of perhaps one atom per cubic centimetre in the general interstellar space between the clouds.

M. G. Edmunds is in the Department of Applied Mathematics and Astronomy at University College, Cardiff.

Infectious provirus synthesised *in vitro*

from Robin Weiss

ON page 122 of this issue of *Nature* Ellen Rothenberg and her colleagues describe the *in vitro* synthesis of infectious DNA of murine leukaemia virus. Although this feat of high fidelity reverse transcription was first announced more than a year ago, and this paper comes as no surprise, it does mark the culmination of several years investigation of reverse transcriptase activity *in vitro*. The successful transfection of a retrovirus by DNA extracted from infected cells was first achieved by Hill and Hillova (*Nature new Biol.* **237**, 35; 1972) and it constituted a formal proof of Temin's DNA provirus hypothesis. Now the MIT laboratory's improved techniques have yielded complete DNA transcripts *in vitro* which show single-hit dose response for infectivity.

The infectivity of the transcripts is resistant to protease and ribonuclease but is completely destroyed by deoxyribonuclease. The structure of the infectious DNA molecule is curious. It appears that a full-length negative strand is synthesised and shorter segments of positive-strand DNA are formed in duplex with it. Transcripts synthesised in the presence of actinomycin D are not infectious; whether this is attributable to the absence of positive-strand DNA or to incompleteness of the negative-strand transcripts is not clear. The data and their significance are so clearly presented that any further commentary here is superfluous.

The importance of the clouds lies not only in their being easily the most massive individual objects in the galactic disk, but also because they are regions of very active star formation.

The actual number of these clouds in the Galaxy was a matter of some contention, depending to some extent on whether the giant clouds are regarded as a single gravitationally bound system, with complex subcondensations, or as a superposition of smaller independent subunits as proposed by M. Gordon (US National Radio Astronomy Observatory). Most participants were content to accept that each cloud really is a single large complex, and the probable number would then be of order 1,000-4,000, constituting the major part of the mass of interstellar material in the Galaxy. The clouds are not uniformly distributed, showing a very marked 'ring' structure with a concentration of the clouds between 4 to 8 kpc from the centre. It is inter-

esting that the distribution of clouds has not yet been shown convincingly to indicate spiral structure in the disk, although A. Pedlar (Jodrell Bank) claimed some slight evidence from a formaldehyde molecule survey. The Galactic Centre itself is the other major concentration of molecular clouds, a fascinating complex reviewed by N. Scoville (University of Massachusetts) containing as much as 10^7 to 10^8 solar masses, with recent data indicating that a model with an expanding spiral pattern extending right in to the centre may be more attractive than previously proposed expanding disk models.

The formation of stars in the molecular clouds is clearly demonstrated by the existence of infrared sources, caused by the inevitable heating of gas and dust as it collapses under its mutual gravitational interaction, and further heating once the stars have started nuclear energy generation. One obvious site for star formation in the clouds is the characteristic small 'cores' where higher than average temperatures and densities indicate that collapse may be taking place. Much evidence was presented that although low mass stars may form throughout the clouds, the high mass stars over about 12 solar masses form only near the edge of the clouds. B. Elmegreen (Harvard University) and C. Lada (Center for Astrophysics, Cambridge, Massachusetts) claimed this as good evidence for a mechanism of inducing the collapse of further protostars at the edges of the clouds by the effect of compression between shock and ionisation fronts driven into the clouds by radiation, once one bright massive star has formed. The existence of ionised hydrogen regions at the edge of molecular clouds seems very common, and F. Israel (Caltech) presented evidence that most bright, dense regions are of this type, with ionised radiation eating its way into the cloud.

But the mechanism which actually drives the typical subcondensation of a cloud into a state where it will collapse into a star, or cluster of stars, was the subject of much discussion. The effects of the shock wave from a supernova explosion was championed by G. Assousa (Carnegie Institution, Washington), and shocks induced by stellar winds from massive stars maintained as a possibility by L. Blitz (Columbia University) when presenting CO maps of many local molecular clouds. P. Woodward (Leiden) described his extensive numerical calculations of the collapse of clouds induced by the passage of the large-scale density-wave pattern which is believed to maintain a spiral structure in the Galaxy. He received some support from

Elmegreen's observation of what looks like a temporal star formation sequence along the Galactic plane from an infrared source at one end of a giant molecular cloud to a young ionised hydrogen region M17 at the other end of the cloud. But the star formation in many other clouds, such as Orion, shows no particular preference for the direction along the Galactic plane.

Probably all of the mechanisms, and simple cooling or collisions, are important in instigating the collapse of the clouds. Once collapsing it is possible that the collapse phase may be fairly insensitive to the inducing mechanism. Attempts to follow the subsequent dynamical and chemical evolution described by A. Glassgold (University of Oxford) and others are idealised and have not yet reached the stage where they can be reliably compared with observation. The effects of magnetic fields are beginning to be incorporated, but the particular physical and chemical conditions peculiar to the molecular clouds must also be incorporated.

Very small scale structure in the star-forming areas of the clouds can be obtained by observations of radio lines of molecules, such as H_2O , SiO and OH , formed under maser conditions. D. Downes (Bonn) showed some beautiful very-long-baseline-interferometry H_2O maser maps of several molecular clouds compiled by American-German-Russian-Swedish collaboration. The maps show detail down to milliarc s, corresponding to sub-Solar System sizes in molecular clouds. Downes proposed that the best interpretation of the motion of the clusters of masering regions is as the effect of the shell containing the masers expanding and breaking up after the formation of a star at the centre. Scoville, in looking at the high velocity gas observed in ^{13}CO mapping of the Orion molecular cloud, proposed on energetic grounds that an explosion must have occurred (perhaps a supernova) since radiation pressure from a massive star would be insufficient to drive the matter outwards without the infrared luminosity of the source exceeding its observed value.

Complex molecules may be of great relevance for the composition of interstellar dust. F. Hoyle and N. C. Wickramasinghe (University College, Cardiff) proposed polysaccharides, possibly formed by surface polymerisation of the common formaldehyde molecules, as excellent candidates to explain the 10, 3 and $3.4\text{ }\mu\text{m}$ features observed in the infrared spectra of compact sources. They also suggested that organic ring structures may explain the $2,200\text{ }\text{\AA}$ ultraviolet feature in the interstellar extinction curve. Support for the idea that a fairly volatile component

does indeed produce the $2,200\text{ }\text{\AA}$ feature was provided by Israel, who described a preliminary analysis of ultraviolet satellite observations of the galaxy M101 which suggests that the feature vanishes near young hot stars, an effect already noticed in the 30 Doradus nebula in the Large Magellanic Cloud.

Not all star-forming molecular clouds in the Galaxy are massive. Elmegreen described a complex in NGC 7023 of about 1,000 solar masses, and M. Walmsley (Bonn) a molecular cloud in Taurus of only about 1 solar mass. In both these clouds, low mass star formation seems to be occurring.

Many questions were necessarily left unanswered. How do the massive clouds form? Why are they such long-lived structures, with their estimated lifetimes greatly exceeding the expected gravitational collapse time, yet allowing some star formation to proceed? Giant Molecular Clouds have established themselves as a primary component of the Galaxy, and their study will provide many clues in understanding the mechanism of star formation. □

Actin cables

from Roger Craig

THE view that muscles contract by a sliding of the actin filaments past the myosin filaments is firmly entrenched in our minds. So beautifully does it explain both structural and biochemical observations, that the possibility that actin or myosin in non-muscle cells might perform in any different way has, until recently, hardly been countenanced. But actin, for one, does now seem to function in some cells in a distinctly non-muscular fashion.

In muscle, actin occurs as filaments—helices of globular actin monomers—which function in two intimately related ways: as structural components able to bear tension, and as activators of the energy-releasing ATPase cycle of myosin. In non-muscle cells actin can occur in different forms depending on the immediate and local needs of the cell. One of the most important of these seems to be cables of parallel filaments, which are found in intestinal microvilli, in tissue culture cells, in the acrosomal process of some invertebrate sperm, in the cytoplasm of plant cells, and in many other situations.

Although myosin occurs in association with actin in many non-muscle cells (although generally in minute

amounts), there are some cells rich in actin where myosin has not been detected. The question therefore arises: does actin perform a non-motile role in some cells, or does it perform a motile function without the participation of myosin? Actin cables should provide some answers to these questions: since they are a different structural state of actin from that found in muscle, it would not be surprising if they also differed functionally.

What are the structural advantages of a tightly packed cable of actin filaments? The most obvious is one of rigidity: filaments bound together laterally will form a much more rigid structure than an array of unconnected filaments. Such a structure should be able to support a compressive load in addition to the tensile forces actin can sustain in muscle contraction. The finding of a criss-cross network of such cables in many cells has led several authors to suggest that actin in some instances may fulfil a purely structural role, as part of a cytoskeleton which maintains cell shape and strength (see Clarke & Spudis *A. Rev. Biochem.* **46**, 797; 1977). An instance where actin seems to have a motile ability not dependent on myosin is the acrosomal reaction of some invertebrate sperm (Tilney in *Molecules and Cell Movement* (eds Inoué & Stephens), Raven Press, New York, 1975). The acrosomal process, which penetrates the outer layers of an egg during fertilisation, forms rapidly, growing up to $90\text{ }\mu\text{m}$ in only 10 s; and it consists essentially of a beautifully ordered cable of actin filaments. Two mechanisms for the acrosomal reaction have been demonstrated. In echinoderm sperm the process develops by the organisation of actin into the filamentous form from an amorphous state. In the horseshoe crab *Limulus*, the cable is preformed but coiled in the head of the sperm. The process is extended by a rearrangement of the filaments in the cable, which emerges from the tip of the sperm.

Cables of filaments are clearly an important form of actin in non-muscle cells. A recent paper by DeRosier *et al.* (*J. molec. Biol.* **113**, 679; 1977) is therefore significant since it provides the first detailed insight into the molecular structure of cables from two different sources: the acrosomal process of horseshoe crab sperm, and extracts from sea urchin oocytes. Filaments lie parallel to each other, closely packed in a hexagonal array, and in both cases occur in association with another protein of molecular weight 55,000 (not tubulin) which crosslinks adjacent filaments. The cables are extremely regularly organised, appearing like the filtered images of actin paracrystals, and are thus well suited

Roger Craig is a Muscular Dystrophy Association postdoctoral fellow at Brandeis University.

METEOR streams are elliptical rings of dust containing a myriad of particles all moving with orbits very similar to that of the comet which ejected them as it decayed. The stream particles move through the general Solar System dust cloud which is made up of particles with random orbits. Cloud particles were once in streams but they have been perturbed from their specific orbits by interparticle collisions, close approaches to planets and radiation effects.

The Earth passes through many of these streams each year at relative velocities of between 11 and 74 km s⁻¹. Stream particles which hit the atmosphere burn up, forming visual meteor trains (shooting stars) if they are 0.1 g or more in mass, or radio meteor trains (detectable as reflectors of radar pulses) if they have masses greater than 10⁻⁷ g. Smaller stream particles are more difficult to observe. Some space-vehicle experiments have reported large increases in microparticle fluxes associated with streams. However, extrapolations of radio meteoroid fluxes to smaller masses indicate that streams contain very few of these particles. It has been suggested that the satellite borne detectors are registering micrometre-sized lunar ejecta produced by the impact of larger stream meteoroids.

Over the past few years a new method of observing meteor streams has been suggested which relies on detecting the solar radiation reflected by the orbiting stream dust particles. These should scatter radiation in a similar way to the particles in the Solar System dust cloud. The scattering from this cloud produces the zodiacal light and the streams should produce localised enhancements in this light. The problem can be approached in two ways. A. C. Levasseur-Regourd and J. E. Blamont (*Space Res.* **15**, 295; 1975) measured the zodiacal light brightness using the French satellite D2A. They found localised enhancements of brightness at specific points in the Earth's orbit. They concluded that these variations were caused by a

New look at meteor streams?

from David W. Hughes

local excess of scatters produced by the Earth's passage through a meteor stream. The photometer can point in nine directions which are all in a plane perpendicular to the Earth-Sun line. The field of view is about 3° × 3°. Sometimes the enhancement only occurred in one direction, at others in two diametrically opposite directions and in certain cases the enhancement could be detected all around the satellite. From year to year enhancements were found at the same time and in the same directions. The brightness usually increases by about 25% (that is, by between 10 and 50 S₁₀(V) units on a signal of 140 S₁₀(V) this lasting for between 1 and 17 days). (A brightness of 1 S₁₀(V) unit is equivalent to the brightness produced by having one star of visual magnitude +10.0 per square degree of the field of view or an energy flux of 1.31 × 10⁻⁸ erg cm⁻² s⁻¹ star⁻¹ Å⁻¹). The authors conclude that the average size of the inhomogeneity in the plane of view is about 0.15 AU. By assuming that the particles in the meteor stream have the same size and reflecting properties as sporadic zodiacal cloud particles and by measuring the spatial extent of the stream of scatters Levasseur-Regourd and R. Dumont (*COSPAR, XX Plenary meeting, Tel Aviv, paper III C.3.2.*) could estimate the ratio of the particle number density in streams to that outside. This ratio was found to be as high as 18.

W. J. Baggaley of the University of Canterbury, New Zealand, approaches the problem from another direction. He uses the known properties of the particles in meteor streams and considers how effectively they will produce zodiacal light enhancements. His theoretical calculations are given in a recent edition of *The*

David W. Hughes is a Lecturer in the Department of Physics, University of Sheffield.

Observatory (**97**, 123; 1977). Meteor stream dust is optically thin, the particle number density being so low that mutual eclipsing is rare. During an Earth-stream interception (which occurs every time we have a meteor shower) an observer looking in the direction of the true geocentric shower radiant will be observing tangentially to the stream orbit and his line of sight will intersect a large volume of dust particles. The numbers of micrometre-sized particles present have been estimated by extrapolating from particle size distribution measurements made using radar techniques. Using experimentally determined phase functions and an albedo of 0.1 Baggaley integrates along the tangent and calculates the brightness that should be observed in the geocentric radiant direction. For the Quadrantid, Perseid and Geminid streams these brightnesses are 0.57, 3.44 and 1.09 S₁₀(V) units respectively. The total sky brightnesses in these directions, caused by zodiacal light, integrated starlight and atmospheric airglow are 178, 222 and 229 S₁₀(V) units. So the streams theoretically produce an increase of at most 1.5%. The bright patches of light should be elliptical in shape because meteor streams are more dispersed in the plane of the orbit than normal to this plane (by a factor of about 10). The Quadrantids, Perseids and Geminids would produce bright patches of maximum angular extent around 10, 10 and 5 degrees respectively.

Baggaley concludes that the major annual meteor streams would not produce any observable radiant glow, assuming of course that the extrapolation of flux *versus* size data is applicable and that the measured spatial particle-number density (about 10⁻¹⁸ cm⁻³) is correct. The fact that Levasseur-Regourd and Blamont do see streams in the zodiacal light indicates that the radio meteor flux and size distribution data must be re-examined. Either that or the collisional fragmentation which perturbs stream particles into the sporadic background changes their reflecting properties.

to analysis by optical diffraction and three-dimensional reconstruction.

Since the filaments lie in a hexagonal lattice, exactly equivalent bonds could be made between each filament and its neighbours if actin subunits were to occur at azimuthal intervals of 60°. Although the non-integral helical symmetry of the actin filaments does not satisfy this condition, the symmetry is such that actin subunits present many different azimuths during one axial

repeat of the structure. By building models of filaments with the symmetries established by optical diffraction, DeRosier *et al.* have shown that the departure from 60° is small at certain points along the filament. Placing these filaments on a hexagonal lattice in the arrangement deduced from the diffraction patterns, they conclude that bonds between filaments can be made at these points if distortions are allowed. The distortions required are

small—of the same order as those discussed by Caspar and Klug for quasi-equivalent bonding between the subunits of spherical viruses (*Cold Spring Harbor Symp. quant. Biol.* **27**, 1; 1962). As pointed out by these authors, such an ordered structure need not have all identical molecules in exactly identical environments. The important point is that the lowest energy structure will have the maximum number of most stable bonds formed—and this may be

physically realised . . . by quasi-equivalent bonding of identical units.'

Support for the quasi-equivalent bonding scheme comes from the oocyte cables. Optical diffraction shows that the filaments repeat after 19 turns of the actin helix containing 41 subunits. The model predicts that 9 of the 41 subunits are appropriately oriented for quasi-equivalent bonding, and the cables reveal nine transverse stripes at the predicted axial positions in each repeat. These are thought to be due to the 55,000 molecular weight protein attaching to and crosslinking actin subunits of adjacent filaments at these points.

In the case of the acrosomal process no transverse stripes are seen. But here every actin is associated with the 55,000 component, not just those involved in crosslinking, so that all morphological subunits look alike. A bonding scheme analogous to that in the oocyte bundles has been proposed, with only those 55,000 components attached to appropriately oriented actins actually participating in crosslinking. The model seems plausible since the bond distortions in this case are less than those required in the oocyte bundles. DeRosier *et al.* have carried out a three-dimensional reconstruction of the acrosomal bundle. This reveals that the 55,000 molecular weight protein is bound to the actin subunit near the myosin binding site. While this may not be significant in a cell thought not to have myosin, it could prove relevant in other systems in controlling the interaction of actin cables with myosin.

This is the first time that actin filament cables from cells have been subjected to such analysis. The results, showing how the 55,000 molecular weight protein binds the filaments into an organised cluster, may be applicable to bundles from other sources—but with caution, since different or additional controls may prevail elsewhere.

Free calcium measurement in cells

from T. J. Lea

An EMBO Workshop on Ca Measurement in Cells was held in Oxford on 8-10 July, 1977.

It was in 1928 that H. Pollack reported that a saturated solution of alizarin sulphonate when injected into amoebae produced a shower of red crystals (Ca alizarinate) in the cyto-

plasm on the initiation of pseudopod formation. This pioneering attempt to examine cellular changes in free Ca was mentioned at the workshop during discussions of more modern methods. In a three-cornered fight between the users of photoproteins (such as aequorin), metallo-chromic indicators (such as arsenazo III) and Ca-sensitive microelectrodes, limitations of these more recently developed methods were critically examined and it became apparent that no single technique satisfied all the requirements of a good indicator of free Ca in cells.

On the evidence of results achieved, aequorin has until now led the field. Among recent advances obtained with this photoprotein was the demonstration by C. C. Ashley (University of Oxford) and colleagues of a transmitter-like action of L-glutamic acid on barnacle muscle fibres, and Ca-transients in cardiac muscle (D. G. Allen, University College London). The related indicator obelin has been used in sarcoplasmic reticulum vesicle by D. W. Yates (University of Bristol) and A. K. Campbell (Welsh National School of Medicine, Cardiff) in an attempt to measure Ca uptake by the vesicles. This method does suffer from the disadvantage of a fairly rapid consumption of the photoprotein by Ca entering the vesicles. An undoubted highlight of the meeting was the presentation by L. Jaffe (Purdue University, Indiana) on aequorin-injected eggs of the medaka, a freshwater fish. Jaffe and his colleagues succeeded in showing that immediately following fertilisation, a Ca-wave crosses a single egg (1 mm diameter) from the point of entry of the sperm and reaches the opposite side within two minutes. He concluded that because the wave is unaffected by Ca-free media, its mechanism involves a calcium-induced Ca release from some internal sites, and this idea was certainly of interest to muscle physiologists present in the audience.

The problem of quantifying the Ca measuring methods especially in relation to resting free Ca in cells was an important theme throughout the meeting. Allen produced evidence of a Ca-independent light emission from aequorin *in vitro* which he claimed gave a theoretical detection limit for free Ca in cells of 6×10^{-8} M with a practical limit of 3×10^{-7} M. Since A. Scarpa (University of Pennsylvania) and P. F. Baker (King's College, London) estimated the free Ca in squid axons using aequorin to be 2×10^{-8} M and 6×10^{-8} – 15×10^{-8} M respectively, this particular claim aroused some objections. However, in

general, new data given by Allen and Ashley on the shape of the aequorin–Ca calibration curve and its dependence on cytoplasmic ions, such as Na^+ , K^+ , H^+ and Mg^{2+} , should improve the accuracy of free Ca determinations made with this photoprotein.

In the opinion of I. Parker (University College, London), the absorbance indicator arsenazo III is superior to aequorin for measuring rapid transient changes in free Ca, such as those in the sarcoplasm and nerve endplate of a frog muscle fibre during a single twitch. The response time is faster, although how much faster seemed to be disputed, and the free Ca is linearly related to the absorbance change. For longer term events such as muscle tetani, he felt arsenazo to be inferior because of large movement artefacts, drift in the optical record and unexplained 'tail effects' at the end of the event. L. H. Pinto (Purdue) reported the successful use of purified arsenazo in *Limulus* photoreceptors, but found that concentrations of the dye greater than 3 mM in the cell caused changes in the membrane response to light pulses, which could be explained by arsenazo buffering of cell Ca. The strong dependence of the arsenazo absorbance on Mg^{2+} and H^+ was also emphasised by several speakers. The limit of Ca detection by arsenazo was put at $\sim 10^{-7}$ M by J. D. Owen (University of Utah, Salt Lake City).

Of the three methods, the Ca-selective microelectrode has achieved least in the field so far, but it has much to offer, particularly in the measurement of free Ca in small cells. The use of new compounds embedded in PVC membranes gives the electrodes a linear response down to 10^{-6} M free Ca, while as low as 10^{-8} M can be detected (Owen). In fact R. Meech (University of Cambridge) reported a value for the free Ca in snail neurones very similar to that of Owen's ($\sim 10^{-7}$ M corrected for Mg^{2+}) without the help of a Ca microelectrode, using instead the reversal potential of the Ca-dependent K current. Attempts to measure Ca changes during a single action potential have not so far been successful (G. Christoffersen, Copenhagen) partly because of the slow response time of these electrodes (1–2 s). □

Correction

In the article 'Meteorite research old and new' (*News and Views* 268, 691; 1977) column 3, paragraph 2, 6 lines from end should read: "Serra de Magé minerals probably did not become closed systems until 4.42 Gyr ago," not "4.52 Gyr ago."

T. J. Lea is MRC research assistant, University Laboratory of Physiology, Oxford.

review article

The mean lifetime of orthopositronium in vacuum

T. C. Griffith & G. R. Heyland*

Various measurements and calculations of the vacuum lifetime of ortho-positronium have given widely divergent values. There are indications that these differences are now gradually being resolved.

POSITRONIUM, the hydrogen-like atom formed when an electron and a positron are bound with a ground state binding energy of 6.8 eV, has lately become the subject of intensive study in several laboratories. It is readily formed when positrons emitted from a radioactive source are moderated to energies comparable with those of the valence electrons in atomic and molecular gases or in solid surfaces especially those of certain oxide powder grains such as MgO or SiO₂. The positronium atoms exist as either *para*-positronium, *p*-Ps, the singlet state with total spin zero or *ortho*-positronium, *o*-Ps, the triplet state with unit spin. Since the positron is the antiparticle of the electron positronium has no permanent existence; the two particles eventually annihilate one another and the probability of annihilation from the bound states can be evaluated. It is found that *p*-Ps is very short lived with a mean lifetime for decay of its ground state into two collinear γ rays, each of energy 0.511 MeV, of only 0.125 ns. Its short lifetime renders it more difficult to observe directly than *o*-Ps whose ground state, to conserve spin, has to decay into three coplanar γ rays of total energy 1.022 MeV with a mean lifetime of about 140 ns. Apart from the vacuum lifetime several other properties of positronium have been extensively investigated. After several attempts, spanning a period of about twenty years, the characteristic Lyman- α radiation from the 2P-1S transition in *o*-Ps was eventually detected by Canter *et al.*¹. There has also been a number of observations on the fine structure (or hyperfine structure) splitting of the ground state of positronium.

Theoretical background

Theoretical and experimentally-determined values of the mean lifetime for the decay of *o*-Ps in free space, which is the main subject of this article, have recently undergone considerable variation. Evaluation of this parameter involves the basic principles of quantum electrodynamics, because to high order the constituent particles interact only through the electromagnetic interaction. Its evaluation and measurement provide a clean test of quantum electrodynamics. A full formulation of the problem was presented by Strosio and Holt² and by Strosio³ and an expression was given for this mean lifetime, which includes the radiative corrections corresponding to all order terms in the fine structure constant α_f , in the form

$${}_o\lambda_p = {}_o\lambda_p(0)[1 + (\alpha_f/\pi)(1.86 \pm 0.45)] \quad (1)$$

where ${}_o\lambda_p(0)$ is the lowest order rate given as

$${}_o\lambda_p(0) = \frac{2\pi\alpha_f^6 m_e c^2}{h} \frac{2(\pi^2 - 9)}{9\pi} \quad (2)$$

and ${}_o\lambda_p$, the decay constant, is the reciprocal of the *o*-Ps mean lifetime. m_e is the electronic mass, c the velocity of light and h is Planck's constant.

Before these calculations only the lowest order rate had been evaluated and the initial value of $7.14 \mu\text{s}^{-1}$ given by Ore and Powell⁴ was used until the improved accuracy of α_f resulted in its upgrading to $7.2112 \mu\text{s}^{-1}$ about ten years ago. The all order calculation, Strosio³, gave ${}_o\lambda_p = (7.242 \pm 0.008) \mu\text{s}^{-1}$ which remained as the best theoretical value until the recent calculations of Caswell *et al.*⁵ who reported a much lower value of ${}_o\lambda_p$, their expression being

$$\begin{aligned} {}_o\lambda_p &= {}_o\lambda_p(0)[1 - (\alpha_f/\pi)(10.348 \pm 0.070)] \\ &= (7.0379 \pm 0.0012) \mu\text{s}^{-1} \end{aligned} \quad (3)$$

The historical sequence of events relating the above calculations to progress with the laboratory measurements, given in Table 1, has been an interesting demonstration of the importance of cross-checking and verification of results by independent research groups.

Measurement techniques—gas method

None of the measurements of ${}_o\lambda_p$ carried out before those of Beers and Hughes^{6,7} and of Coleman and Griffith⁸ were sufficiently precise to warrant higher order calculations but the improvement of experimental techniques since that time has been such that measurements to an accuracy approaching 0.01% can now be contemplated. The measurements of ${}_o\lambda_p$ performed before 1976 were taken using methods based on a linear extrapolation of the *o*-Ps decay rates, λ_p , in a given gas at different densities, to zero density according to the relationship

$$\lambda_p = {}_o\lambda_p + q\rho \quad (4)$$

where ρ is the gas density in amagats (1 amagat = 2.69×10^{19} molecules cm^{-3}) and q the quenching coefficient which defines

Table 1 Values for the decay rate of positronium

Year	Value of ${}_o\lambda_p$ (μs^{-1})	Comment
1949	7.14	Lowest order calculation ⁴
~1970	7.2112	Upgrading of the lowest order calculation to allow for improved values of α_f
1968	7.275 ± 0.015	Measurement of Beers and Hughes ^{6,7}
1973	7.262 ± 0.015	Measurement of Coleman and Griffith ⁸
1974	7.242 ± 0.008	All order calculation of Strosio ³
1976a	7.104 ± 0.006	Precision measurement of Gidley <i>et al.</i> ⁹
1976b	7.09 ± 0.02	New measurement of Gidley <i>et al.</i> ¹⁰
1977a	7.0379 ± 0.0012	All order calculation of Caswell <i>et al.</i> ⁵
1977b	7.058 ± 0.015	Measurement of Griffith <i>et al.</i> unpublished

*Department of Physics and Astronomy, University College London, Gower Street, London WC1, UK

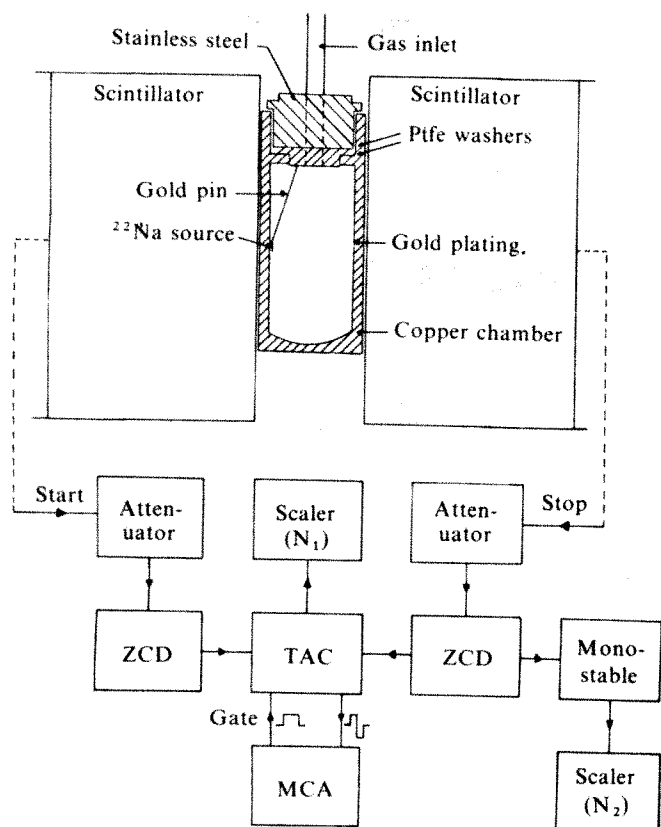


Fig. 1 Positron lifetime apparatus of Coleman *et al.*¹¹ with block diagram of the electronics. (ZCD, zero crossing discriminator; TAC, time-amplitude-converter; MCA, multi-channel analyser. N_1 and N_2 are the 'start' and 'stop' counting rates).

the increase of the decay rates, λ_p , due to collisions between the *o*-Ps atoms and gaseous molecules. The constancy of q has been established for many gases so that a linear extrapolation of equation (4) at low densities is justified.

The main features of the gas extrapolation methods are explained by reference to Fig. 1, where a schematic diagram of the lifetime apparatus of Coleman *et al.*¹¹ is given. Positrons from a ^{22}Na source were moderated in the gas contained in a small copper vessel internally gold plated to enhance back-scattering of positrons into the gas. The time interval between the emission of a positron and its subsequent annihilation is measured for a large number of events to give the positron lifetime spectrum. The 1.28 MeV γ ray emitted simultaneously with the positron is detected with one plastic scintillation counter and provides the start pulse for a timing sequence which is terminated by a pulse from the second counter which detects one of the annihilation γ rays. The lifetime spectrum of about 10^8 events accumulated in 24 hours will generally have components corresponding to the annihilation of free positrons and *p*-Ps as well as the decay of *o*-Ps. The experimental conditions for the determination of λ_p are arranged so that the component due to *o*-Ps is dominant with all the other components compressed into a narrow prompt peak near $t = 0$. This is achieved by choosing a gas, or mixture of gases, for the experiment to satisfy the following criteria: (1) high probability for formation of positronium (2) rapid thermalisation of those positrons which have not formed positronium (3) fast decay rate for the thermalised free positrons (4) small value of q (5) large molecular weight to ensure high stopping power. A mixture of ammonia-Freon 12-helium in the proportion 42%, 42% and 16% respectively, used by Griffith *et al.* (unpublished) with the above apparatus, satisfied most of these requirements and it was found that 50% of the positrons in the gas formed *o*-Ps. Even at the lowest densities the free positrons decay so rapidly that the lifetime spectrum, as shown in Fig. 2, consists of a narrow

prompt peak followed by a single exponential due to *o*-Ps decay. λ_p is derived from a distribution $D(t)$ expressed, to a good approximation, as

$$D(t) dt = [A \exp(-\lambda_p t) + B] dt \quad (5)$$

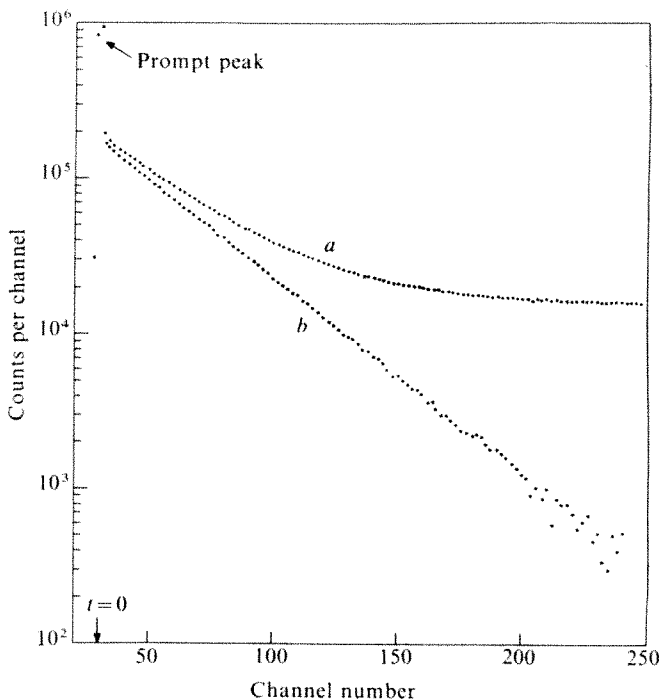
where $D(t) dt$ is the observed number of events in the time interval between t and $t+dt$ and both A and B are constants. In situations where this approximation is not valid Coleman *et al.*^{12,13} have given an exact formulation of the problem. Accurate background subtraction is very important especially for data at low densities where the signal to background, defined at A/B at $t = 0$, is low and typically varied from 31 at high density to 7 at low density in the experiment of Griffith *et al.* (unpublished).

The earlier measurements of Beers and Hughes⁶ were taken with a similar type of apparatus to that described above. They used Freon-12 alone and also a mixture of Freon-12 and argon but points below 1 amagat were not consistent with a linear plot. This non-linearity was attributed to *o*-Ps quenching at the vessel walls. Coleman and Griffith⁸ also used Freon-12 in a similar type of experiment but did not experience any difficulties at their low density points. Their ratio of A/B varied from 5 to 2 and the method of background subtraction, although more detailed than for earlier experiments, was not as accurate as that of Griffith *et al.* (unpublished). The use of the method of 'direct detection', described by Coleman *et al.*¹², to give high counting rates was a new feature in the system of Coleman and Griffith⁸. Instead of using the prompt 1.28 MeV γ ray to produce the start pulse for the timing sequence they detected the fast positrons from the ^{22}Na source in a thin plastic scintillator (of thickness 0.015 cm) as they entered the gas in the chamber. This method gave a value of λ_p which was in agreement with that of Beers and Hughes^{6,7} and also with the calculations of Strosio and Holt².

Other methods

The method of Gidley *et al.*⁹, illustrated in Fig. 3 was also based on the principle of 'direct detection'¹². Instead of producing the *o*-Ps by moderation of fast positrons in a gas they used very fine grains of SiO_2 powder as moderator. A high yield of posi-

Fig. 2 Typical lifetime spectrum for an ammonia-freon-helium mixture at a density of 0.79 amagat units. Curve (a) represents the raw data and curve (b) the *o*-Ps component after background subtraction. For clarity only alternate data points have been plotted. $A/B = 9$, $\lambda_p = 7.396 \pm 0.007 \mu\text{s}^{-1}$. Total number of *o*-Ps events = 6×10^6 .



tronium from positrons moderated in the oxide powders MgO , SiO_2 and Al_2O_3 was first reported by Paulin and Ambrosino¹⁴ when they measured a decay constant of value close to λ_p for such powders in vacuum. This observation suggested that there was very little quenching of the o -Ps trapped in the interstices between the powder grains. Gidley *et al.*⁹ used grains of uniform size and assumed that the quenching was proportional to the collision rate of the o -Ps atoms with the grains. A parameter analogous to density in the gas experiments was defined as $\rho_s[\rho/\rho_s - \rho]$ and represented the mass per unit free volume of the powder. Accordingly λ_p was plotted as a function of

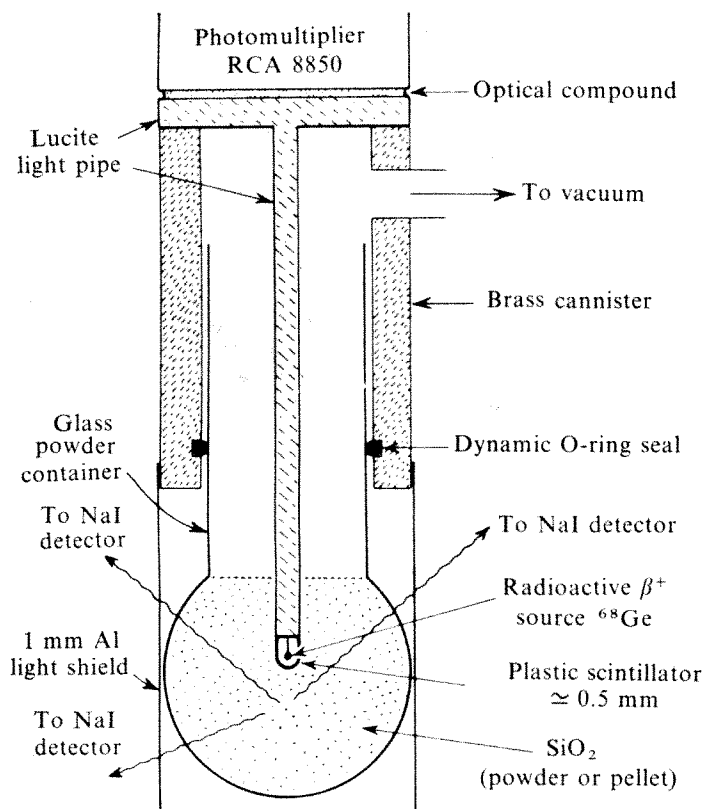


Fig. 3 Schematic diagrams of the positron "direct detection" system of Gidley *et al.*⁹ for the SiO_2 powder lifetime experiment.

$\rho/(\rho_s - \rho)$ where ρ_s is the solid aggregate density of 2.20 g cm^{-3} and ρ the powder density which was varied by compression over a range from 0.03 to 0.26 g cm^{-3} . Their results (see Fig. 4), show straight lines for two different grain sizes extrapolating to the same zero density value of $\lambda_p = 7.104 \pm 0.006 \mu\text{s}^{-1}$ which was substantially lower than all the earlier measurements and theoretical predictions. The timing sequence in this experiment was similar to that used in the gas experiments. Their efficient detection of all the positrons entering the powder through a 0.5-mm -thick plastic scintillator from a ^{68}Ge source ensured values of A/B ranging from 300 to $2,000$ depending on the method adopted for producing the stop pulse from the annihilation γ rays. Analysis of the data using equation (5) was therefore accomplished to a high degree of accuracy. In common with the experiments at other laboratories precise methods were used for the time calibration of the system and a variety of tests were devised to ensure that their data was not affected by systematic errors.

The large discrepancy with previous measurements and theoretical calculations revealed by the above experiment prompted Gidley *et al.*¹⁰ to design an experiment for the direct measurement of λ_p in vacuum. The apparatus for this new experiment is illustrated in Fig. 5 and the method is based on the production of positronium from a beam of low-energy positrons as was done by Canter *et al.*¹ in their experiment to detect the positronium Lyman- α radiation. About 500 slow

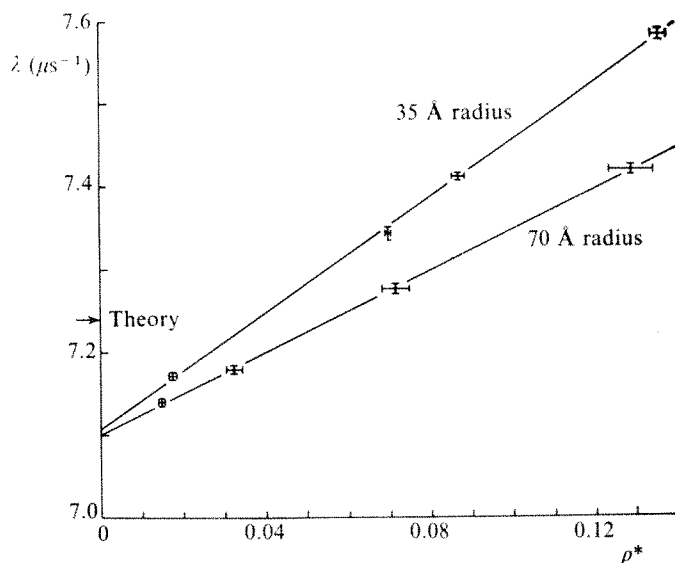


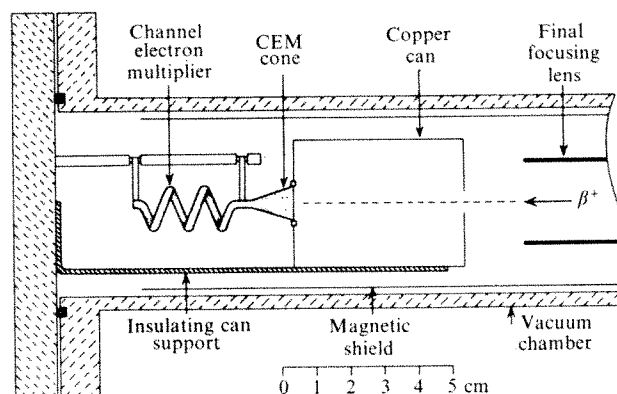
Fig. 4 O -Ps decay rates in SiO_2 powder, by Gidley *et al.*⁹, as a function of the parameter $\rho^* = \rho/(\rho_s - \rho)$ defined in the text. Curves are given for two different grain size. All samples were evacuated to 10^{-6} torr. and outgassed at 350°C for 4 h .

positrons per second, from a $46 \text{ mCi } ^{58}\text{Co}$ source followed by a gold- MgO moderator, were accelerated to 400 eV and transported down a 1 m flight path and focused through a 6-mm diameter aperture on to the MgO -coated cone of a channeltron electron multiplier. Positronium is formed on the cone with 15% efficiency and allowed to diffuse back into the evacuated copper can whose inside walls were also coated with a thin layer of MgO to reduce quenching and confine the positronium to a region for which the efficiency of detection of the annihilation γ rays is approximately uniform. The detectors were shielded from γ rays directly from the source. Backscattered positrons from the cone were stopped from entering the can by biasing the cone to -400 V and the can to -50 V . The stop pulse of the timing sequence was generated from the annihilation γ rays in the same way as for the earlier experiment⁹ but the start pulse was derived from the secondary electrons emitted simultaneously with the positronium from the channeltron cone. The lifetime spectrum was again represented by equation (5) with λ_p now replaced by λ_p measured directly and, depending on the method used to derive the stop pulse, the A/B ratio ranged from $1,500$ to $9,000$. Their data, of the same form as illustrated in Fig. 2, gave a mean value of $\lambda_p = 7.09 \pm 0.02 \mu\text{s}^{-1}$ which was in good agreement with their previous measurement⁹.

The meaning of recent results

The recent calculated value of $\lambda_p = 7.0379 \pm 0.0012 \mu\text{s}^{-1}$ by Caswell *et al.*⁵ strongly suggested that the measurements of

Fig. 5 Apparatus used by Gidley *et al.*¹⁰ for the direct determination of λ_p for o -Ps in vacuum.



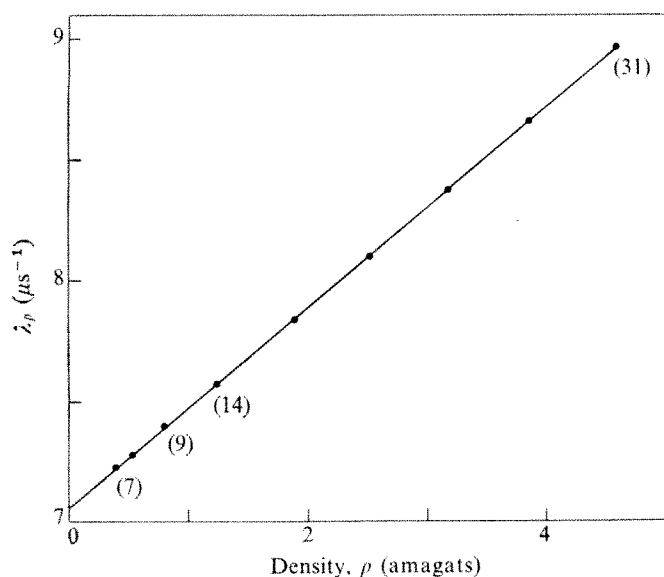


Fig. 6 The *o*-Ps decay rate λ_p as a function of gas density, ρ , for an ammonia–freon–helium mixture measured by Griffith *et al.* (unpublished). The errors are smaller than the circles enclosing the points. The numbers in parentheses represent the ratio A/B at those densities.

Gidley *et al.*^{9,10} could be closer to the true value than those obtained in the earlier experiments. This view is now reinforced by the new low value obtained in the improved gas extrapolation method of Griffith *et al.* (unpublished). Their values of λ_p , shown in Fig. 6, carry very small statistical errors and lie on a good straight line extrapolating to $7.058 \pm 0.15 \mu\text{s}^{-1}$, a value which is smaller than all the previous measurements. The gold plating on the inner walls of their vessel, by increasing the number of *o*-Ps events, has improved the A/B ratios by a factor of 5 in comparison with those of Coleman and Griffith⁸. It is probable that the very low values of A/B in the latter experiment⁸ contributed a systematic error which gave a high value of λ_p and a similar argument may apply to the measurement of Beers and Hughes⁶.

The new value of $7.058 \pm 0.015 \mu\text{s}^{-1}$ is currently being subjected to a series of tests to establish that the measurement is

free of systematic errors. At present there is no evidence for nonlinear behaviour even at densities as low as 0.05 amagat units. This implies a negligible amount of quenching of the *o*-Ps on the gold surface. There is some uncertainty about the assignment of gas density from the pressure and temperature of mixtures of molecular gases because the virial coefficients are not accurately known, but at low density this should not be a serious error. Corrections for the mass per unit free volume have not been applied in the gas experiments because the appropriate value of ρ_s is not known. There is no evidence of deviation from a straight line at high densities which would be expected if this correction was significant.

All recent measurements of λ_p lie within the range 7.05 to $7.11 \mu\text{s}^{-1}$ and the true value is probably within these limits or lower. In common with the new gas extrapolation method the experiments of Gidley *et al.*^{9,10} may also be subject to error. In their first experiment⁹ the determination of the powder density is subject to some uncertainty and the way in which the powder packs under compression has not been investigated. Some error might also be expected in their second experiment¹⁰ due to uncertainty about the quenching of *o*-Ps at the surface of the can.

Gidley *et al.*¹⁵ have recently reported that they are also performing a gas extrapolation experiment and their preliminary values of λ_p are reported to be in agreement with their earlier values. It is clear that very accurate values of λ_p will soon be available from more than one laboratory and that the recent theoretical calculations of Caswell *et al.*⁵ will be closely tested.

- 1 Canter, K. F., Mills, A. P. & Berko, S. *Phys. Rev. Lett.* **34**, 177–180 (1975).
- 2 Strosio, M. A. & Holt, J. M. *Phys. Rev.* **10**, 749–755 (1974).
- 3 Strosio, M. A. *Phys. Rep.* **22**, C, 217–277 (1975).
- 4 Ore, A. & Powell, J. L. *Phys. Rev.* **75**, 1696–1699 (1949).
- 5 Caswell, W. E., Lepage, G. P. & Sapirstein, J. *Phys. Rev. Letts.* **38**, 488–491 (1977).
- 6 Beers, R. H. & Hughes, V. W. *Bull. Amer. Phys. Soc.* **13**, 633 (1968).
- 7 Hughes, V. W. *Phys. 1973, Plenarvortr. Physik-ertag 37th* 123–155 (Physik Verlag, Weinheim, 1973).
- 8 Coleman, P. G. & Griffith, T. C. *J. Phys. B: Atom. Molec. Phys.* **6**, 2155–2161 (1973).
- 9 Gidley, D. W., Marko, K. A. & Rich, A. *Phys. Rev. Lett.* **36**, 395–398 (1976).
- 10 Gidley, D. W., Zitzewitz, P. W., Marko, K. A. & Rich, A. *Phys. Rev. Lett.* **37**, 729–732 (1976).
- 11 Coleman, P. G., Griffith, T. C., Heyland, G. R. & Killeen, T. L. *Appl. Phys.* **3**, 271–273 (1974).
- 12 Coleman, P. G., Griffith, T. C. & Heyland, G. R. *J. Phys. E.* **5**, 376–378 (1972).
- 13 Coleman, P. G., Griffith, T. C. & Heyland, G. R. *Appl. Phys.* **5**, 223–230 (1974).
- 14 Paulin, R. & Ambrosino, G. J. *Phys. (Paris)* **29**, 263–270 (1968).
- 15 Gidley, D. W., Rich, A., Zitzewitz, P. W. & Paul, D. A. L. *Bull. Amer. Phys. Soc.* **22**, 586 (1977).

articles

Positions of galactic X-ray sources: $20^\circ < l^{\text{II}} < 55^\circ$

R. E. Doxsey, K. M. V. Apparao*, H. V. Bradt, R. G. Dower & J. G. Jernigan

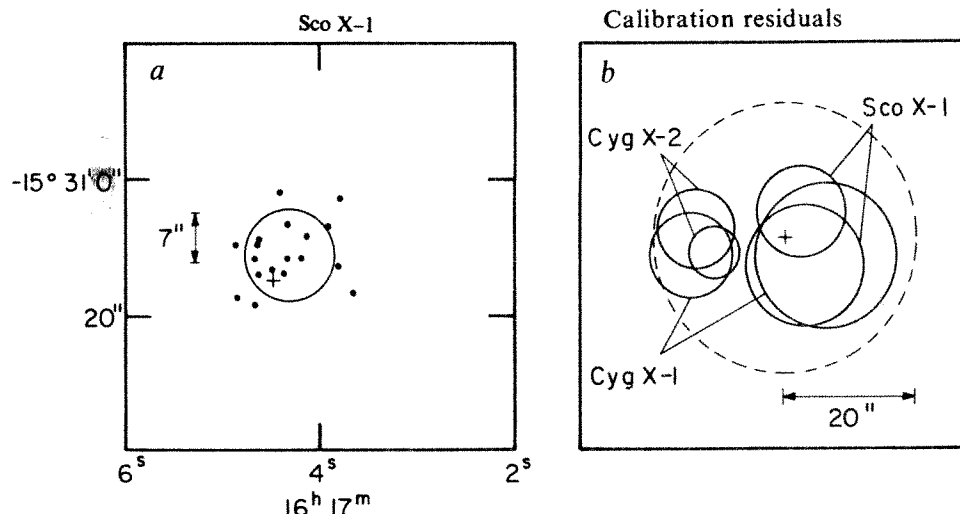
Department of Physics and Center for Space Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Precise positions, determined with data from the SAS-3 X-ray observatory, are presented for eight galactic plane X-ray sources (*Aq1 X-1*, *Ser X-1*, *3U1956+11*, *3U1822-00*, *3U1915-05*, *A1845-02*, *A1850-08*, *A1905+00*). Error radii for the positions range from 20 to 50 arc s. Previously proposed optical identifications of three of the sources (*Ser X-1*, *3U1956+11*, *A1850-08*) are supported by these results. Three (*Ser X-1*, *A1905+00*, *3U1915-05*) have been identified as X-ray burst sources.

THE rotating modulation collimator (RMC) system on the SAS-3 X-ray observatory has been used to carry out a systematic survey of the galactic plane ($|b^{\text{II}}| \lesssim 10^\circ$). The aim of the survey is to identify galactic X-ray sources by the precise measurement of their positions (error radii $< 60''$). A few preliminary results based on small amounts of quick-look data have been previously reported¹. The availability of production data and a more complete calibration of the instrument now allows a more thorough, sensitive,

*Permanent address: Tata Institute of Fundamental Research, Bombay, India.

Fig. 1 *a*, Sco X-1 calibration of 2.3' collimator. Each dot represents the position determined for Sco X-1 from one orbit of data. The circle is centred at the average position and has a radius equal to the r.m.s. scatter of the points (7"). + indicates the position of the optical counterpart. *b*, Calibration residuals for the 2.3' collimator. The data for each calibration observation has been analysed and plotted as in *a*. The circles representing the r.m.s. scatter of points about the average positions have been overlayed with the true position used as a registration point. The r.m.s. scatter of the average positions from the true position is 12". The 20" dashed circle represents the error radius for a source position limited only by systematic errors.



and more accurate survey than previously possible. We discuss here the instrument calibrations and present results of the analysis for the region $20^\circ < l < 55^\circ$. This region has been previously surveyed with the UHURU (ref. 2) and Ariel V (ref. 3) satellites.

The SAS-3 RMC system consists of two independent modulation collimators^{4,5}. One has a periodic triangular response function with a FWHM of 2.3', the other, 4.5'. Both modulation collimators have $12^\circ \times 12^\circ$ FWHM coarse collimators to limit the field of view. Proportional counters sensitive in the range 2–11 keV are used as X-ray detectors. The centre of the overall field of view is aligned with the spacecraft spin axis. RMC survey data are taken with the satellite rotating uniformly about its axis at ~ 1 –2 revolutions per 95-min orbit. Star trackers provide data from which the motion of the spacecraft relative to the celestial sphere can be reconstructed to an accuracy of 5–10".

Accuracy of position determinations

The calibration of the RMC system was determined from observations of several bright, optically identified X-ray sources; in particular Sco X-1, Cyg X-1, and Cyg X-2. These observations have been made periodically throughout the lifetime of the satellite and are designed to cover the range of parameters normally encountered (spin rate and direction, temperature, and location of source in field of view). These data have also been used to investigate the short and long term stability of the star tracker-collimator system.

Two orientation angles and the collimator periodicity define completely the transmission response of a modulation collimator over its entire field of view. The two angles required are the rotational orientation of the collimator about the spacecraft spin

axis and the phase of the spin axis perpendicular to the periodic transmission bands. If the true celestial position of the source in the field of view is known, then a set of linearised least square fits to the data can be used to determine these angles. The data from two observations of Sco X-1 (19–20 May 1975 and 9–10 July 1975) were used in this way. Each satellite orbit of data was independently analysed and fit to determine the three parameters. The average value of each parameter for each collimator was then fixed for use in the subsequent analyses.

X-ray source positions are obtained from a computation of the cross-correlation between the data and the expected response for a two-dimensional grid of sky points⁶. A grid separation of $\sim 1'$ is used to compute a 'map' of the central region ($15^\circ \times 15^\circ$) of the field of view. Precise positions are obtained from a high resolution map ($\sim 1''$ grid) in the local region of a source. Subtraction of the Bessel function patterns of the bright sources often reveals fainter sources, not noticeable in the original map⁶.

The two Sco X-1 observations were used to test the mapping algorithm and the reproducibility of the position determination on a short (~ 1 d) time scale. Sco X-1 is sufficiently bright so that the effect of counting statistics on the measured position is negligible ($< 1''$) for a one-orbit exposure. The correlation map technique was applied independently to each orbit of data. The r.m.s. scatter of positions relative to the average position was 7" for one Sco X-1 observation (Fig. 1a) and 9" for the other. We believe that this scatter of the positions is due to small random errors in the star tracker attitude solution since the orbit-to-orbit variations for the two collimators are highly correlated. The offset (5") between the average position and the optical counterpart reveals a systematic error.

Fig. 2 Line drawings of regions containing X-ray sources reported in this paper. U, UHURU error boxes²; A, Ariel V error boxes³; S, SAS-3 error circles. In cases where two SAS-3 error circles are shown, the larger is the previously reported quick-look result.

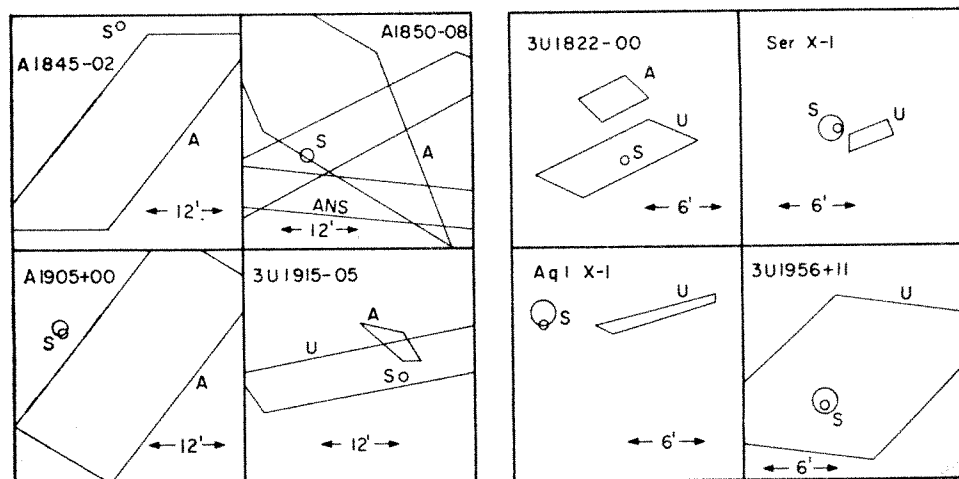


Table 1 Celestial positions

SAS-3 designation	Other designations	Position α (1950)	δ	μ^a b^a	Error radius (90%)	Flux density* (2–11 keV)	Comments
2S1822–000	3U1822–00 A1822+00	18h 22min 48.5s 275.7021°	–00°02'24" –0.0400°	29.9° 5.8°	20"	30 μ Jy	
2S1837+049	Ser X–1 3U1837+04	18 37 29.8 279.3742	04 59 23 4.9897	36.1 4.8	20	250	Optical candidate ⁸ Burst source MXB1837+05 (refs 10–12)
2S1845–024	A1845–02	18 45 41.1 281.4213	–02 28 37 –2.4769	30.4 –0.4	35	11	
2S1850–087	A1850–08	18 50 21.6 282.5900	–08 45 36 –8.7600	25.4 –4.3	50	5	Globular cluster NGC6712
2S1905+000	A1905+00	19 05 54.9 286.4788	00 05 37 0.0936	35.0 –3.7	35	10	Burst source MXB1906+00 (refs 11, 18)
2S1908+005	Aql X–1 3U1908+00	19 08 43.6 287.1817	00 30 05 0.5014	35.7 –4.1	20	45	
2S1916–053	3U1915–05 A1916–05	19 16 08.5 289.0354	–05 19 51 –5.3308	31.4 –8.5	35	20	Burst source ^{20,24}
2S1957+115	3U1956+11 A1956+11	19 57 02.9 299.2621	11 34 14 11.5706	51.3 –9.3	20	24	Optical candidate ²¹

*Averaged over the observation and accurate to $\sim 10\%$. We quote the flux density at 5.2 keV of an incident power-law energy spectrum with slope $\alpha=1.08$ (for example, the Crab nebula²³). 1.0 μ Jy corresponds to 2.2×10^{-11} erg s^{–2} (2–11 keV). $I=1.060$ μ Jy and 1.0 RMC count s^{–1} = 2.7 μ Jy.

The systematic errors were further evaluated with a comparison of the average positions determined for Cyg X-1 and Cyg X-2 and the true positions (Fig. 1b). We find an r.m.s. scatter of 12" in the difference between true and measured positions for both collimators. This is larger than can be accounted for either by counting statistics or the random attitude solution error. The errors are not consistent with a correlation between the calibration parameters and temperature, satellite age, or spin rate. There is no set of values for the calibration parameters which will reduce this scatter. Therefore, we adopt the calibration parameters determined from the Sco X-1 data for the analysis of the survey data.

The error radii for X-ray source locations are determined from an evaluation of four sources of error: counting statistics, random aspect errors, systematic errors, and errors resulting from the subtraction of bright sources. Each of these errors is modelled as a Rayleigh distribution (two-dimensional Gaussian). The contribution due to counting statistics is $\sigma_1 = 1.7 \delta N_T^{1/2} N_S^{-1}$, where δ is the FWHM of the collimator (2.3' or 4.5'), N_T is the total number of counts recorded, and N_S is the number of X rays detected from the source. The factor 1.7 is calculated from the specifics of the cross-correlation computation and has been verified empirically. The contribution due to systematic errors is $\sigma_2 = 12''/2^{1/2} = 8.5''$ (for a Rayleigh distribution, $\sigma = \theta_{r.m.s.} 2^{-1/2}$). The random aspect error is $\sigma_3 = 10''(2n)^{-1/2}$, where n is the number of orbits of data in the observation. Since n is generally greater than 10, this term is usually negligible. The error resulting from the subtraction of a bright source is $\sigma_4 = 0.6 \delta/f$, where f is the ratio of the intensity of the faint source to the intensity in the same region of the residual ripples from the bright source after subtraction. This ratio must be evaluated for each source; in most cases $f > 30$ and $\sigma_4 < 5''$.

The 90% confidence error radius for the combined error is then $E_r = 2.15 (\sigma_1^2 + \sigma_2^2 + \sigma_3^2 + \sigma_4^2)^{1/2}$. This radius ranges from $\sim 60''$ for a source near the detection threshold to $\sim 20''$ for a source limited only by systematic errors. A source position and its associated error are calculated for each collimator from all the data taken with the satellite spin axis at one location relative to the source.

We usually obtain several pairs of independent position determinations by observing each source from a number of different spin axis locations. The quoted position is the weighted average of the positions determined for each observation. The quoted error radius is representative of the error radii computed for the

individual observations. An empirical error radius, E_r' , is calculated from the scatter of the determined positions. We have found so far good agreement between E_r' and our chosen error radius. If, occasionally, we do not find them to be comparable, we will adopt the larger value.

Results

The region of the galactic plane between longitudes 20° and 55° was observed 11–16 July 1975, immediately after one of the observations of Sco X-1, and also on 13 September 1975. The spin axis was pointed for 1 d at each of six 'targets' in the region. The overall

Table 2 Stellar astrometry and magnitudes

Source	Star	m_{pk}^*	Position (1950)† α δ
2S1822–000	1	16.9	
	4	16.5	18h 22min 46.7s –00°04' 26"
	5		18 22 54.0 –00 03 03
2S1837+049 (Ser X–1)	D	18.5‡	
	10	17.9	
	11	15.1	18 37 24.4 05 00 47
2S1845–024	2	16.2	18 45 41.6 –02 28 34
	3	18.6	18 45 43.3 –02 29 10
2S1905+000	3	18.9	
	12	17.4	
	27	14.8	19 05 52.1 00 05 01
2S1908+005 (Aql X–1)	3	17.9	
	13	12.7	19 08 42.7 00 31 07
	14		19 08 46.1 00 28 33
2S1916–053	2	17.9	
	19	12.9	19 16 07.4 –05 21 06
	20		19 16 13.0 –05 18 51
2S1957+115	M	19.0††	
	8	17.2	
	10		19 56 55.4 11 34 01
	11	16.0	19 57 07.6 11 34 30

*Estimated from stellar diameters⁷ to ~ 0.5 mag.

†Precise to $\lesssim 2''$.

‡ m_B from other work^{8,21}.

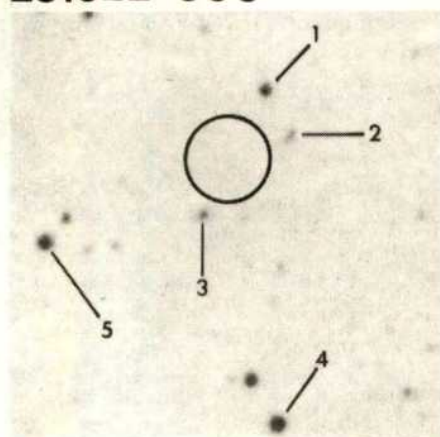
field of view was larger than the separation between targets, so each source was detectable in the data from at least two independent observations. Earth blockage, South Atlantic anomaly penetration, and partial loss of daytime star tracker data reduced the usable data in each of these observations to $\sim 30,000$ s.

A total of eight previously known X-ray sources were detected in this region. The positions determined for these sources are given in Table 1. Error circle radii were determined as discussed above. The contribution to the total error radius from the random aspect and

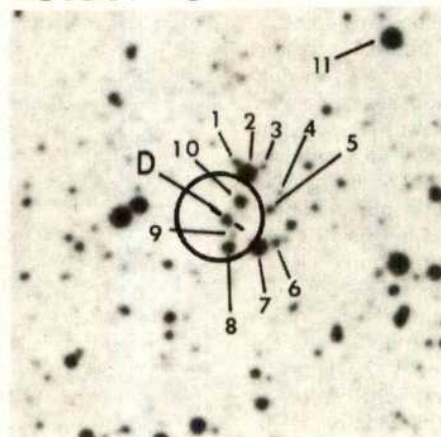
source subtraction errors was negligible for all of these sources. In all cases, the scatter in the positions determined from independent observations was less than the computed error radius. Source intensities (2–11 keV) are averaged over the one day observation and are accurate to $\sim 10\%$. The positions are plotted relative to previous results in Fig. 2. Finding charts for each of the sources are provided in Fig. 3. Table 2 contains celestial positions and magnitudes estimated from image diameters² for several stars in each finding chart.

Fig. 3 Finding charts for the SAS-3 positions. The charts are taken from the Palomar Sky Survey blue plates (National Geographic Society). North is up and east is to the left. The indicated scale factor applies to all charts. Stars are numbered to ease communication between observers. Letter designations indicate proposed identifications (see text).

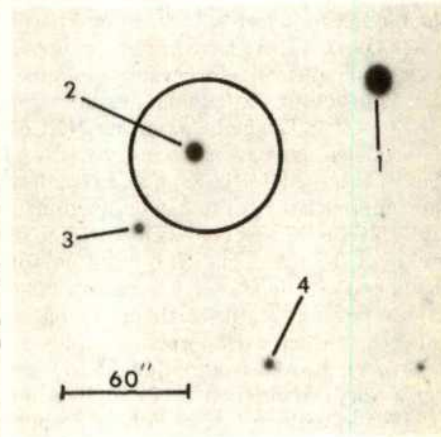
2S1822-000



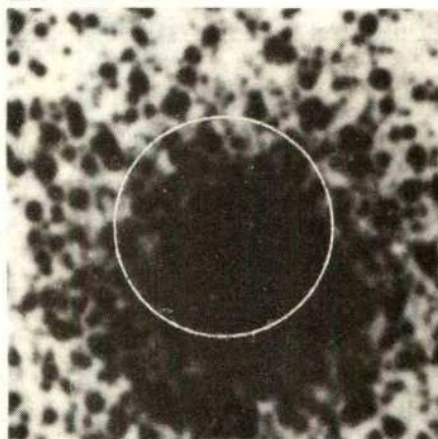
2S1837+049



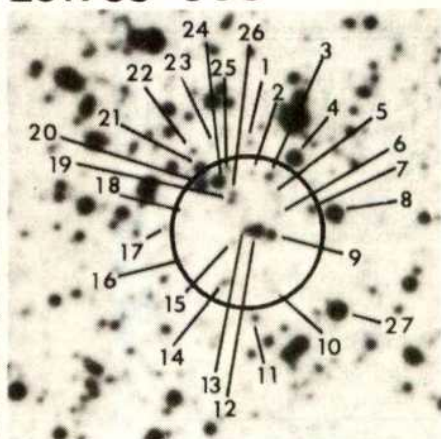
2S1845-024



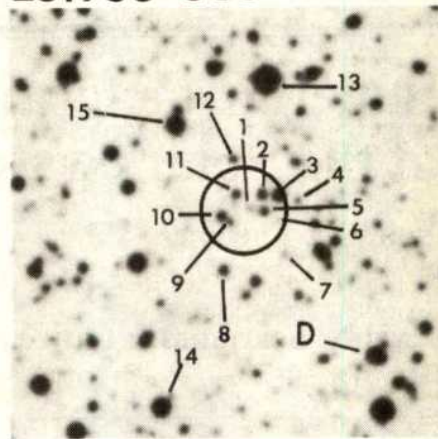
2S1850-087



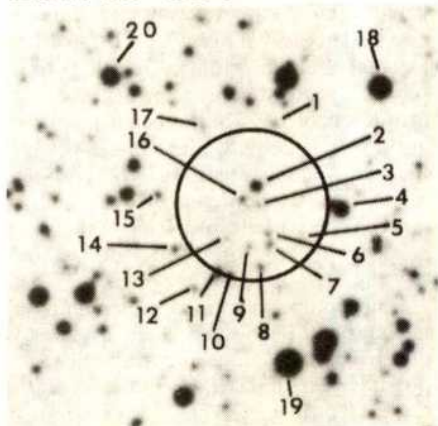
2S1905+000



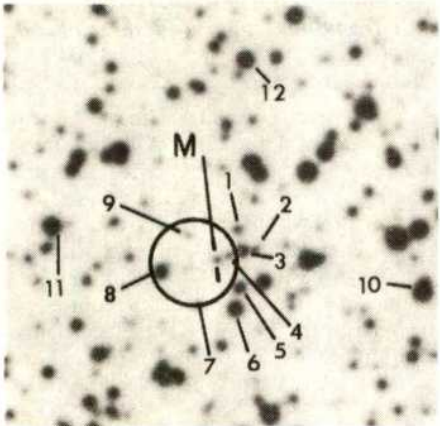
2S1908+005



2S1916-053



2S1957+115



There are 11 sources detected in the UHURU and Ariel V surveys of this region which we have not seen. If these sources were at their catalogued intensities, then only one, 3U1901+03, should have been clearly above our detection threshold. Another one, 3U1912+07, would have been above our threshold if it were at the intensity reported in the 3U catalogue², but below our threshold at the intensity reported by Seward³.

The current status, with regard to the identification of these objects, is as follows:

3U1822-00: there have been no proposed candidates for this source. There are no stars visible on the Palomar Sky Survey blue plate in the error circle and only a few on the red plate.

Ser X-1: Davidsen⁸ proposed a candidate for Ser X-1 based on the SAS-3 quick-look results⁹. The object is a faint ($B \approx 18.5$) star with an ultraviolet excess. It is $\sim 4''$ from the centre of our refined error circle. Ser X-1 is also a source of X-ray bursts¹⁰⁻¹², the first burst source for which a stellar counterpart has been proposed.

A1845-02: There have been no proposed candidates for this source. The source lies in a heavily obscured region. There is only one star visible on the Palomar Sky Survey inside the error circle.

A1850-08: The globular cluster NGC6712 was proposed by Seward³ *et al.* as a possible counterpart for A1850-08. Cominsky *et al.*¹³, using UHURU data, and Grindlay *et al.*¹⁴, using ANS data, improved the position determination of this source. In both cases, NGC6712 was consistent with the data, but field objects could not be ruled out. Our position for this source clearly identifies it with the cluster. The centre of the cluster is $\sim 30''$ from our best position, well inside the error circle. Of the known variable stars in the cluster, two long period variables, V2 and V8, lie within the error circle. Also lying within the error circle is V17, which was seen to vary by Harwood¹⁵, but not by Sandage¹⁶ or Rosino¹⁷.

A1905+00: there are currently no proposed candidates for this object. The source has been identified as an X-ray burst source^{11,18}.

Aql X-1: a candidate for Aql X-1 was proposed by David-

sen¹⁹ based on the UHURU error box. This candidate is ruled out by our results.

3U1915-05: The star 26f Aql, suggested as a possible counterpart in the 3U catalogue² is not consistent with our results. This source was first proposed as a burst source candidate by Becker *et al.*²⁰ based on OSO-8 observations (error box area $\sim 13 \text{ deg}^2$) and has been confirmed with recent SAS-3 observations (error box area $\sim 0.1 \text{ deg}^2$ (ref. 24)).

3U1956+11: Margon *et al.*²¹ have proposed a candidate for this source based on the SAS-3 quick-look results²². It has a B magnitude of 19.0 with a spectrum similar to that of Sco X-1. It lies $10''$ from our present refined position.

We thank the staffs of the Center for Space Research at M.I.T. and the Goddard Space Flight Center. Stellar coordinates were measured at facilities of the Kitt Peak National Observatory. This work was supported in part by the US NASA. This is the second in the series of articles on positions of galactic X-ray sources with SAS-3 (ref. 1 being the first).

Received 5 July; accepted 2 August 1977.

¹ Bradt, H. V. *et al.* *Nature* **269**, 21-25 (1977).

² Giacconi, R. *et al.* *Astrophys. J. Suppl.* **27**, 37-64 (1974).

³ Seward, F. D., Page, C. G., Turner, M. J. L. & Pounds, K. A. *Mon. Not. R. Astr. Soc.* **175**, 39P-46P (1976).

⁴ Dossay, R. *et al.* *Astrophys. J. Lett.* **203**, L9-L12 (1976).

⁵ Schnopper, H. W. *et al.* *Astrophys. J. Lett.* **210**, L75-L77 (1976).

⁶ Schnopper, H. W. *et al.* *Astrophys. J. Lett.* **161**, L161-L167 (1970).

⁷ Liller, M. H. & Liller, W. *Astrophys. J. Lett.* **199**, L133-L135 (1975).

⁸ Davidsen, A. *I.A.U. Circ.* No. 2824 (1975).

⁹ Dossay, R. *I.A.U. Circ.* No. 2820 (1975).

¹⁰ Swank, J. H., Becker, R. H., Pravdo, S. H. & Serlemitsos, P. J. *I.A.U. Circ.* No. 2963 (1976).

¹¹ Li, F. & Lewin, W. H. G. *I.A.U. Circ.* No. 2983 (1976).

¹² Li, F. K. *et al.* *Mon. Not. R. Astr. Soc.* **179**, 21P-25P (1977).

¹³ Cominsky, L., Forman, W., Jones, C. & Tananbaum, H. *Astrophys. J. Lett.* **211**, L9-L13 (1977).

¹⁴ Grindlay, J. E. *et al.* *Astrophys. J. Lett.* **212**, L67-L71 (1977).

¹⁵ Harwood, M. *Ann. Sterrewacht Leiden* **21**, 387 (1962).

¹⁶ Sandage, A., Smith, L. L. & Norton, R. H. *Astrophys. J.* **144**, 894-902 (1966).

¹⁷ Rosino, L. *Astrophys. J.* **144**, 903-915 (1966).

¹⁸ Lewin, W. H. G. *et al.* *Mon. Not. R. Astr. Soc.* **177**, 93P-100P (1976).

¹⁹ Davidsen, A., Malina, R. & Bowyer, S. *Astrophys. J.* **203**, 448-454 (1976).

²⁰ Becker, R. H. *et al.* *Astrophys. J.* (in the press).

²¹ Margon, B., Thorstensen, J. & Bowyer, S. *Bull. Am. astr. Soc.* **9**, 330 (1977).

²² Bradt, H., Dossay, R., Jernigan, J. G. & Spada, G. *Bull. Am. phys. Soc.* **21**, 544 (1976).

²³ Toor, A. & Seward, F. D. *Astr. J.* **79**, 995-999 (1974).

²⁴ Lewin, W. H. G., Hoffman, J. A. & Doty, J. *I.A.U. Circ.* No. 3087 (1977).

Supernovae, grains and the formation of the Solar System

James M. Lattimer

Department of Astronomy, University of Illinois, Urbana, Illinois 61801

David N. Schramm & Lawrence Grossman

The Enrico Fermi Institute, University of Chicago, Chicago, Illinois 60637

The observed ^{26}Mg and ^{16}O anomalies in meteorites can be consistently understood if a supernova occurred within a few million years of the condensation of the Solar System. Grains condensing in the ejecta from this supernova may be an integral aspect of this process.

THE detection^{1,2} of excess ^{26}Mg which correlates with the Al/Mg ratio in a Ca-rich inclusion in the C3 chondrite Allende offers strong evidence that a nucleosynthetic event preceded the formation of the Solar System by at most a few million years. Two explanations for the production of ^{26}Al , which decayed to form the observed excess ^{26}Mg , have been presented. (1) An irradiation in the early Solar System³ could synthesise the observed amount of ^{26}Al throughout a solar nebula of $1 M_{\odot}$, but the energy required is greater than the binding energy of the Sun. This seems unreasonable large. In addition, excess amounts of other rare isotopes such as ^{40}K and ^{50}V , as well as neutron-induced isotopic anomalies in elements such as Gd, would be produced. No such anomalies have been observed to date. (2) One or more super-

novae, which could produce ^{26}Al in either explosive carbon burning or in a high temperature carbon burning shell source immediately preceding the explosion, occurred near the protosolar cloud⁴. This supernova would have had to occur within a few ^{26}Al decay half lives (7.2×10^5 yr). The fact that the protosolar cloud condensed within such a short time of this supernova may not be accidental; the supernova trigger hypothesis of the formation of the Solar System has been considered by several authors (ref. 4 and S. Margolis, in preparation). We present here the results of our supernova grain condensation calculations and relate them to the hypothesis that a 'last event' supernova was indeed related to the formation of the Solar System and thus might have created the observed isotopic anomalies in magnesium, oxygen, neon and xenon.

Four components of presolar material are envisaged for the formation of the Solar System. The first two, interstellar gas and dust, have been enriched by supernova and nova debris (gas and possibly dust) and stellar winds, among other processes, throughout galactic history. The average composition of the dust may be chemically and isotopically different from the gas. The last two

Table 1 Isotopic anomalies and condensing minerals in supernova ejecta

Supernova zone	Non-solar isotopic abundance peculiarities	Major condensible species at 10^{-8} atm which also condense in the solar nebula* C/O < 1	C/O > 1
(H)	$^{13}\text{C}(+)$, $^{15}\text{N}(\pm)$, $^{17}\text{O}(+)$	Al_2O_3	C, TiC, SiC, Fe_3C
(He)	$^{13}\text{C}(-)$, $^{15}\text{N}(\pm)$, $^{17}\text{O}(-)$ $^{18}\text{O}(\pm)$, Mg(n), Si(n) S(n), Ca(n), Ti(n), Fe(n)	$\text{Ca}_2(\text{Al}_2, \text{MgSi})\text{SiO}_7$ MgAl_2O_4 CaTiO_3 $\text{CaMgSi}_2\text{O}_6$	AlN, CaS, Fe Al_2O_3 , MgAl_2O_4 $\text{CaMgSi}_2\text{O}_6$
(C)	$^{12}\text{C}(+)$, $^{16}\text{O}(+)$, $^{25,26}\text{Mg}(\pm)$ $^{26}\text{Al}(+)$, $^{29}\text{Si}(+)$, $^{30}\text{Si}(\pm)$ S(n), Ca(n), Ti(n), Fe(n)		C/O > 1 C, TiC, SiC, Fe CaS, Al_2O_3 MgAl_2O_4 , CaTiO_3
(O)	$^{16}\text{O}(+)$, $^{24}\text{Mg}(+)$, $^{28}\text{Si}(+)$ $^{36}\text{S}(-)$, $^{40,42}\text{Ca}(+)$ $^{40}\text{Ti}(+)$, Fe(n)	(S + Si)/O < 1 Al_2O_3 , CaTiO_3 $\text{Ca}_2(\text{Al}_2, \text{MgSi})\text{SiO}_7$ Fe, $\text{CaMgSi}_2\text{O}_6$, CaS	(S + Si)/O > 1 CaS, Fe, Al_2O_3 $\text{Ca}_2(\text{Al}_2, \text{MgSi})\text{SiO}_7$ $\text{CaMgSi}_2\text{O}_6$
(Si)	$^{32}\text{S}(+)$, $^{40}\text{Ca}(+)$		Fe, CaS

(-) Possible depletion relative to other stable isotopes and solar abundances; (+), possible enhancement relative to other stable isotopes and solar abundances; (n), possible enhancement of neutron-rich isotopes due to neutron capture. (\pm), Indicates uncertainty in the production relative to other stable isotopes.

*Solar nebula conditions: 10^{-3} atm pressure and either $T > 1,430$ K, C/O < 1 or $T > 1,300$ K, C/O > 1 and otherwise solar¹² composition.

components, 'last-event' supernova gas and dust, may be mixed into the protosolar cloud when the shock wave from this supernova collides with the cloud and initiates its collapse. We note here, in reference to the problem of producing observable isotopic anomalies, that it may be easier for the 'shrapnel-like' grains from the supernova to penetrate to the interior of the cloud than for supernova gas. This gas, part of which mixes by Rayleigh-Taylor 'interfingering' at the interface between the expanding supernova shock front and the protosolar cloud, may mostly just pass around the exterior of the collapsing presolar nebula. In addition, the time required for injected supernova gas to completely mix with nebular gas may be so short that production of non-radiogenic isotopic anomalies with supernova gas alone might be precluded.

The currently accepted model⁶ of presupernova stars pictures them to be composed of several layers. The outer layer has roughly 'solar' composition, while successive interior shells have compositions corresponding to the products of static hydrogen, helium, carbon, oxygen and silicon burning, respectively. The supernova occurs when the presupernova core collapses, and a shock wave explosively burns and ejects the outer layers of the star. In some stars, however, the shock wave may not cause significant explosive processing. Because the most complete estimates of the composition of supernova ejecta are explosive burning calculations, we assume that these ejecta contain zones composed, successively, of the products of explosive hydrogen, helium, carbon, oxygen and silicon burning. These zones are hereafter referred to as the (H), (He), (C), (O) and (Si) zones.

Table 1 shows, for each explosive burning zone, the isotopic compositions⁷⁻⁹ of the major elements that are decidedly non-solar. Entries are of three types: first, the oversynthesis of one or more isotopes of an element, relative to the others, compared with solar isotopic abundances (namely ^{32}S , ^{33}S , ^{40}Ca and ^{42}Ca in the (C) zone); second, the survival of only one isotope of an element (namely ^{16}O and ^{24}Mg in the (O) zone); or, third, the enhancement of the neutron-rich isotopes of an element relative to solar abundances due to neutron capture during explosive burning or the static helium burning¹⁰ phase that preceded explosive carbon and oxygen burning (namely Ti in the (He) zone).

To discover what minerals could condense in supernova ejecta, equilibrium condensation calculations for each of these zones have been performed¹¹. The results for 10^{-8} atm pressure and different C/O and (S + Si)/O ratios are also shown in Table 1. We have listed, for reasons discussed below, only those minerals that are also condensible at 10^{-3} atm, assuming either that $T > 1,430$ K and C/O < 1 or $T > 1,300$ K and C/O > 1, and otherwise solar¹² abundances. The calculations are not very sensitive to pressure

changes. The most important compositional parameter is the C/O ratio, or, in the (Si) zone, the (S + Si)/O ratio. Grains condensed from a given zone will have an isotopic composition characteristic of that zone and are hence capable of carrying that composition into the presolar nebula. But it is not at all certain that grains can form in expanding supernova ejecta. Preliminary estimates¹¹ suggest that grain condensation is only possible if the shock wave that explosively burns and ejects the matter is relatively weak. If grains do form these results indicate their probable compositions.

As the collapse of the presolar nebula proceeds, the pressure and temperature rise. If isotopic anomalies are to be carried into the Solar System in grains, these grains must be able to survive, that is, their composite minerals must be condensible in this physical environment. We are primarily interested in Ca-rich inclusions of C3 chondrites, which seem to have formed in a nebular region that had a maximum pressure of about 10^{-3} atm and a roughly solar¹² composition. The maximum temperature, T_{max} , that this region reached must have been greater than the condensation temperature of forsterite¹¹, 1,430 K, because the bulk chemical compositions of the Ca-rich inclusions are similar to those calculated for the pre-forsterite condensate and dramatically different from the post-forsterite composition. If $T_{\text{max}} > 1,742$ K, no major O-bearing mineral could survive. But corundum (Al_2O_3), perovskite (CaTiO_3), melilite [$\text{Ca}_2(\text{Al}_2, \text{MgSi})\text{SiO}_7$], spinel (MgAl_2O_4) and diopside ($\text{CaMgSi}_2\text{O}_6$) could survive if $T_{\text{max}} < 1,742, 1,681, 1,625, 1,530$ and $1,435$ K, respectively. Because all these minerals could condense in at least two supernova zones, isotopic anomalies in the elements O, Mg, Si, Ca and Ti may eventually be observable in Ca-rich inclusions.

Excess ^{16}O (ref. 13) and ^{26}Mg have been observed in these inclusions and could have been carried into the solar nebula in grains condensed in the (C) zone (Table 1). In addition, excess ^{16}O is also produced in the (O) zone. Lee *et al.*² found that there is a positive linear correlation between the excess ^{26}Mg and the Al/Mg ratio, that is, the data define an Al-Mg isochron. Clayton *et al.*¹³ found the amount of excess ^{16}O to be fairly constant within a given phase, even from samples taken from different inclusions. Both observations may be easier to understand on the basis of a gaseous rather than a crystalline source of these anomalies. But textural¹⁴ and trace element¹⁵ evidence imply that these inclusions were at least partially melted during or after their formation. Since diffusion in silicate melts is more rapid than in crystalline silicates, all phases crystallising from or equilibrating with a melt would tend to have the same isotopic composition, thus obliterating any previous distribution that could be used to ascertain the source of these anomalies. Those phases which did not melt would be

expected to have different isotopic compositions. Experiments¹⁶ have shown that spinel and pyroxene have higher minimum melting temperatures in this system than melilite and anorthite. Observations¹³ show that the spinel and pyroxene of Ca-rich inclusions have greater concentrations of excess ¹⁶O than melilite, by about a factor of four. This could be explained by a partial melting of the inclusions at a time when there was less excess ¹⁶O in the ambient gas than in the inclusion. On the same grounds, one could expect deviations from the Mg-Al isochron to occur. But, the higher melting point phases (spinel, pyroxene) have such small Al/Mg ratios that relatively large differences in their initial ²⁶Al/²⁷Al ratios would, considering the experimental uncertainty², be unobservable. Thus, the determination of the source of the Mg and O anomalies will be difficult since we may not see the original distribution of ²⁶Al and excess ¹⁶O.

One other distinction remains between the Mg and O anomalies. While the ²⁶Al has to be associated with a nucleosynthetic event that occurred within a few million years of the Solar System's formation, the observed excess ¹⁶O does not. Since the (C) and (O) zones produce excess ¹⁶O, either 'old' presolar grains or 'last-event' supernova grains could have carried excess ¹⁶O into the presolar nebula. But, if grains cannot form in supernova ejecta, the only remaining way, in our scenario, to inject excess ¹⁶O is through last-event supernova gas.

The ²²Ne anomaly¹⁴ observed in CI chondrites may have been produced by 2.6 yr ²²Na-bearing presolar grains condensed in the (He) zone¹⁹. In the solar nebula these grains vaporise if $T_{\max} > 1,000$ K. But evidence²⁰ suggests that CI chondrites may have formed in nebular regions where $T_{\max} < 400$ K; if decay-produced ²²Ne can be retained in or on these grains at this temperature, incorporation of excess ²²Ne into CI chondrites may occur. But, irradiation¹⁷ in the early solar nebula could also produce excess ²²Ne with energy requirements far less stringent than those for the production of ²⁶Mg by irradiation.

What minerals could survive in the presolar nebula if the composition was solar except for a C/O ratio > 1 ? Enstatite chondrites apparently condensed in such a region²¹. Unfortunately, less is known about the mineralogical history of enstatite chondrites, so no limits on T_{\max} can at present be imposed. Calculations¹¹ show that if $T_{\max} > 2,025, 1,930, 1,745, 1,458, 1,400$ and $1,390$ K, respectively, TiC, C, SiC, Fe₃C, AlN and CaS could survive: all of these phases can be condensed in at least two supernova zones (if C/O > 1 in the appropriate supernova zones). Thus, C, N, S and Fe, in addition to Mg, Si, Ca and Ti, isotopic measurements could potentially be key indicators of which supernova zones have been sampled. The C¹²/C¹³ ratio could prove very important in this respect, since this ratio should be less than its solar value if the (H) zone is responsible, while if presolar grains from the (He) and/or (C) zones are incorporated in enstatite chondrites this ratio should be greater than its solar value.

Before the discovery of the ²⁶Mg anomaly, it was thought that

the last nucleosynthetic event affecting the Solar System occurred about 10⁸ yr before its formation²², which is the interval between the production of the r-process radioactive parents (¹²⁹I, ²⁴⁴Pu) of Xe and the retention of daughter Xe in the meteorites. Assuming that the anomalous ²⁶Mg is the *in situ* decay product of ²⁶Al, the Mg timescale is about 10⁶ yr. There are at least two explanations for this discrepancy. First, ²⁶Al, ¹²⁹I and ²⁴⁴Pu may not have been produced in the same nucleosynthetic event, but in separate events 10⁸ yr apart. The latter event made ²⁶Al, but no r-process nuclei (or at least its r-process material did not get into the solar nebula). The incorporation of Al, I and Pu occurred about 10⁶ yr later. The second explanation is that these three nuclei were all made in the same (last) event, but somehow Xe was unable to be retained by meteorites until 10⁸ yr later, possibly because this region of the presolar nebula or the meteorite parent bodies remained too hot. A search for ²³⁵U anomalies due to the decay of ²⁴⁷Cm might resolve this problem²³. ²⁴⁷Cm (half life 1.54×10^7 yr), ¹²⁹I (1.7×10^7 yr) and ²⁴⁴Pu (8.3×10^7 yr) are all r-process nuclei. The U timescale's turning out to be of the order of the Xe timescale would be evidence in favour of the first possibility since U is a refractory element and does not diffuse out of meteoritic minerals as readily as gaseous Xe.

It seems, therefore, that a last-event supernova provides a consistent explanation of the observed isotopic anomalies. Condensation sequences for supernova ejecta imply that presolar grains may be a useful part of this picture.

We thank E. Anders, D. Arnett, R. Clayton, S. Falk and J. Truran for useful discussions. J.M.L. expresses appreciation for the hospitality of both the Institute of Astronomy, Cambridge, England, and NORDITA, Copenhagen, Denmark, where part of this research was done. This work was supported in part by the US NSF (J.M.L., D.N.S.), NASA (J.M.L., D.N.S., L.G.) and by the Alfred P. Sloan Foundation (L.G.).

Received 29 April; accepted 27 June 1977.

- ¹ Gray, C. M. & Compston, W. *Nature* **251**, 495 (1974).
- ² Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Astrophys. J. Lett.* **211**, L107 (1977).
- ³ Schramm, D. N. *Astrophys. Space Sci.* **13**, 249 (1971); *Bull. Am. astr. Soc.*, Hawaii (1977).
- ⁴ Sabu, D. D. & Manuel, O. K. *Nature* **262**, 28 (1976); Cameron, A. G. W. & Truran, J. W. *Icarus* **30**, 447 (1977).
- ⁵ Woodward, P. R. *Astrophys. J.* **207**, 484 (1976).
- ⁶ Schramm, D. N. & Arnett, W. D. *Mercury* **4**, 16 (1975).
- ⁷ Howard, W. M., Arnett, W. D. & Clayton, D. D. *Astrophys. J.* **165**, 495 (1971).
- ⁸ Pardo, R. C., Couch, R. G. & Arnett, W. D. *Astrophys. J.* **191**, 711 (1974).
- ⁹ Woosley, S. E., Arnett, W. D. & Clayton, D. D. *Astrophys. J. Suppl.* **26**, 231 (1973).
- ¹⁰ Couch, R. G., Schmeidekamp, A. B. & Arnett, W. D. *Astrophys. J.* **190**, 95 (1974).
- ¹¹ Lattimer, J. M., Schramm, D. N. & Grossman, L. *Astrophys. J.* (1978).
- ¹² Cameron, A. G. W. in *Explosive Nucleosynthesis* (eds Schramm, D. N. & Arnett, W. D.) (University of Texas Press, Austin, 1973).
- ¹³ Clayton, R. N., Onuma, N., Grossman, L. & Mayeda, T. K. *Earth planet. Sci. Lett.* **34**, 209 (1977).
- ¹⁴ Blander, M. & Fuchs, L. H. *Geochim. cosmochim. Acta* **39**, 1605 (1975).
- ¹⁵ Grossman, L. & Ganapathy, R. *Geochim. cosmochim. Acta* **40**, 331 (1976).
- ¹⁶ Seitz, M. G. & Kushiro, I. *Science* **183**, 954 (1974).
- ¹⁷ Black, D. C. *Geochim. cosmochim. Acta* **36**, 377 (1972).
- ¹⁸ Eberhardt, P. *Earth planet. Sci. Lett.* **24**, 182 (1974).
- ¹⁹ Clayton, D. D. *Nature* **257**, 36 (1975).
- ²⁰ Grossman, L. *Scient. Am.* **232**, 30 (1975).
- ²¹ Larimer, J. W. *Geochim. cosmochim. Acta* **39**, 389 (1975).
- ²² Schramm, D. N. & Wasserburg, G. J. *Astrophys. J.* **162**, 57 (1970).
- ²³ Blake, J. B. & Schramm, D. N. *Nature phys. Sci.* **243**, 138 (1973).

Three-dimensional model of membrane-bound ribosomes obtained by electron microscopy

P. N. T. Unwin

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

A low-resolution three-dimensional map has been obtained from crystalline arrays of membrane-bound eukaryotic ribosomes. It shows both ribosomal subunits to be adjacent to the membrane surface, attached to it by a part protruding from the large subunit.

Most eukaryotic cells have two populations of actively synthesising ribosomes which are distinguishable morphologically according to whether they exist free in the cytoplasm or are bound to membranes, normally the endoplasmic reticulum. In general, the two populations synthesise different sets of proteins. One function of membrane-bound ribosomes, originally discovered from

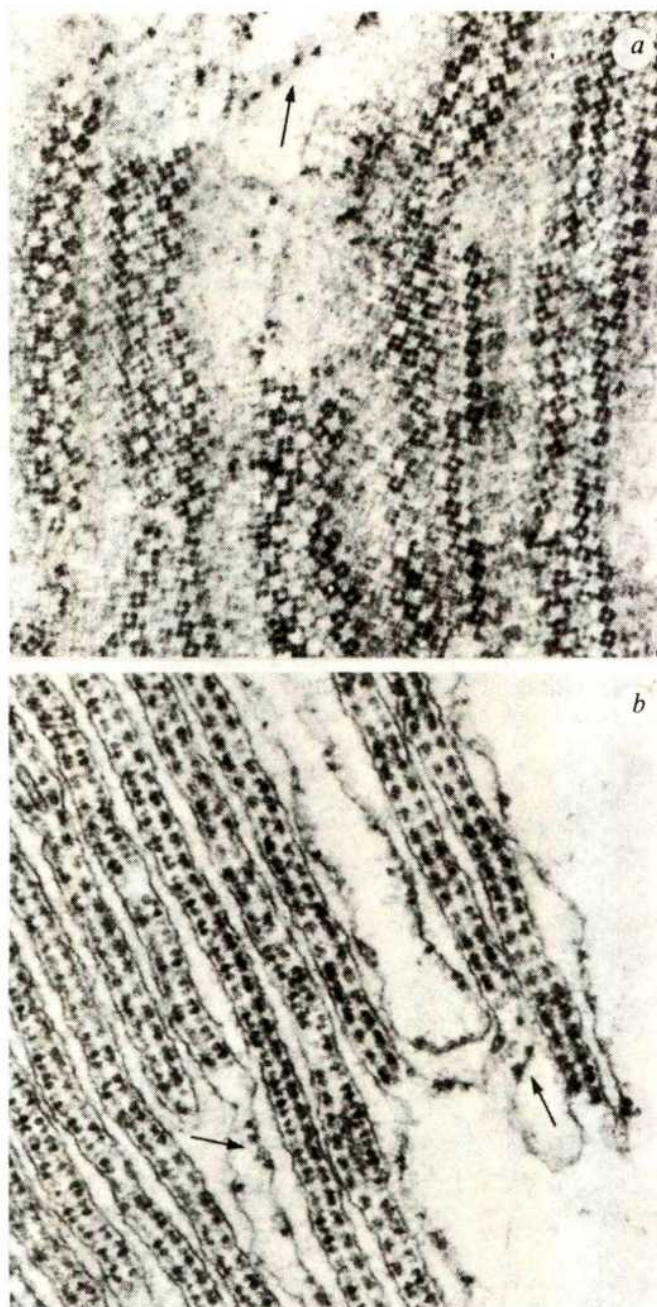


Fig. 1 Organisation of crystalline ribosomes and membranes as in the oocyte of *Lacerta sicula* during winter. *a*, Face-on view; *b*, edge-on view. Membrane-bound ribosomes which are separate from those in the crystals (arrows) are also evident ($\times 50,000$). To bring out details clearly, the ribosome-membrane complexes have been isolated from the cytoplasm and suspended in 100 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose, 50 mM triethanolamine-HCl, pH 7.6. All thin sections were prepared by fixing in glutaraldehyde, post-fixing in OsO_4 , embedding in Araldite, then staining after cutting with uranyl acetate and lead citrate.

experiments with secretory cells¹, is to synthesise proteins for export from the cell. These proteins are vectorially discharged through the membrane during synthesis².

Ribosomes are attached to the membranes by two types of interaction³; one is sensitive to ionic strength and the other to the antibiotic puromycin. The salt-sensitive interaction occurs directly between the large ribosomal subunit and the membrane, but its significance is not yet understood. The puromycin-sensitive interaction is indirect and is thought to be due to an anchoring effect by the membrane on the nascent polypeptide chain. It is therefore associated with proteins which are to be transferred across

the membrane. Recent findings indicate that the specificity for the interaction between the nascent polypeptide and the membrane resides in the amino-terminal sequence of the protein⁴⁻⁷.

It is generally assumed that the large ribosomal subunit lies between the small subunit and the membrane surface^{8,9} and that the protein being synthesised passes into and through the membrane through a protected region^{10,11} within the large subunit. But the electron microscopical and biochemical experiments on which these ideas are based do not provide definitive evidence, and the exact configuration of the ribosome with respect to the membrane is uncertain.

This study is based on a three-dimensional analysis of crystalline arrays of ribosomes which bind to membranes through a salt-sensitive linkage. The commonly assumed configuration is not confirmed. In the crystals, both subunits lie close to the membrane surface, attached to it by a part protruding from the large subunit.

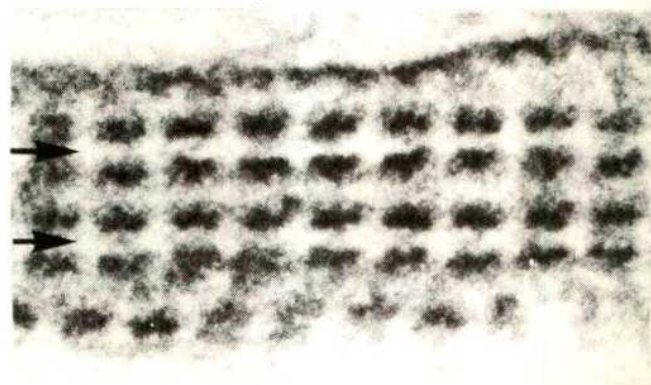
Lizard ribosome crystals

The crystalline sheets of ribosomes which develop in the oocytes of the lizard *Lacerta sicula* during its winter hibernation period¹², were used for this study. Each sheet is composed of two layers of ribosomes which are continuous with the cytoplasm and are bordered on either side by extended, flattened, vesicular membranes similar in appearance to the cisternae of the endoplasmic reticulum. In each layer of a sheet the ribosomes present the same surface to the membrane against which they are juxtaposed, and are arranged as tetramers in the space group P4 ($a=595 \text{ \AA}$) (ref. 13). The crystalline material is inactive *in vivo*, but seems to be competent in protein synthesis when isolated and tested in a cell-free system¹⁴.

Face-on and edge-on views of the sheets are shown in Fig. 1. A detailed description of the packing of the ribosomes seen there and of the crystallographic relations between the layers of a sheet has been given elsewhere¹³. Face-on views (Fig. 1*a*) looked at from an appropriate glancing angle, show either tetramers or single ribosomes to line up in rows. When, in the edge-on views (Fig. 1*b*), the tetramers or the single ribosomes superimpose, they give rise to characteristic patterns of dashed (tetramer) or dotted (single ribosome) lines.

The membranes are most obvious in the edge-on views. They are separated from the densely staining parts of

Fig. 2 Edge-on view of a six-layer stack of sheets formed by aggregation of three single sheets (each of two layers of ribosomes) in a medium of low ionic strength (50 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose, 50 mM triethanolamine-HCl, pH 7.6) containing 0.25% Triton. The zones from which the membranes have been extracted are indicated by the arrows. The other, darker, zones correspond to the region between the two layers of a sheet. Section $\sim 2000 \text{ \AA}$ thick ($\times 150,000$). Stacks of sheets formed artificially in this way have the same appearance as the 'three-dimensional crystals' of ribosomes which develop, in the absence of membranes, in live chick embryos when they are cooled¹⁵.



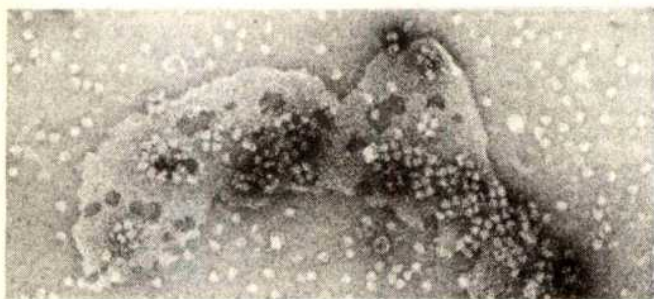


Fig. 3 Single ribosomes and tetramers attached to membrane fragments after high salt treatment; negatively stained in uranyl acetate ($\times 55,000$). The following steps were carried out at 4°C . The cytoplasm from about 50 selected previtellogenic oocytes were carefully opened out under a dissecting microscope into about $50\ \mu\text{l}$ of low salt medium ($50\ \text{mM KCl}$, $5\ \text{mM MgCl}_2$, $0.25\ \text{M sucrose}$, $50\ \text{mM triethanolamine-HCl}$, $\text{pH } 7.6$), stirred vigorously, then centrifuged at $\sim 300g$ for 10 min to produce a pellet consisting almost entirely of large aggregates of ribosome sheets and membranes. This pellet was resuspended in $50\ \mu\text{l}$ of high salt medium ($500\ \text{mM KCl}$, $5\ \text{mM MgCl}_2$, $0.25\ \text{M sucrose}$, $50\ \text{mM triethanolamine-HCl}$, $\text{pH } 7.6$), held for 15 min, and then centrifuged at higher speed to remove any large contaminating material. The supernatant, containing the freed ribosomes and membranes was divided into two equal portions, one of which was dialysed against the low salt medium for several hours. Both portions were subsequently examined together in the microscope. Only the dialysed portion contained membrane-bound ribosomes. The few mitochondrial membranes present in these solutions, and erythrocyte ghosts which had been added to them before dialysis, showed no signs of attachment.

the crystalline ribosomes by a narrow, but distinct, zone relatively free of stained material. A zone of the same width is also observed when the membranes have been partially dissolved, indicating that osmotic effects are not responsible for its appearance or its width. No gap corresponding to this zone is found, however, between ribosomes and membranes in the non-crystalline regions. These ribosomes always seem to be more closely attached, as do those on the endoplasmic reticulum.

Biochemical findings

The stain-depleted zone could be taken to indicate that the ribosome crystals are not attached to the membranes but are held apart, for example, by electrostatic forces. Other evidence favoured direct attachment, however: it was not possible to separate the components in detergent-free media of near physiological composition by normal mechanical means. In addition, the appearance of membrane-extracted stacks of sheets, which sometimes form in the presence of the detergent, Triton X-100 (see Fig. 2), seemed to imply that this zone contained some matter.

Two types of experiment gave more compelling evidence. In the first (Fig. 3) the KCl concentration was varied. It was found that the ribosome sheets, which seemed to be strongly attached to the membranes in solutions containing $5\ \text{mM MgCl}_2$ at KCl concentrations of about $100\ \text{mM}$ or less, were all released without difficulty, and dissociated into single ribosomes and subunits, when it was raised to $500\ \text{mM}$. Furthermore, the released ribosomes were competent in attaching specifically to fragments of the same membrane when the KCl concentration was brought down again. As Fig. 3 shows, many, but not all, of the ribosomes which attached on lowering the KCl concentration tended to associate to form tetramers; the crystal bonding between tetramers was not re-established. In the second experiment (Fig. 4), ribosome sheets which had been isolated from the membranes by detergent treatment¹³, were incubated with vesicles of lizard liver endoplasmic reticulum from which

the bound ribosomes had been removed. A large proportion of the surfaces of all sheets then became covered by membranes, as would be expected if the ribosomes composing them were orientated so that their salt-sensitive attachment sites were exposed and available for interaction. A strong association is suggested by the observations that most of the vesicles are drawn out in single membrane layers and that in places where they are only on one side they cause the crystal to bend.

I conclude that the ribosomes are directly attached to the membranes through a salt-sensitive linkage which may well be the same linkage that binds them to the endoplasmic reticulum.

Analysis of isolated sheets

To investigate the three-dimensional structure of the ribosomes in the sheets they were isolated from the membranes as in ref. 13, placed on electron microscope grids and negatively stained with uranyl acetate. Micrographs were then taken of them tilted at angles between 0° and 76° to the incident 100-kV electron beam.

Fourier methods^{17,18} were used to analyse and combine the data from the micrographs. The Fourier transform of one layer of ribosomes is a two-dimensional lattice of lines along which the phases and amplitudes vary continuously. The Fourier transform of an isolated sheet therefore consists of two such lattices, one of which is

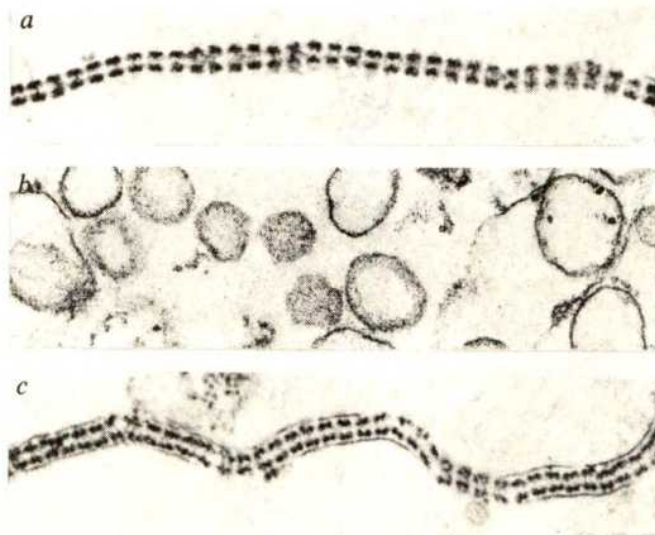


Fig. 4 Isolated crystalline sheets of ribosomes, (a), were incubated with vesicles of endoplasmic reticulum (b), giving the result, (c) ($\times 40,000$). Vesicles of endoplasmic reticulum were prepared at 4°C by a procedure similar to that given in ref. 16. Two lizard livers were homogenised in about twice the volume of a medium containing $0.25\ \text{M sucrose}$, $50\ \text{mM triethanolamine-HCl}$, $\text{pH } 7.6$, and centrifuged twice at $15,000\ \text{r.p.m.}$ ($\sim 20,000g$) in a M.S.E. 3X6.5 titanium swing-out rotor, discarding the pellet and scum at the top on each occasion. The supernatant was then layered on to a discontinuous gradient of $1.5\ \text{M}$ and $2.0\ \text{M sucrose}$ in $50\ \text{mM KCl}$, $5\ \text{mM MgCl}_2$, $50\ \text{mM triethanolamine-HCl}$, $\text{pH } 7.6$, and spun at $42,000\ \text{r.p.m.}$ ($\sim 150,000g$) for 20 h, using the same rotor. The slightly opalescent 'rough microsome fraction' was withdrawn from the region near the $1.5\ \text{M}/2\ \text{M sucrose}$ interface and mixed with an equal volume of a high salt medium containing puromycin so that the final concentration was $500\ \text{mM KCl}$, $5\ \text{mM MgCl}_2$, $0.75\ \text{M sucrose}$, $50\ \text{mM triethanolamine-HCl}$, $1\ \text{mM puromycin}$, $\text{pH } 7.6$. This mixture was incubated at 22°C for 30 min to release the ribosomes and then passed through the same sucrose gradient as previously. The ribosome-stripped vesicle fraction was removed from the second centrifugation at a concentration of $\sim 4\ \text{mg}$ of membrane protein per ml. The vesicle preparation was mixed with an equal volume of isolated ribosome sheets in $50\ \text{mM KCl}$, $5\ \text{mM MgCl}_2$, $0.25\ \text{M sucrose}$, $50\ \text{mM triethanolamine-HCl}$, $\text{pH } 7.6$, prepared as before¹³, and incubated for 6 h at 0°C before fixation, embedding and sectioning.

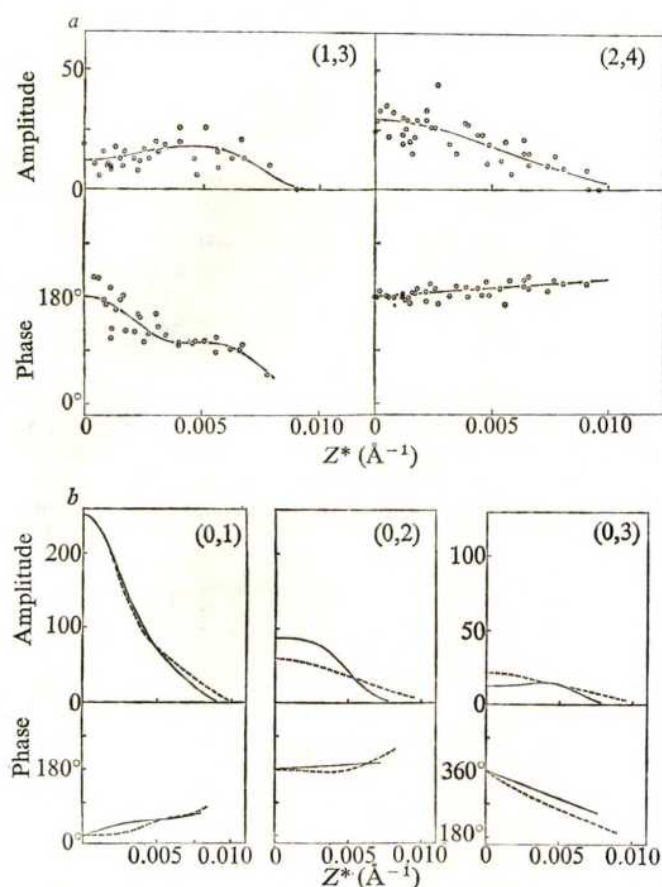


Fig. 5 *a*, Values of calculated amplitudes and phases along two typical lattice lines, plotted as a function of distance (Z^*) from their centres. In this space group the Friedel relationship gives the corresponding values for negative Z^* . Continuous curves drawn through the points and sampled every 0.002 \AA^{-1} give the data on which the three-dimensional map is based. The average phase error, based on comparison of single phase measurements against all others within a small range of Z^* , is less than 20° . Micrographs were taken with a goniometer stage, using bent support grids to achieve the highest tilt angles. They were densitometered and analysed as in ref. 13. The information was combined as in ref. 18. No corrections for effects of defocus were needed at this resolution. *b*, Amplitudes and phases calculated for the $(0,k)$ lattice lines from the tilting experiments (full line) and from edge-on views of superimposed tetramers in $\sim 2,000\text{-\AA}$ thick sections (broken line). The data obtained from the edge-on views have been adjusted to give the best fit with the data from the tilting experiments, the difference between positive and negative staining being accounted for by a 180° phase change. The amplitude scale factor and phase origin adjustment needed to fit the two sets of data were used to correct the variation along the $(0,0)$ lattice line, which the edge-on view gives directly.

upside-down and rotated by a specific amount with respect to the other, depending on the crystallographic relationship between the two layers. Hence, each micrograph gives two sections through the three-dimensional Fourier transform of one layer.

Sixteen micrographs (32 different views of one layer) were needed to give continuous curves of phase and amplitude along all of the 25 crystallographically independent lattice lines, except for the line through the origin, out to a resolution of 90 \AA . Two such curves are shown in Fig. 5*a*. Several micrographs from a given tilt series were suitable for data collection since radiation damage was not a limiting factor.

Optical diffraction patterns from micrographs of negatively stained and (sectioned) positively stained isolated sheets were similar, indicating that at this low resolution, except for the reversal in contrast, positively stained and negatively stained sheets would provide roughly

equivalent maps of the structure. The approximate form of the lattice line through the origin could therefore be determined from sectioned material by calculating the Fourier transform of an edge-on view of one layer. The correct scale of the amplitudes and the origin adjustment for the phases along this lattice line were then found by comparing data, as in Fig. 5*b*.

By sampling the continuous variation of the complete transform at suitably fine intervals (0.002 \AA^{-1}) along the lattice lines, a three-dimensional map of the ribosomes in one layer was calculated.

Three-dimensional map

Figure 6 shows a tetrameric unit from the three-dimensional map, viewed so that the juxtaposed membrane, if it were present, would lie underneath, and also a single ribosome from this unit, viewed parallel to the plane of the array. The contours map out the negative stain-excluding regions only. Therefore they are insensitive to differences of composition within the ribosome and do not show up areas of RNA or protein into which the negative stain may have penetrated.

Particularly noticeable in Fig. 6*a* is a region within the ribosomes which looks like a hole, and so probably represents that part where the large and small ribosomal subunits are most widely separated. The shape and dimensions of the matter surrounding this region resemble features observed in micrographs of isolated eukaryotic ribosomes and of their isolated subunits^{19,20}. A detailed comparison, and arguments based on molecular weight estimates²¹, indicate that the two subunits lie side by side, and suggest that the ribosome can be partitioned as shown, with the large subunit closest to the centre of the tetramer. The line dividing the subunits seems to follow the path in which the inner contours come closest together and connect the 'hole' to the outside, but its exact position cannot be determined from this low resolution map.

Figure 6*b*, the view of a single ribosome along the direction of this path, emphasises another feature of the other map—a narrow protrusion on the large subunit

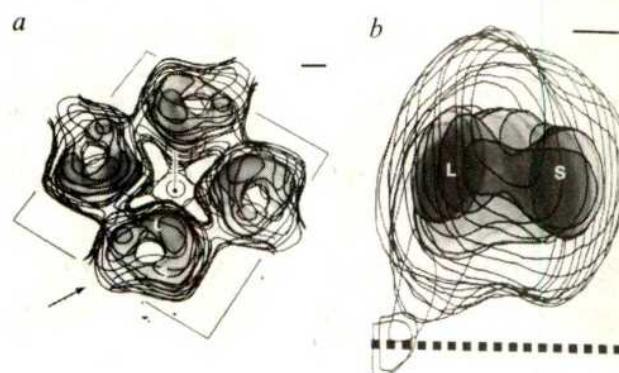


Fig. 6 Views of *a*, a tetramer and *b*, a single ribosome emphasising the dominant features of the three-dimensional map. The negative contours, representing the stain excluding parts, have been drawn at two levels (-2 and -6 in a scale 0 to -10) and the region within the inner contours has been shaded. The tetramer is orientated so that the juxtaposed membrane would lie underneath (right-handed configuration¹³); it is upside-down in comparison with the usual orientation on microscope grids of isolated tetramers and tetramers in p4 arrays²², where the ribosomes present 'left featured frontal' views^{19,20}; thus the small subunit (S) is on the anticlockwise side, and the large subunit (L) on the clockwise side, of the path (arrowed) connecting the 'hole' to the outside. The single ribosome is viewed from the direction indicated by the arrow; the feature protruding from it was estimated, independently of the map, to extend as far ($\pm 15 \text{ \AA}$) as the dotted line. Scale bars represent 50 \AA .

extending towards the central fourfold axis. From the configuration of the group of four of them, it seems that the ribosomes of a tetramer touch in the region close to the central fourfold axis, as well as at a higher radius. These protrusions are unlikely to be something peculiar to ribosomes which crystallise, since the tetramers can be formed *in vitro* from ribosomal subunits released from polysomes²². Nor are they likely to be membrane proteins, since another explanation would then be required for the identical packing of ribosome tetramers in the non-membrane associated stacks of sheets found in chick embryos when they are cooled¹⁵. They are therefore most probably normal components of the large subunits.

Membrane attachment

Edge-on views of stacks of sheets (Fig. 2) were used to derive, independently of the three-dimensional map, the distance reached by the protrusions in the direction of the membrane. In the membrane-extracted zones of these stacks the protrusions show up as slightly denser areas between the dashed lines formed by the tetramers, and presumably extend halfway into the zones. The half-way distance was estimated from projection maps calculated from Fourier transforms of these views, using the data on which the three-dimensional map is based to obtain the correct scale and to define the centre of a layer. The result of the estimate, the furthestmost distance reached by the protrusions in the direction of the membrane, is indicated by the dotted line drawn in Fig. 6b. The position of this line is consistent with the details in the map.

The line in Fig. 6b could also represent the surface of the membrane with which the crystalline ribosomes are associated *in vivo*, since the open space between the protrusions would then give a zone of the same width as seen in Fig. 1b. No additional material is required to span the space. Therefore the protrusions must be the links by which the crystalline ribosomes establish contact with sites on the membrane surface.

It seems unnecessary to draw a distinction in mode of attachment between the crystalline and non-crystalline membrane-bound ribosomes. The closer association with

the membrane of the latter could be due to some freedom of movement of the ribosomes about their attachment site when they are no longer constrained by the crystal bonding.

It is possible that membrane-bound ribosomes are attached by the same mode—some distance from the body of the ribosome—during the initial stages of protein synthesis. A wide separation between the site where the large subunit binds and the emerging polypeptide should allow the polypeptide freedom of motion relative to the highly fluid^{23,24} endoplasmic reticulum, without greatly disturbing its proximity or orientational disposition. Thus, the main function of this type of attachment could be to give the molecules in the membrane and the protein being synthesised a good chance to interact.

I thank Nichol Thompson for cutting the sections, Dr L. Amos for some computer programs and Drs C. Taddei, R. Henderson and M. Bretscher for helpful discussions. The Philips Application Laboratory Cambridge, kindly made available the goniometer version of their EM400 electron microscope for the tilting experiments.

Received 13 May; accepted 8 July 1977.

- 1 Siekevitz, P. & Palade, G. E. *J. biophys. biochem. Cytol.* **7**, 619–644 (1960).
- 2 Redman, C. M., Siekevitz, P. & Palade, G. E. *J. Biol. Chem.* **241**, 1150–1158 (1966).
- 3 Adelman, M. R., Sabatini, D. D. & Blobel, G. *J. Cell Biol.* **56**, 206–229 (1973).
- 4 Milstein, C., Brownlee, G. G., Harrison, T. M. & Mathews, M. B. *Nature new Biol.* **239**, 117–120 (1972).
- 5 Blobel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 835–851 (1975).
- 6 De Villiers-Thierry, A., Kindt, T., Scheele, G. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5016–5020 (1975).
- 7 Wirth, D. F., Katz, F., Small, B. & Lodish, H. F. *Cell* **10**, 253–263 (1977).
- 8 Sabatini, D. D., Tashiro, Y. & Palade, G. E. *J. molec. Biol.* **19**, 503–524 (1966).
- 9 Redman, C. M. & Sabatini, D. D. *Proc. natn. Acad. Sci. U.S.A.* **56**, 608–615 (1966).
- 10 Malkin, L. I. & Rich, A. *J. molec. Biol.* **26**, 329–346 (1967).
- 11 Blobel, G. & Sabatini, D. D. *J. Cell Biol.* **45**, 130–145 (1970).
- 12 Taddei, C. *Expl Cell Res.* **70**, 285–292 (1972).
- 13 Unwin, P. N. T. & Taddei, C. *J. molec. Biol.* **114**, 491–506 (1977).
- 14 Taddei, C., Gambino, R., Metafora, S. & Monroy, A. *Expl Cell Res.* **78**, 159–169 (1973).
- 15 Byers, B. *J. molec. Biol.* **26**, 155–167 (1967).
- 16 Adelman, M. R., Blobel, G. & Sabatini, D. D. *J. Cell Biol.* **56**, 191–205 (1973).
- 17 DeRosier, D. J. & Klug, A. *Nature* **217**, 130–134 (1968).
- 18 Henderson, R. & Unwin, P. N. T. *Nature* **257**, 28–32 (1975).
- 19 Nonomura, Y., Blobel, G. & Sabatini, D. D. *J. molec. Biol.* **60**, 303–323 (1971).
- 20 Lake, J. A., Sabatini, D. D. & Nonomura, Y. in *Ribosomes* (eds Nomura, M., Tissières, A. & Lengyel, P.) 543–557 (Cold Spring Harbor Laboratory Press, New York, 1974).
- 21 Wool, I. G. & Stöffler, G. in *Ribosomes* (eds Nomura, M., Tissières, A. & Lengyel, P.) 417–460 (Cold Spring Harbor Laboratory Press, New York, 1974).
- 22 Byers, B. *Proc. natn. Acad. Sci. U.S.A.* **68**, 440–444 (1971).
- 23 Rogers, M. J. & Strittmatter, P. *J. biol. Chem.* **249**, 895–900 (1974).
- 24 Ojakian, G. K., Kreibich, G. & Sabatini, D. D. *J. Cell Biol.* **72**, 530–551 (1977).

In vitro synthesis of infectious DNA of murine leukaemia virus

Ellen Rothenberg, David Smotkin,
David Baltimore & Robert A. Weinberg

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

DNA synthesised in vitro by purified virions of murine leukaemia virus is infectious. Neither RNA nor protein is required for infectivity. Transfection with reverse transcriptase product shows a single-hit dose response and results in the production of complete, infectious virus.

THE discovery of reverse transcriptase in virions of retroviruses^{1,2} provided a system for studying the viral DNA synthesis which was known to be essential early in infection^{3,4}. Until recently, however, most groups studying reverse transcription *in vitro* found the DNA products to be small relative to the size of the RNA templates. Thus it was not known whether virions could make a complete copy of the

viral genome without the intervention of cellular factors.

Recent studies have improved reaction conditions so as to allow detergent-disrupted virions to synthesise DNA molecules up to 8,000–9,000 nucleotides long, the length of the viral genome RNA which serves as template^{5–7}. It has been possible to synthesise very long DNA transcripts in the 'endogenous reaction' without sacrificing the specificity of initiation: the longest products made by virions of Moloney murine leukaemia virus (M-MuLV) apparently share the unique initiation site and tRNA^{Pro} priming mechanism used by the shorter transcripts^{8,9}. Since the longest transcripts also migrate as a discrete species close to genome length in Agarose gel electrophoresis, it seemed possible that they could be biologically active. We report here that infectious DNA can be synthesised *in vitro* by purified virions of M-MuLV.

Conditions for synthesis of infectious molecules

The conditions used for DNA synthesis were essentially the same as described previously⁶ with a few modifications. Actinomycin D was omitted except where indicated below. A higher yield of the longest DNA molecules was obtained when the ionic strength was reduced to a minimum by omission of NaCl from the reaction mixture and when the incubation was carried out at temperatures between 39 and 41 °C. In most of the DNA preparations used for these studies, each deoxyribonucleoside triphosphate (dNTP) was present at 2 mM. In early studies, DNA was synthesised with each dNTP at 5 mM, but a higher absolute yield of the longest transcripts was obtained with the lower substrate concentration. The lower yield at high substrate concentration was presumably due to the inhibitory effects of the 40–60-mM Na⁺ ions contributed by 20 mM nucleotides. The concentration of magnesium acetate used in these studies was 6.6–7.2 mM, just lower than the total dNTP concentration of 8 mM. Synthesis of long DNA molecules proceeded for 12–20 h, and the incubation could be continued for at least 40 h without detectable degradation of the product DNA.

The products of reverse transcriptase reactions were assayed for infectivity by the Ca₃(PO₄)₂ coprecipitation method of Graham and van der Eb^{10,11} as previously described^{12,13}. The total nucleic acid was extracted from an incubation mixture with sodium dodecyl sulphate, phenol, and chloroform, passed over a column of Sephadex G-50, and ethanol precipitated. In most experiments, virion RNA was not removed before transfection. After exposure to the nucleic acid, NIH/3T3 cells derived from NIH/Swiss mice were scored for successful infection using the syncytium-forming assay with an XC cell overlay as previously described^{12,14}.

Unincubated virions and virions incubated in suboptimal conditions for DNA synthesis were consistently devoid of infectious nucleic acid. After 3–4 h of incubation in the conditions described above, however, infectivity could be detected in the reaction product and increased thereafter for at least 12 h (Fig. 1).

Transfection yields complete virus

To determine whether the transfected cells forming syncytia with XC cells were producing complete virions, the culture fluids of NIH/3T3 cells transfected with endogenous reaction product were assayed for infectious virus. Titres of 10²–10³ PFU per 10 ml culture fluid were found within 3 d of transfection in every culture yielding even one or two infective centres by primary XC assay. After 15 d culture, the supernatants of transfected cells yielded titres of over 10⁶ PFU ml⁻¹. Mock-transfected cells always remained virus free. Therefore transfection with reverse transcriptase products resulted in the production of infectious virus.

The fact that virus spread efficiently through the transfected culture suggested that it was not an endogenous virus, since the only reported endogenous virus of NIH/Swiss mice is xenotropic, that is, incapable of infecting murine cells¹⁵. To confirm the origin of the transfection-derived virus, it was tested for the distinctive host range of the parent M-MuLV. M-MuLV grows equally well on NIH/3T3 (N-type) and BALB/3T3 (B-type) cells, but no known endogenous virus of mice can grow on both. When the transfection-derived virus and M-MuLV were titred on NIH/3T3 and BALB/3T3 cells, the ratio of the titre on N cells to the titre on B cells was 2.9 for M-MuLV and 2.4 for the transfection-derived virus. As controls, an N-tropic virus stock was shown to have a 100-fold preference for the N-type cells, while a B-tropic virus stock had a 10,000-fold preference for B-type cells. Thus the transfection-derived virus displayed the host range of the M-MuLV used in the reverse transcriptase reaction, and not of any known endogenous virus. Unless every infective centre resulted from recombination of M-MuLV fragments specifying host range with an endogenous viral genome, the product of incubated virions contained all the essential genetic information of M-MuLV.

Structure of infectious molecules

The biochemical nature of the infectious molecules was studied by subjecting them to various treatments (Table 1). The infectivity was unaffected by protease, and resistant to RNase at low ionic

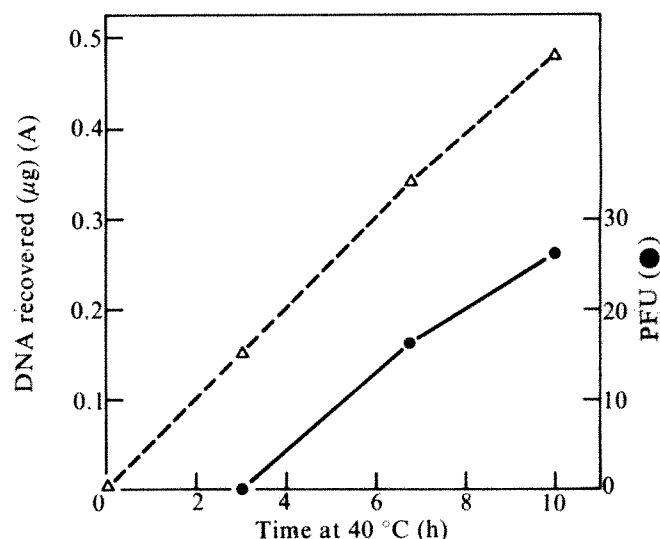


Fig. 1 Synthesis of infectious DNA *in vitro*. Virions were incubated in an endogenous reaction mixture containing 320 c.p.m. pmol⁻¹ ³H-dCTP. At the indicated times, the nucleic acid was extracted from a sample, freed of unincorporated substrate, and used to transfect two plates of 1 × 10⁶ NIH/3T3 cells. The amount of DNA in each sample was determined by monitoring the radioactivity immediately before the addition of CaCl₂. Each point shows the total number of XC plaques obtained with a DNA sample corresponding to about 90 μg of virus protein. Δ, DNA recovered; ●, PFU.

strength, a treatment which will solubilise even RNA hybridised to DNA. Deoxyribonuclease completely abolished the infectivity. Therefore the infectivity must depend on DNA but not on a DNA-protein or DNA-RNA complex. The infectivity was not affected by heating to 68 °C in low salt to disrupt aggregates or by denaturation at 37 °C in 0.1 M NaOH. Complete resistance of the infectivity to alkaline denaturation was observed in the four independent DNA preparations tested. The implications of this result for the structure of the infectious molecules are discussed below.

Since the specific infectivity of the total endogenous reaction product was at least three orders of magnitude lower than that of purified proviral DNA (ref. 12) relatively high concentrations of reverse transcriptase product had to be applied to the cells. Thus it was conceivable that much or most of the infectivity was due to complementation among different partial transcripts. Two different DNA preparations of similar specific infectivity were used to study the dose-response of the transfection. In both cases (Fig. 2)

Table 1 Infectivity of reverse transcriptase product after different treatments

Treatment	XC plaques	DNA (μg)	PFU μg ⁻¹
None	54	0.32	167
68 °C	43	0.31	137
0.1 M NaOH	45	0.29	154
Proteinase K	45	0.25	178
RNase	15	0.14	105
DNase	0	<0.02	—

Virus at 1.6 mg ml⁻¹ was incubated in an endogenous reaction containing 15 mM Mg acetate, 60 mM NaCl, and each dNTP at 5 mM. Incubation was for 7 h at 37 °C. After deproteinisation and gel filtration, six portions were ethanol precipitated. The control was used for transfection in 1 ml of HEPES-buffered saline in standard conditions. One sample was dissolved in 50 μl of 2 mM Tris-HCl, pH 8, 2.2 mM EDTA, and incubated for 5 min at 68 °C before addition of HEPES-buffered saline. One sample was dissolved in 20 μl 0.1 M NaOH and incubated for 15 min at 37 °C, then chilled, diluted with HEPES-buffered saline, and adjusted to pH 7.05 with acetic acid. RNase digestion was for 30 min at 37 °C with 50 μg ml⁻³ pancreatic RNase in 2 mM Tris-HCl pH 7.5, 1 mM EDTA. DNase treatment was for 30 min at 37 °C with 20 μg ml⁻¹ DNase in 2 mM Tris-HCl, pH 7.5, 10 mM MgCl₂. Nuclease-treated samples anti control were subsequently treated with 1 mg ml⁻¹ proteinase K for 10 min at 37 °C and extracted twice with chloroform iso-myl alcohol. They were ethanol precipitated again before transfection.

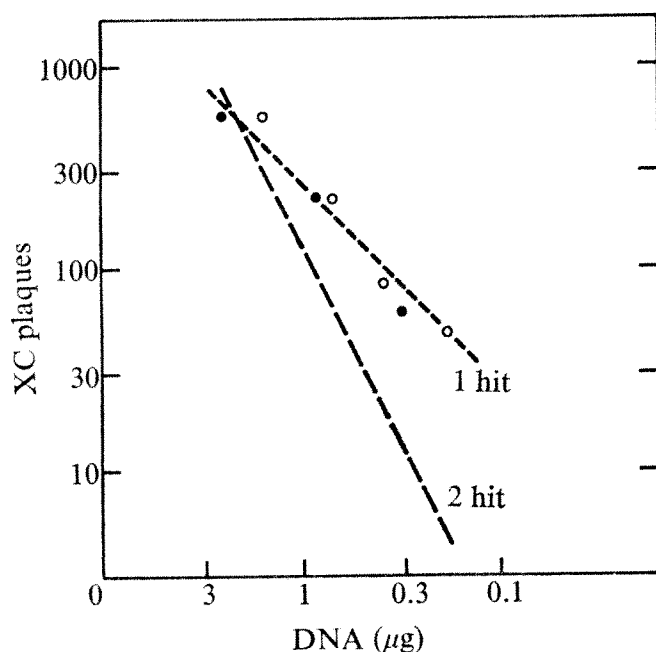


Fig. 2 Dose-response of transfection with endogenous reaction product. ^3H -DNA with a specific activity of $42,400 \text{ c.p.m. } \mu\text{g}^{-1}$ was synthesised from two different harvests of M-MuLV, as described in the legend to Fig. 1. Incubation was for 13.7 h at 40°C . One DNA preparation (\bullet) was subjected to serial threefold dilutions, and the other (\circ) was used in twofold dilutions. Both sets of samples were then ethanol precipitated and used to transfect duplicate plates of cells. The concentration of DNA in each dilution was ascertained by measuring the radioactivity in an aliquot. The broken lines represent the theoretical curves expected for one-hit and two-hit processes giving 500 plaques per $2 \mu\text{g}$ DNA.

the titration showed single-hit kinetics. On a double-logarithmic plot, the slopes of the lines giving the best least-squares fit through each set of points were 1.09 ± 0.10 and 1.22 ± 0.10 . Thus it is most likely that all the genetic information of M-MuLV was carried in individual molecules or molecular complexes resulting from reverse transcription.

Effects of actinomycin D

The alkali resistance of the infectivity, noted in Table 1, suggested that duplex structure might not be required. If single strands of DNA were infectious, then actinomycin D, which selectively blocks synthesis of the second (positive) strand, might be expected to allow infectious molecules to be made. Pairs of DNA samples synthesised in parallel with and without the inhibitor were therefore tested for infectivity (Table 2). In each case, the sample made in the presence of the drug lacked detectable infectivity, whereas the parallel sample made in the absence of the drug was highly infectious. The difference was not due to persistence of small amounts of actinomycin D through the phenol extraction, which might inhibit transfection directly, because a sample made in the absence of the drug remained infectious after addition of $100 \mu\text{g ml}^{-1}$ actinomycin D followed by phenol extraction. Thus actinomycin D prevented the synthesis of infectious molecules.

DNA preparations made with or without actinomycin D were compared in an attempt to explain the difference in biological activity. After 12 h of synthesis, the DNA made in the absence of the drug consistently banded in isopycnic gradients of NaI as almost fully double stranded¹⁶. Actinomycin D, at $100 \mu\text{g ml}^{-1}$, completely suppressed the formation of double-stranded molecules, allowing only the synthesis of negative-strand DNA (not shown). This result is consistent with previous reports^{17,18}. Another difference was found, however, when the ^3H -labelled products of reactions with and without the drug were fractionated by electrophoresis through alkaline Agarose gels, and the DNA displayed either by staining with ethidium bromide (Fig. 3a) or by

fluorography of incorporated tritium (Fig. 3b). In completely denaturing conditions¹⁹, products of both reaction mixtures migrated as an array of species with molecular weights of up to 2.5×10^6 – 3.0×10^6 . The majority of the additional DNA made in the absence of actinomycin D was smaller than about 2×10^6 daltons. This size range is consistent with the size of the majority of positive-strand DNA found *in vivo*²⁰. As previously reported⁶, the longest molecules in each sample formed a discrete band (arrows in Fig. 3b). In the absence of the inhibitor, however, the most extensive transcripts were about 0.2×10^6 MW (600 nucleotides) larger than the largest species made in the presence of the drug (compare lanes 1 and 2, Fig. 3). Both species were capable of hybridising to ^{125}I -labelled virion RNA, and so both represented the first (negative) strand of DNA (F. Yoshimura & E. Rothenberg, unpublished). Thus actinomycin D apparently blocked the addition of about 600 nucleotides to the longest negative-strand polymerase products. The biological deficiency of DNA made in the presence of the drug could therefore be attributed to either of two effects: the absence of positive-strand DNA, or the incompleteness of the negative-strand transcripts.

Sedimentation of infectious molecules

To relate the infectivity more closely to the structure of the DNA, the total endogenous reaction product was fractionated by sedimentation through neutral and alkaline sucrose gradients and the fractions were assayed for infectivity. In the neutral sucrose gradient, the pattern of infectivity was comparable with that reported for the linear cytoplasmic viral DNA extracted from infected cells^{12,21}. A peak of infectivity was observed sedimenting at 18–20S, with no infectivity in material sedimenting more slowly than linear SV40 DNA (Fig. 4a). This is consistent with double-stranded linear molecules of full genome size, 8,000–9,000 nucleotides long. In contrast to viral DNA found *in vivo*¹², the endogenous reaction product contained a relatively high proportion of infectivity sedimenting rapidly from 20S to about 60S. These molecules might be DNA concatemers or DNA still associated with remnants of the 70S RNA complex. Since DNA synthesised in these conditions was almost completely double stranded, it was unlikely that any appreciable fraction of the rapidly-sedimenting infectious material might be single-stranded DNA of genome length.

If denatured endogenous reaction products were infectious, full infectivity should be retained by a sample fractionated on an alkaline sucrose gradient. This was not the case: in three separate attempts, only about 10% of the infectivity could be recovered from these gradients (Fig. 4b). Virtually no DNA was lost during

Table 2 Effect of actinomycin D on synthesis of infectious DNA

Sample synthesised with	DNA (μg)	PFU	PFU μg^{-1}
1 No actinomycin D	1.9	172	92
$100 \mu\text{g ml}^{-1}$ actinomycin D	0.57	0	<1.8
2 No actinomycin D	0.90	92	102
$100 \mu\text{g ml}^{-1}$ actinomycin D	0.35	0	<2.9
3 No actinomycin D	0.78	309	396
$100 \mu\text{g ml}^{-1}$ actinomycin D	0.28	0	<3.8
4 No actinomycin D	1.5	558	365
No actinomycin D; $100 \mu\text{g ml}^{-1}$ actinomycin D added after incubation	1.5	338	224

Pairs of DNA samples labelled with ^3H -dCTP were synthesised in parallel with or without $100 \mu\text{g ml}^{-1}$ actinomycin D. Different virus preparations were used for different experiments, in incubations of 19 h (experiment 1) or 16 h (experiments 2 and 3) at 40°C . The DNA sample used in experiment 4 was one of the samples described in the legend to Fig. 2. Each sample was used to transfect duplicate plates of NIH/3T3 cells, monitoring the input of DNA by radioactivity. In experiment 4, actinomycin D was added to a sample of the purified DNA preparation to a final concentration of $100 \mu\text{g ml}^{-1}$; the sample then underwent two extractions with phenol and one with chloroform before ethanol precipitation. The control sample in experiment 4 was precipitated directly without extraction.

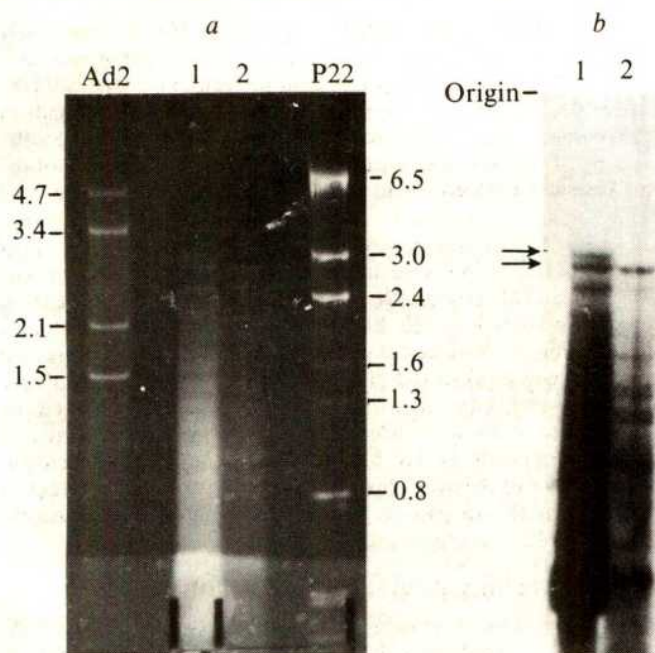


Fig. 3 Effect of actinomycin D on size of reverse transcriptase products. ^3H -labelled DNA samples synthesised in the absence and presence of actinomycin D (a gift of Merck, Sharp and Dohme), as described in the legend to Table 2 (experiment 3), were analysed by electrophoresis on a slab gel of 1% Agarose in the denaturing conditions described by McDonnell *et al.*¹⁹. The gel was cast in 30 mM NaCl, 2 mM EDTA on a plug of 2% Agarose in the same buffer; the samples were applied in 2 mM Tris-HCl, pH 7.5 1 mM EDTA for 13 h at 25 V. The gel was stained with $0.5 \mu\text{g ml}^{-1}$ ethidium bromide²² and photographed during ultraviolet excitation (a). The same gel was treated for fluorography²³ and exposed to film for 6 d at -70°C . Lane 1: DNA synthesised in the absence of the drug. Lane 2: DNA synthesised in the presence of $100 \mu\text{g ml}^{-1}$ actinomycin D. The single-strand molecular weights ($\times 10^{-6}$) of the marker DNAs, adenovirus type 2 cleaved with *Bam*HI endonuclease and phage P22 cleaved with *Eco*RI endonuclease, were as given by H. Delius, R. Greene, and C. Mulder (unpublished), and E. N. Jackson, H. I. Miller, and M. L. Adams (submitted for publication), respectively. Two additional single-strand fragments in the P22 digest were previously reported⁶.

the fractionation, and control samples not submitted to fractionation retained their infectivity. The longest DNA molecules made *in vitro* had previously been shown to resediment on alkaline sucrose gradients without evidence of degradation⁶ so that it seemed unlikely that breakage was responsible for the loss of infectivity. Furthermore, the remaining biological activity sedimented not as full-length transcripts, which should be in fractions 7 and 8 (Fig. 4b), but as molecules of only 4,000–5,000 nucleotides (fractions 9–10), between the full-length single strands and the bulk of the DNA (fractions 11–12, Fig. 4b). Similarly, when a sample identical to that shown in Fig. 4a was denatured in alkali before fractionation on a neutral sucrose gradient, once again only 10% of the infectivity was recovered. As observed with the alkaline sucrose gradient fractionation, the residual infectivity in these neutral sucrose gradients was found in material sedimenting more rapidly than the bulk of the DNA but more slowly than full-length single strands (not shown).

These results present two striking paradoxes: first, that infectious molecules should sediment in alkali similarly to DNA of only half genome length, and second, that DNA which quantitatively retained its infectivity through complete alkaline denaturation should lose biological activity if fractionated by size before transfection.

Discussion

The drastic loss of infectivity in these experiments suggested that size fractionation of the denatured DNA could be separating components which were jointly required for biological activity. The infectivity recovered from the alkaline sucrose gradient could

then result from reassociation of these components. The nature of these cooperating components is suggested by the observation, noted above, that the genome-length transcripts seem to represent the negative strand, whereas the positive strand molecules seem to be distinctly shorter. Size fractionation of the denatured reverse transcriptase products would thus result in the separation of all positive strand DNA from the only molecules which were genetically complete. We therefore believe it most likely that for a molecule to be infectious, the cooperation of a full-length negative strand and some shorter positive-strand DNA is required. On this interpretation, the small peak of infectivity in Fig. 4b would represent the limited overlap of the peaks of full-length negative strands and incomplete positive strands, and the quantitative resistance of the infectivity to simple alkaline denaturation (Table 1) would be an artefact of rapid reassociation. The positive-strand

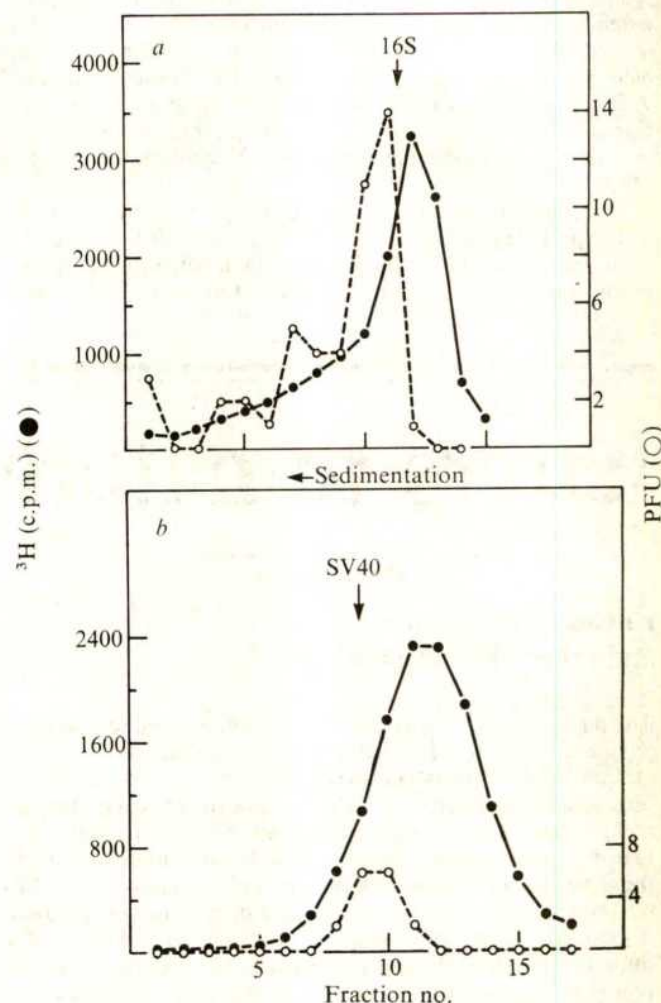


Fig. 4 Sucrose gradient sedimentation of reverse transcriptase product. a, Total nucleic acid from an endogenous reaction was centrifuged through a 4.8-ml 15–30% sucrose gradient in 0.1 M NaCl, 0.02 M Tris-HCl, pH 8.3, 0.002 M EDTA, for 2 h at 48,000 r.p.m., 4°C , in the SW 50.1 rotor. The 16S marker, ^{14}C -labelled SV40 DNA cleaved with *Eco*RI endonuclease, was centrifuged in a parallel bucket. Fractions of 0.32 ml were collected from the bottom and the ^3H -labelled DNA was detected by liquid scintillation counting of 30 μl aliquots (●). Each fraction was used to transfect a single plate of NIH/3T3 cells (○). b, A sample of one of the DNA preparations described in the legend to Fig. 2 was centrifuged through a 5-ml gradient of 5–20% sucrose in 0.3 M NaOH, 0.7 M NaCl, 0.005 M EDTA, 0.1% sarkosyl, for 4.5 h at 49,000 r.p.m., 4°C , in the SW 50.1 rotor. Fractions of 0.26 ml were collected from the bottom, neutralised with an equal volume of 100 mM Tris, 0.3 M acetic acid, and used to transfect single plates of cells as above (○, PFU). A portion of the unfractionated DNA was transfected in parallel, yielding a value for the input infectivity of 150 PFU. The DNA was detected by counting 50 μl portions of the neutralised fractions before ethanol precipitation (●, ^3H). The marker was as described in (a). In both experiments the recovery of DNA after ethanol precipitation was greater than 80% in every fraction.

fragment might be necessary as a primer for cellular DNA polymerase to complete the proviral DNA duplex before integration.

This interpretation does not directly address the question of the unexpected size difference between the longest strands made in the presence and absence of actinomycin D. If the longest DNA made in the presence of the drug were in fact a complete transcript, then the extra sequences might be part of a self-complementary 'hairpin' structure initiating the positive strand. Alternatively, the additional sequences might represent a transcript of the 5'-proximal 600 nucleotides of the RNA template, untranscribed in the presence of the inhibitor.

In summary, we have shown that detergent-disrupted virions of M-MuLV can synthesise DNA which is infectious. DNA made in the endogenous reverse transcriptase reaction can specify the synthesis of complete, infectious virus with the host range of the parent stock; this genetic information is probably carried on individual duplex molecules and not provided by complementation among partial transcripts. Thus it is likely that the virions contain all activities necessary for the synthesis of unintegrated proviral DNA. Reverse transcription in purified virions may accurately reflect the mechanism of synthesis of the viral DNA in infected cells.

A preliminary account of these findings was presented at the Cold Spring Harbor meeting on RNA tumour Viruses, May 30, 1976. We would like to thank Daniel Donoghue for thoughtful advice and valuable criticism throughout this work. This research

was supported by grants from the National Cancer Institute and the American Cancer Society and by a contract from the Virus Cancer Program of the National Cancer Institute. During part of this work, E.R. was supported by an NSF graduate fellowship. R.A.W. is a Cancer Research Scholar of the American Cancer Society, Massachusetts Division. D.B. is a Research Professor of the American Cancer Society.

Received 5 May; accepted 20 June 1977.

- ¹ Baltimore, D. *Nature* **226**, 1209-1211 (1970).
- ² Temin, H. M. & Mizutani, S. *Nature* **226**, 1211-1213 (1970).
- ³ Boettiger, D. & Temin, H. M. *Nature* **228**, 622-624 (1970).
- ⁴ Balduzzi, P. & Morgan, H. R. *J. Virol.* **5**, 470-477 (1970).
- ⁵ Junghans, R. P., Duesberg, P. H. & Knight, C. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4895-4899 (1975).
- ⁶ Rothenberg, E. & Baltimore, D. *J. Virol.* **21**, 168-178 (1977).
- ⁷ Hu, S., Davidson, N. & Verma, I. M. *Cell* **10**, 469-477 (1977).
- ⁸ Haseltine, W. A., Kleid, D. G., Panet, A., Rothenberg, E. & Baltimore, D. *J. molec. Biol.* **106**, 109-131 (1976).
- ⁹ Peters, G., Harada, F., Dahlberg, J. E., Panet, A., Haseltine, W. A. & Baltimore, D. *J. Virol.* **21**, 1031-1041 (1977).
- ¹⁰ Graham, F. L. & van der Eb, A. J. *Virology* **52**, 456-467 (1973).
- ¹¹ Graham, F. L. & van der Eb, A. J. *Virology* **54**, 536-539 (1973).
- ¹² Smotkin, D., Gianni, A. M., Rozenblatt, S. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4910-4913 (1975).
- ¹³ Smotkin, D., Yoshimura, F. K. & Weinberg, R. A. *J. Virol.* **20**, 621-626 (1976).
- ¹⁴ Rowe, W. P., Pugh, W. E. & Hartley, J. *Virology* **42**, 1136-1139 (1970).
- ¹⁵ Levy, J. A. *Science* **182**, 1151-1153 (1973).
- ¹⁶ Birnie, G. D. *FEBS Lett.* **26**, 19-22 (1972).
- ¹⁷ Manly, K. F., Smoler, D. F., Bromfield, E. & Baltimore, D. *J. Virol.* **7**, 106-111 (1971).
- ¹⁸ Garapin, A.-C., Varmus, H. E., Faras, A. J., Levinson, W. E. & Bishop, J. M. *Virology* **52**, 264-274 (1973).
- ¹⁹ McDonnell, M. W., Simon, M. N. & Studier, F. W. *J. molec. Biol.* **110**, 119-146 (1977).
- ²⁰ Gianni, A. M. & Weinberg, R. A. *Nature* **255**, 646-648 (1975).
- ²¹ Fritsch, E. & Temin, H. M. *J. Virol.* **21**, 119-130 (1977).
- ²² Sharp, P. A., Sugden, B. & Sambrook, J. *Biochemistry* **12**, 3055-3063 (1973).
- ²³ Laskey, R. A., Mills, A. D. & Knowland, J. S. *Eur. J. Biochem.* **56**, 335-341 (1975).

letters to nature

Pulsar interpulses and other off-pulse emission

THE pulse emission from pulsars is usually confined to a window having a width of a few per cent of the pulsar period. Seven out of the 149 known pulsars, however, exhibit secondary pulses known as interpulses¹ which follow the relatively stronger main pulses by about half of the pulsar period. While it seems that only a few pulsars have observable interpulses, not all of the known pulsars have been carefully examined for this phenomenon. This is mainly because once a pulsar has been discovered, observers tend to concentrate on studies of the pulse window rather than the whole pulsar period. Consequently it would be statistically incorrect to compare the fraction of pulsars known to have interpulses with the fraction predicted by a proposed pulsar model. In order to check this possibility we conducted an interpulse search on the pulsars that are visible from Jodrell Bank. We report here the results of this search in which one new interpulse was discovered. These observations also revealed the presence of weak emission from regions other than the main pulse and interpulse windows in the cases of three pulsars.

The search took place between July 1975 and April 1976. Observations were made on approximately 100 pulsars at 327, 408 and 610 MHz and of about 50 of these pulsars at 151 and 240 MHz. For each pulsar the full period was observed for a total time of about 90 min. Dispersion removing techniques² were used to keep dispersion smearing comparable with the bin size of 1/400th of the pulsar period. At each frequency observations were made simultaneously, using two contiguous frequency bands of widths 2 MHz at 408 MHz and 610 MHz and 0.5 MHz, at the other frequencies. This enabled an interpulse to be distinguished from interference since the pulse arrival

times would be different in the two bands due to dispersion. The observation of an interpulse at several frequencies provided further checks on the validity of an identification.

Out of all the pulsars observed only PSR1822-09 revealed a previously unknown interpulse. The full period profile of this pulsar is shown in Fig. 1. The interpulse, whose intensity is ~10% of the main pulse, has been seen at 327, 408 and 610 MHz with approximately 170° of longitude separation from the main pulse (where 360° represents the whole pulsar period). Note that PSR1822-09 has several features in common with the Crab Nebula pulsar, PSR0531+21. Besides having interpulses both pulsars have a precursor to the main pulse and both have relatively large values of *P* (the period time derivative). Observations³ of PSR1822-09 indicate that this pulsar also emits pulsed γ rays like PSR0531+21. We suggest that there may be a causal connection between these phenomena.

Figure 1 also shows the profile of PSR0740-28 for which a new component has been detected at 240 MHz. This component has ~4% of the intensity of the main pulse and follows the main pulse by about 70° of longitude. The component is below the limit of detection at 327 MHz and is obscured by interstellar scattering at 151 MHz. The separation between this component and the main pulse is possibly too small to identify it as an interpulse and we regard it as part of the main pulse window which, therefore, has a width of about 70° longitude.

From the survey, therefore, it seems that interpulses are a property of only a few pulsars; of the 105 pulsars studied only four, PSR0531+21, PSR0950+08, PSR1822-09 and PSR1929+10, showed interpulses. Of the remaining pulsars with known radio interpulses, PSR0904+77, PSR0823+26 and PSR1055-52, two were too weak to be detected and one is not visible from Jodrell Bank. (The interpulse in the Vela pulsar, PSR0833-45, is only seen at optical and γ wavelengths). The pulsars with interpulses have, with one exception (PSR0904

+77), shorter than average periods and three of the six that have determined values of \dot{P} have very large values for this. These are thought to be characteristic of young pulsars⁴ which suggests that interpulses may also be a property of young neutron stars. This accords with theory since interpulses are generally thought to indicate that the observer is seeing the effects of both magnetic poles of the neutron star. This occurs when the magnetic axis is almost orthogonal to the rotation axis of the star and Jones⁵ has suggested that this may in fact be the case for the youngest pulsars. According to this model the axes begin to realign after $\sim 10^6$ yr and consequently interpulses should not be seen for the older pulsars. A completely opposite view of pulsar magnetic field alignment has been put forward by Flowers and Ruderman⁶. They also propose an alternative explanation of interpulses and our results may be understood in terms of either model.

Our observations also provided a second result: three pulsars exhibited low frequency emission with a wide longitudinal distribution. Figure 2 shows the full period profiles of these pulsars at several frequencies. This weak emission was confirmed by the presence of a time delay between its components in the two adjacent frequency bands caused by dispersion. Observations⁷⁻⁹ at other wavelengths have shown similar features to be present in other pulsars but greatly enhanced. From Fig. 2 we deduce that this emission has a steeper spectrum than the main pulse radiation. This fact and the wide distribution in longitude suggest that we are dealing with a different part of

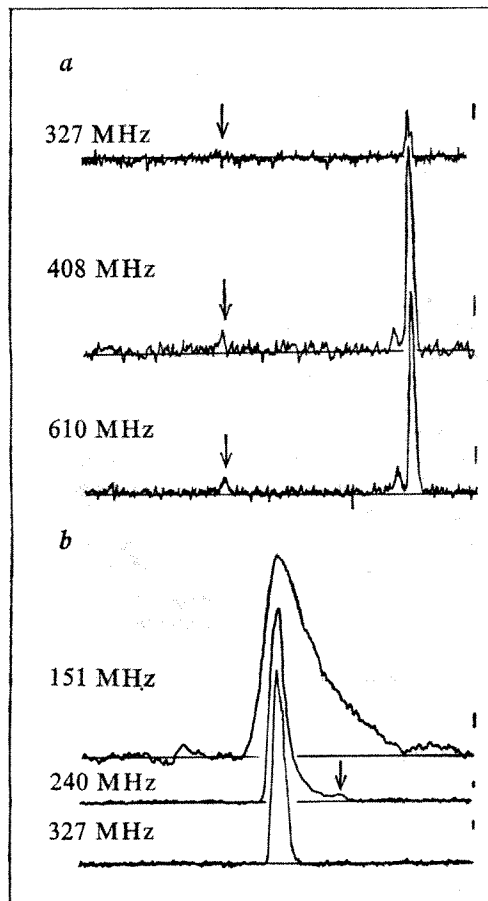


Fig. 1 Integrated profiles of *a*, PSR 1822-09 and *b*, PSR 0740-28. The positions of newly discovered interpulse and pulse components are marked by arrows. The intensity scale is linear but in arbitrary units and the length of the vertical bar at the top right hand of each figure represents the r.m.s. noise of the observation and corresponds to the uncertainty of the position of the fitted baseline. The width of the vertical bar shows the time resolution.

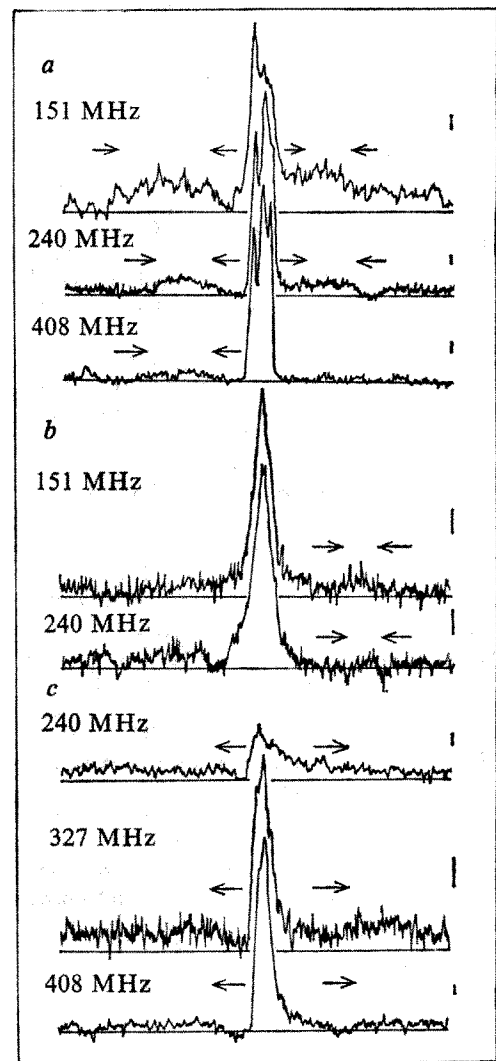


Fig. 2. The integrated profiles of three pulsars exhibiting low frequency emission with a broad longitude distribution: *a*, PSR 0450-18; *b*, PSR 1541+09; *c*, PSR 1718-32. The extent of the emission is shown by delimiters.

the magnetosphere to that responsible for the main and interpulse emissions which tend to have similar widths and spectra.

Future observations of the polarisation of this phenomenon may help distinguish between these two emission regions and reveal further information about the magnetospheric structure of pulsars.

We thank Dr A. G. Lyne for much help and we acknowledge financial support from the SRC.

E. B. CADY
R. T. RITCHINGS*

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank,
Macclesfield,
Cheshire, UK

Received 15 July; accepted 26 July 1977.

*Present address: Department of Medical Biophysics, University of Manchester, UK.

- 1 Manchester, R. N. & Lyne, A. G. *Mon. Not. R. astr. Soc.* (in the press).
- 2 Lyne, A. G. & Thorne, D. J. *Mon. Not. R. astr. Soc.* **172**, 97 (1975).
- 3 Kanbach, G. et al. *Proc. 12th ESLAB Symp.* (1977).
- 4 Lyne, A. G., Ritchings, R. T. & Smith, F. G. *Mon. Not. R. astr. Soc.* **171**, 579 (1975).
- 5 Jones, P. B. *Mon. Not. R. astr. Soc.* **178**, 87 (1977).
- 6 Flowers, E. & Ruderman, M. A. *Astrophys. J.* **215**, 302 (1977).
- 7 Bruck, Y. M. & Ustimenko, B. Y. *Nature phys. Sci.* **242**, 58 (1973).
- 8 Bruck, Y. M. & Ustimenko, B. Y. *Nature* **260**, 766 (1976).
- 9 Backer, B. C., Boriakoff, V. & Manchester, R. N. *Nature phys. Sci.* **243**, 77 (1973).

Observable gravitational effects on polarised radiation coming from near a black hole

THE effects of general relativity on the degree and plane of linear polarisation of X rays emitted by an accretion disk orbiting a black hole (such as Cyg X-1 might contain), were described in a previous letter¹ where we showed that the effects were large. Using a constant of motion along null geodesics in the Kerr metric, discovered by Penrose and Walker², we have since been able to calculate analytically polarisation rotations along any null ray. We present here more extensive results obtained by this method, for two different kinds of disk model and for different black hole angular momenta.

The alteration in observed polarisation properties from their Newtonian values³⁻⁶ is due to the strong bending of light rays coming from the accretion disk. The effects of gravitational redshifts and focusing are to strongly 'pick-out' some regions of the disk to contribute most to the observed properties. To show these effects we have made calculations for 'one'- and 'two'- temperature disk models^{7,8}. These models differ in that the former has a radial temperature gradient, whereas in the latter the inner region, closest to the black hole, has an essentially constant temperature. Although no completely satisfactory disk model exists, our calculations give some idea of the effects to be expected from more refined models.

Figure 1 shows the variation of the plane of polarisation, ϕ , as a function of observing energy for the one-temperature model, seen at a polar angle θ_0 of 41° . An observer above the disk ($\theta_0 < 90^\circ$) sees a clockwise rotation (not anticlockwise as

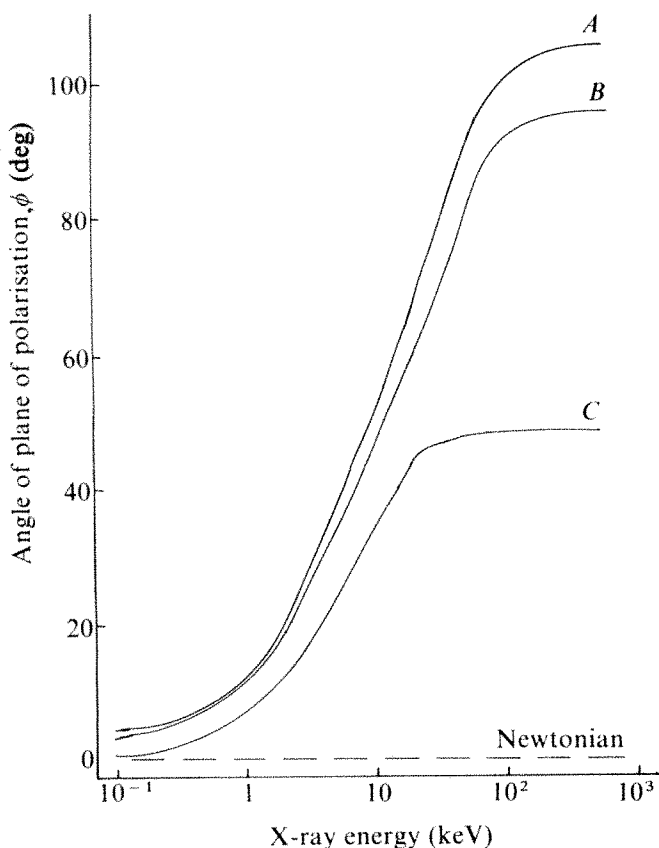


Fig. 1 Variation of plane of polarisation with energy for $\theta_0 = 41.1$ for the one-temperature model with A, $a/m = 0.9981$; B, $a/m = 0.9$; C, $a/m = 0$, where viscosity parameter, $\alpha = 0.1$; mass of black hole, $M = 9M_\odot$; mass accretion rate $= 7 \times 10^{17} \text{ g s}^{-1}$.

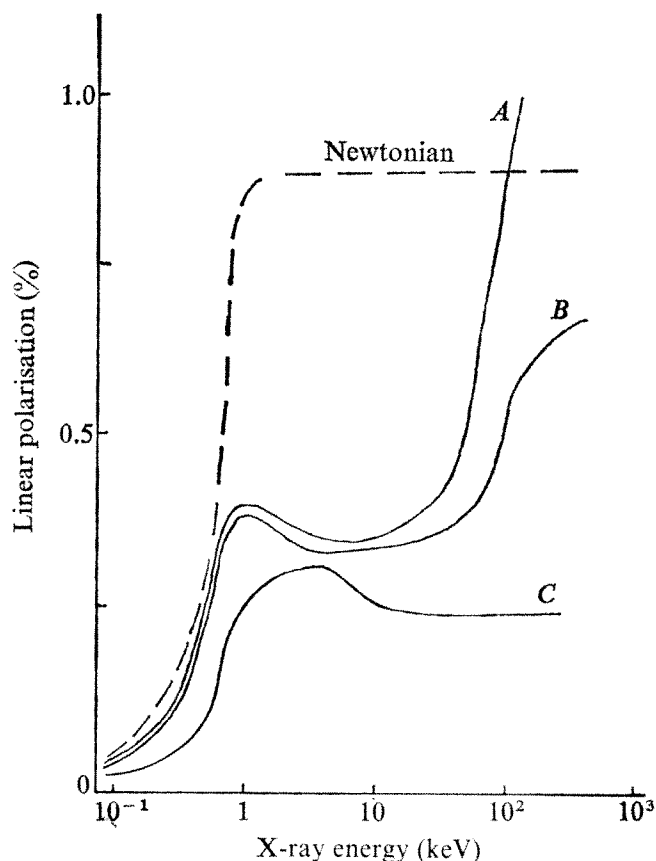


Fig. 2 Variation of linear polarisation with energy for the one-temperature model, with A, $a/m = 0.9981$; B, $a/m = 0.9$; C, $a/m = 0$. Same parameters as Fig. 1.

we stated previously¹). There is still an effect when the black hole is non-rotating ($a/m = 0$). This is because the disk itself is rotating, and the 'pick-out' still operates. Figure 2 shows the degree of polarisation for the same parameters.

The two-temperature model has an outer region with the same structure as the one-temperature model, but it becomes optically thin and hot at a transition radius of $r \lesssim 50$ gravitational radii. Above a threshold energy the radiation from the inner region is, locally, rotated by 90° compared with the optically thick case. So classically one would expect the polarisation vectors of photons coming from the outer and inner regions to be seen as perpendicular. When general-relativistic effects are included, and the 'pick-out' effect is integrated over the inner region, the observed plane of polarisation is not aligned with the projected polar axis, but has an additional jump, of the order of 10° , and the plane then has a constant direction at higher energies. Figure 3 shows the value of this jump in angle as a function of a/m , the angular momentum parameter.

Individual polarisation rotations can be determined analytically using the constant of motion found by Penrose and Walker. This is a complex number, constant along null geodesics in type (2, 2) space-times (the most astrophysically relevant of which is the Kerr metric). Its modulus is the constant of motion found by Carter⁹, and its argument contains the required information about polarisation rotations. The constant is

$$K_{pw} = \kappa^a f^b (l_a n_b) - m_l \bar{m}_b) \Psi_2^{-1/3}$$

where κ^a is the photon wavevector, f^b is the polarisation vector, $(l^a, n^a, m^a, \bar{m}^a)$ is the null tetrad based on the repeated principal null directions of the Kerr metric¹⁰, and Ψ_2 is a complex

function coming from the Weyl tensor. In Kerr we find (using Boyer-Lindquist coordinates¹¹)

$$K_{pw} = (\alpha - i\beta)(r - ia \cos \theta) = K_2 - K_1$$

where $\alpha = (\kappa^0 f^1 - \kappa^1 f^0) + a \sin^2 \theta (\kappa^1 f^3 - \kappa^3 f^1)$

and $\beta = (r^2 + a^2) \sin \theta (\kappa^3 f^2 - \kappa^2 f^3) - a \sin \theta (\kappa^0 f^2 - \kappa^2 f^0)$

Also we have $\mathbf{k} \cdot \mathbf{f} = 0$, and \mathbf{f} is only determined to within a multiple of \mathbf{k} (so we can choose $f^0 = 0$ at any fixed point).

For a given disk model we can evaluate α and β at the point where a null ray leaves the disk, from a knowledge of the initial polarisation vector. We then have to solve three linear equations for the three space-like components of \mathbf{f} at infinity. We find for orthonormal components

$$f^r(\infty) = 0, \quad f^\theta(\infty) = (SK_1 - TK_2)/(S^2 + T^2)$$

$$f^\Phi(\infty) = (-SK_2 - TK_1)/(S^2 + T^2)$$

where $S = (L_z/\sin \theta_0 - a \sin \theta_0)$

and $T = \text{sgn}(k^\theta) \times (Q - L_z^2 \cot^2 \theta_0 + a^2 \cos^2 \theta_0)^{1/2}$

here Q and L_z are constants of motion along the ray. In this way we obtain, after integration over the disk, the results shown in Fig. 1.

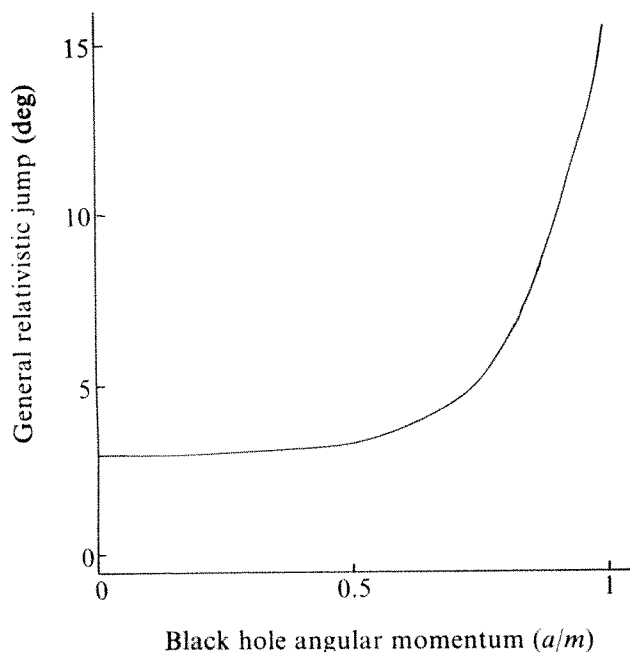


Fig. 3 General-relativistic jump for the two-temperature model, as a function of black hole angular momentum. Same parameters as Fig. 1.

Our previous numerical results for ϕ differ from the exact values by only about 1%.

We thank Roger Penrose for pointing out the analytic constant. Dennis Sciama for his help, and Drs T. Piran, G. Bath, J. Miller and Mr S. J. Rose and D. Pooler for helpful suggestions.

P. A. CONNORS
R. F. STARK

Department of Astrophysics,
South Parks Road,
Oxford, UK

Received 23 June; accepted 22 July 1977.

¹ Stark, R. F. & Connors, P. A. *Nature* **266**, 429-430 (1977).

² Walker, M. & Penrose, R. *Commun. math. Phys.* **18**, 265-274 (1970).

³ Chandrasekhar, S. *Radiative Transfer*, 219-224 (Dover, New York, 1969).

⁴ Angel, J. R. P. *Astrophys. J.* **158**, 219-224 (1969).

⁵ Lightman, A. P. & Shapiro, S. L. *Astrophys. J. Lett.* **198**, L73-L75 (1975).

⁶ Lightman, A. P. & Shapiro, S. L. *Astrophys. J.* **203**, 701-703 (1976).

⁷ Novikov, I. D. & Thorne, K. S. *Les Astres Occlus* (Gordon and Breach, New York, 1973).

⁸ Shapiro, S. L., Lightman, A. P. & Eardley, D. M. *Astrophys. J.* **204**, 187-199 (1976).

⁹ Carter, B. *Phys. Rev.* **174**, 1559-1571 (1968).

¹⁰ Breuer, R. A. *Lecture Notes in Physics* **44**, (Springer, Berlin, 1975).

¹¹ Misner, C. W., Thorne, K. S. & Wheeler, J. A. *Gravitation* (Freeman, San Francisco, 1973).

Quantum uncertainty in the final state of gravitational collapse

THE ratio of the action S to \hbar (Planck's constant/ 2π) determines whether the physical system in question is to be treated classically or quantum mechanically. In the area of classical physics the ratio S/\hbar is large compared with unity, and the governing equations are given by $\delta S = 0$. Quantum mechanics begins to be important when $S \lesssim \hbar$, and the definitive approach of classical physics is replaced by quantum uncertainty. We discuss here the behaviour of a physical system which is initially in the classical domain ($S \gg \hbar$) but whose later development may well take it into the region of quantum uncertainty. We consider a specific example of this—the gravitational collapse of a spherical dust ball. While classically such a dust ball ends up in a space-time singularity, the corresponding quantum mechanical result suggests a range of final states some of which are non-singular.

The explicit solution obtained here ignores pressures which have an important role in the gravitational equilibrium of stars. But classical relativity predicts that a space-time singularity develops in a body undergoing gravitational collapse in a fairly general and 'reasonable' set of physical conditions^{1,2}. Thus pressures satisfying 'physically reasonable' equations of state are not able to prevent this fate for those stars which become black holes towards the end of their evolution. For such stars S eventually becomes small enough for the above quantum considerations to become important. In these cases the above solution gives a qualitative indication of the probable types of final states.

The classical relativistic equations are derived from the variation of an action given by

$$S = \frac{c^4}{16\pi G} \int_V R(-g)^{1/2} d^4x - \sum_a \int m_a da \quad (1)$$

where c is the speed of light, G the gravitational constant, R the scalar curvature of space-time, g the determinant of the metric tensor g_{ik} . The second term of S is the action describing the behaviour of a system of particles labelled by a , with masses m_a . We may add other terms to S to include other possible physical interactions like electromagnetism. V is the 4-volume of space-time under consideration. In the classical approach, we may think of V as the region sandwiched between two chronologically ordered spacelike hypersurfaces Σ_1 and Σ_2 . The specification on Σ_1 of a 3-geometry $^{(3)}G_1$, and $^{(3)}G_2$, together with the field equations obtained from $\delta S = 0$ would lead to the determination of the 3-geometry $^{(3)}G_2$ on Σ_2 (see ref. 3).

The classical approach is a good one so long as $S \gg \hbar$, where $2\pi\hbar = h$ = Planck's constant. For most situations discussed in general relativity this condition is satisfied. But in the gravitational collapse of a compact object S may become small enough to be of the order \hbar close to the singularity. It therefore becomes necessary to examine this problem from the quantum mechanical point of view. Here we adopt the path integral approach.

In this approach, non-classical geometries, that is, those not satisfying $\delta S = 0$ are permitted and the above sandwich problem is rephrased thus. Given two 3-geometries, $^{(3)}G_1$ on Σ_1 and $^{(3)}G_2$ on Σ_2 what is the probability amplitude for the system with the given action (1) to go from one to the other? The answer may be expressed as a propagator $K[^{(3)}G_2, \Sigma_2; ^{(3)}G_1, \Sigma_1]$ which is obtained by the Feynman rule of sum over histories⁴.

In practice the execution of this project is extremely difficult and has not been done in the general situation. It is, however, possible to obtain an exact solution under a very limited form. Since this

form gives an insight into the gravitational collapse problem, it is described below in brief.

Following DeWitt⁵ we take into consideration only the conformal degrees of freedom. Suppose the classical solution of $\delta S = 0$ is described by a metric \bar{g}_{ik} . Consider non-classical solutions which are conformal to the classical one

$$g_{ik} = \Omega^2 \bar{g}_{ik} \quad (2)$$

where Ω is the conformal function. From (2) we get

$$\int_V R(-g)^{1/2} d^4x = \int_V (\Omega^2 \bar{R} - 6\Omega_i \Omega^i)(-\bar{g})^{1/2} d^4x + \text{surface term} \quad (3)$$

where on the right hand side the quantities refer to the classical metric. In the summing over histories we now have only the histories of Ω from Σ_1 to Σ_2 .

The problem is further simplified for the case of a collapsing homogeneous ball of dust in an otherwise empty space-time⁶. In terms of the comoving coordinates r ($\leq r_b$), θ , Φ , t , the classical solution is given by the line element

$$ds^2 = c^2 dt^2 - Q^2(t) \left[\frac{dr^2}{1 - \alpha r^2} + r^2(d\theta^2 + \sin^2\theta d\phi^2) \right] \quad (4)$$

in the interior of the ball. The scale factor $Q(t)$ is taken to be unity at the start of the collapse when the density was ρ_0 . The Einstein field equations $\delta S = 0$ give

$$\dot{Q}^2 = c^2 \alpha \left(\frac{1-Q}{Q} \right), \quad \alpha = \frac{8\pi G \rho_0}{3c^2} \quad (5)$$

The singularity ($Q = 0$) develops in a t -interval $\pi/(2c\alpha^{1/2})$. We will denote this instant by $t = 0$. As we are interested in the final stages of collapse, we will approximate the solution of (5) by

$$Q = \left(\frac{3c}{2} \alpha^{1/2} \right)^{2/3} T^2, \quad T = (-t)^{1/3} \quad (6)$$

Consider now the quantum fluctuations which preserve the symmetries of the problem. These are the conformal fluctuations for which Ω is a function of t only. We denote Σ_1 and Σ_2 by $t = t_1$, ($T = T_1$) and $t = t_2$, ($T = T_2$) respectively. Following the techniques of path integration⁴ it is possible to compute K exactly. The answer is best expressed in terms of the departure from the classical solution ($\Omega = 1$)

$$\Phi = \Omega - 1 \quad (7)$$

Given $\Phi = \Phi_1$ on Σ_1 and $\Phi = \Phi_2$ on Σ_2 we have

$$K(\Phi_2, T_2; \Phi_1, T_1) = \left(\frac{3iV\rho_0 c^2 T_1^2 T_2^2}{4\pi\hbar T_1 - T_2} \right)^{1/2} \times \\ \times \exp \left\{ \frac{3iV\rho_0 c^2}{4\hbar (T_1 - T_2)} [T_1^3 (T_1 - 2T_2) \Phi_1^2 + \right. \\ \left. + (T_2 - 2T_1) T_2^3 \Phi_2^2 + 2T_1^2 T_2^2 \Phi_1 \Phi_2] \right\} \quad (8)$$

where V is the coordinate volume of the ball.

This kernel has the reproducing property and its significance can be seen in the following way. Suppose at Σ_1 the state of the dust ball is described by a wave packet of dispersion Δ_1 in Φ_1

$$\Psi(\Phi_1, \Delta_1) = \left(\frac{1}{2\pi\Delta_1^2} \right)^{1/4} \exp \left(-\frac{\Phi_1^2}{4\Delta_1^2} \right) \quad (9)$$

where $\Delta_1 \ll 1$. Then at Σ_2 , with $|t_2| \ll |t_1|$, the state is described by a wave packet with a mean $\langle \Phi_2 \rangle = 0$ and a dispersion

$$\Delta_2 = \left(\frac{\hbar}{3V\rho_0 c^2 T_1 \Delta_1} \right) \left\{ 1 + \left(\frac{3V\rho_0 \Delta_1^2 c^2}{\hbar} \right)^2 T_1^6 \right\}^{1/2} T_2^{-2} \quad (10)$$

Thus, however small Δ_1 may be, equation (10) shows that Δ_2 diverges as $T_2 \rightarrow 0$, ($t_2 \rightarrow 0$). In other words, although the wave function at t_2 continues to have the classical solution ($\Phi_2 = 0$) as the mean, the spread around this solution gets progressively larger as the so-called classical singular epoch is approached: (10) effectively tells us where the quantum uncertainty begins to dominate. For a solar mass dust ball with $\rho_0 = 1 \text{ g cm}^{-3}$, the linear shrinkage must factor 10^{-43} before the quantum uncertainty takes over.

Although the smallness of this ratio indicates the range over which the classical theory is valid, the ultimate dominance of quantum uncertainty as $t \rightarrow 0$ seems inescapable.

This solution can also be used to discuss the early stages of a Friedmann universe. A few years ago Hoyle and Narlikar⁷ had suggested that the quantum fluctuations in a classical big bang model might produce models without particle horizons (which inhibit the transfer of information). It is interesting to note that the range of uncertainty indicated by Δ_2 permits such models.

I thank Professor J. A. Wheeler for discussions and for hospitality at the Center for Theoretical Physics, University of Texas at Austin. I also thank Professor Cecile DeWitt for discussions on path integrals, and the International Astronomical Union for a travel grant.

J. V. NARLIKAR*

Center for Theoretical Physics
University of Texas at Austin
Austin, Texas 78712

Received 9 May; accepted 23 June 1977.

*Permanent address: Tata Institute of Fundamental Research, Bombay 400 005, India.

¹ Penrose, R. *Phys. Rev. Lett.* **14**, 57 (1965).

² Hawking, S. W. & Ellis, G. F. R. *The Large Scale Structure of Space time* (Cambridge University Press, Cambridge, 1973).

³ Misner, C. W., Thorne, K. S. & Wheeler, J. A. *Gravitation* (Freeman, San Francisco, 1973).

⁴ Feynman, R. P. & Hibbs, A. R. *Quantum Mechanics and Path Integrals* (McGraw-Hill, New York, 1965).

⁵ DeWitt, B. S. *Phys. Rev.* **162**, 1239 (1967).

⁶ Hoyle, F. & Narlikar, V. V. *Proc. R. Soc. A* **278**, 465 (1964).

⁷ Hoyle, F. & Narlikar, J. V. *Nature* **228**, 544 (1970).

Origin of diffuse interstellar lines

SINCE their discovery over 40 years ago, the origin of the diffuse interstellar absorption lines has been a mystery although there have been many suggestions put forward. Developments in spectroscopy and astronomy have intensified rather than reduced the mystery. This letter takes into account some of these recent developments and suggests a rather definite (but unconfirmed) identification of the lines. Excellent surveys of the diffuse interstellar lines have been given by Herbig¹ and by Wu², so the many studies of these lines will not be mentioned again. For our purposes, it is sufficient to note that the strongest line which lies at 4,428 Å has a half width of 20 Å, and that 38 weaker lines of varying strengths and widths lie at longer wavelengths. The strength of the lines is strongly correlated with regions in space containing interstellar grains (particularly small grains) and there is strong evidence that all the lines arise from a single species or from closely related species. The only feature in the spectrum lying to the violet of 4,428 Å which seems to be related to the diffuse lines is a continuum in the 2,200 Å region but this must certainly involve much higher electronic states of the absorbing species.

The line widths of the diffuse lines are the unique characteristics which differentiate them from all identified interstellar lines. The line widths which vary between 1 and

100 cm^{-1} seem to preclude assigning the lines to transitions between discrete states of free atoms or molecules (which at the temperature of the interstellar region give bands narrower than the $40\text{--}100\text{ cm}^{-1}$ widths of most of the stronger lines). Many suggestions have been made regarding the origin of the lines. The line widths have been accounted for by assuming either that the absorbing species is pre-ionised or predissociated in its excited state, or that the absorbing species is on or in an interstellar grain where site inhomogeneities account for the line widths. The first of these hypotheses suffers because in spite of numerous studies of most of the abundant simple molecules and atoms, it has not been possible to identify the absorbing species. A second difficulty with the predissociation hypothesis is that each absorbed photon destroys the absorber and an efficient process must exist to restore the population. The second hypothesis also suffers because it has not been possible to identify the species which is responsible for the absorption. More important, considering the probable wide variation of sizes and compositions of the grains, it is difficult to conceive a system of grains and absorbers which will give the observed discrete set of lines with widths down to a few cm^{-1} and give series of lines extending only to the red of the strongest line at $4,428\text{ \AA}$. Danks and Lambert³ have shown that for a certain reasonable range of molecular moments of inertia, dipole moments and collision rates, the shapes of some of the diffuse lines can be fitted by band contours of heavy molecules. They do, however, have difficulty in fitting the wider lines and probably in accounting for the wide range of bandwidths.

Here I suggest that the lines are caused by the absorption of polyatomic molecules and that the line width is the result of radiationless internal conversion between stable states. Furthermore, I propose that the absorbing species are long chain carbon molecules, C_n where n may lie in the range 5–15. The direct evidence to support this is very meagre indeed but this suggestion seems to account for the observations without straining our present concepts of spectroscopy or astronomy.

A radiationless internal conversion results from an interaction between the levels of an excited electronic state and the higher vibrational levels of a lower state⁴. The process causes each rotational line of an electronic transition to be replaced by a band whose width depends on the interaction energy. This interaction energy varies greatly from one state to another and even from one vibrational level to another in the same electronic state. The band widths can be predicted only by very elaborate calculations but widths in the $1\text{--}100\text{ cm}^{-1}$ range are not unusual. For all but the most simple molecules, the band consists of such a high density of lines that it is for all practical purposes a continuum. Following the absorption of radiation in this band, the molecule will lose its excitation through a series of vibrational transitions. Thus, in molecules displaying this type of radiationless transition, diffuse bands are not accompanied by dissociation. It is worth noting that internal conversions are not rare phenomena but exist in most polyatomic molecules and are particularly important for the larger molecules.

Recently HC_3N (ref. 5), HC_5N (ref. 6), and HC_7N (H. W. Kroto *et al.*, submitted for publication) have been identified by radio observations of dense interstellar clouds. It seems most likely that this series will be extended to at least HC_9N once the spectrum of this molecule has been identified in the laboratory and it is reasonable to assume that long chain acetylenes, which have no dipole moment and thus cannot be observed, are more abundant than the observed cyanoacetylenes. How these long chains of carbon atoms are formed is unknown although their existence is firmly established. It can, therefore, be suggested that similar long chains of carbon atoms, either formed by the same process or released from

clouds, exist in the less dense interstellar regions. In the less dense regions, these chains may lose any hydrogen they had or acquire, by photodissociation and exist as pure carbon chains. We may further postulate that the shortest of the chains (C_2 , C_3 , C_4) are photodissociated but chains beyond a certain length can lose the energy absorbed in the form of radiation by the internal conversion process and are not readily photodissociated.

Pitzer and Clementi's⁷ calculations on the structures and electronic states of long chain carbon molecules agree with the experimentally observed high stability of these molecules, and indicate that the stability of chains with odd numbers of atoms is greater than those with even numbers. These calculations, which give only a qualitative picture of the absorption spectra, indicate that the smallest of the molecules should show strong absorption bands in the 2-eV ($6,000\text{-\AA}$) region with the corresponding absorption shifting to the red with increased chain length. Some alteration between chains with odd and even numbers of atoms is also expected in the position of the absorption spectrum with the odd tending to lie to shorter wavelengths. The simplest polyatomic carbon molecule, C_3 , is known to have a strong absorption band at $4,050\text{ \AA}$ (ref. 8) and there is evidence that C_4 has absorption bands in the $5,000\text{-\AA}$ region⁹. Therefore, if we examine the spectrum of a mixture of C_n molecules in which the abundance of C_n decreases as n increases and n has a certain lower limit such as 5, we should expect to find a series of bands extending to the red from a strong band in $4,000\text{--}5,000\text{-\AA}$ region.

The facts and postulates given above suggest that the strong $4,428\text{ \AA}$ band arises from the absorption of a C_n molecule where n is a number such as 5, 7 or 9. The absorption bands at longer wavelengths result from the various absorptions of longer chains. The known correlation between these absorption bands and small interstellar grains seems reasonable since at least some fraction of these grains may be nothing more than an aggregate of carbon chains. It is possible that some of the interstellar extinction in the ultraviolet, which is usually attributed to small interstellar grains, may be due to absorption into the higher electronic states of long chain carbon molecules.

The present hypothesis has the following merits: (1) It associates the observed absorption with very stable molecules which are composed of one of the most abundant elements and which have absorption bands in the region of the observed lines. (2) It accounts for the absence of lines at wavelengths shorter than the strong $4,428\text{-\AA}$ band. (3) It presents a process which could give the observed line widths and which does not involve the dissociation of a molecule with the absorption of each photon. (4) There is evidence that long chains of carbon atoms exist in at least some interstellar regions. (5) The association of the absorption bands with small interstellar grains seems natural.

The weakness of this suggestion lies in the lack of experimental evidence for the several postulated mechanisms. It has been assumed that radiationless transitions between stable states account for the line widths of the diffuse lines and may also protect the long chain molecules from photodissociation by ultraviolet light. It has been assumed that photodissociation destroys the short chain carbon molecules and removes other atoms, particularly hydrogen, from the chains and it has been assumed that the absorption bands of long chain carbon molecules lie at the positions of the observed diffuse lines. It may be possible to devise experimental test for some of these assumptions. Thompson, DeKock and Weltner¹⁰ have observed the infrared spectra of several long chain carbon molecules in argon matrices and there have been several mass spectrometry studies¹¹. Experimental studies of the electronic spectra of such carbon molecules should be possible in the near future.

I thank Drs G. Herzberg, J. M. MacLeod and T. Oka for their helpful comments.

A. E. DOUGLAS

*Herzberg Institute of Astrophysics,
National Research Council,
Ottawa, Ontario, Canada*

Received 18 April; accepted 13 July 1977.

- ¹ Herbig, G. H. *Astrophys. J.* **196**, 129–160 (1975).
- ² Wu, C. *Astrophys. J.* **178**, 681–699 (1972).
- ³ Danks, A. C. & Lambert, D. L. *Mon. Not. R. astr. Soc.* **174**, 571–586 (1976).
- ⁴ Jortner, J., Rice, S. A. & Hochstrasser, R. M. *Adv. Photochem.* **7**, 149–309 (1969).
- ⁵ Turner, B. E. *Astrophys. J.* **163**, L35–L39 (1971).
- ⁶ Avery, L. W., Broten, N. W., MacLeod, J. M., Oka, T. & Kroto, H. W. *Astrophys. J.* **205**, L173–L175 (1976).
- ⁷ Pitzer, K. S. & Clementi, E., *J. Am. chem. Soc.* **81**, 4477–4485 (1959).
- ⁸ Gausset, L., Herzberg, G., Lagerqvist, A. & Rosen, R. *Astrophys. J.* **142**, 45–76 (1965).
- ⁹ Graham, W. R. M., Dismuke, K. I. & Weltner, W. *Astrophys. J.* **204**, 301–310 (1976).
- ¹⁰ Thompson, K. R., DeKock, R. L. & Weltner, W. *J. Am. chem. Soc.* **93**, 4688–4695 (1971).
- ¹¹ Berkowitz, J. & Chupka, W. A. *J. chem. Phys.* **40**, 2735–2736 (1964).

Carbonaceous compounds in interstellar dust

SEVERAL infrared bands found recently in astronomical sources have not been assigned to specific substances in a convincing way (see refs 1–6). Resonance features in solids seem to be the most plausible emission and absorption mechanisms, but the features cannot be identified with known or suspected compounds in the interstellar dust grains such as silicates, water ice, silicon carbide, or graphite. We propose here a new substance, consisting of carbonaceous material, which may be responsible for some or all of the unidentified infrared features.

The infrared bands for which there are no positive identifications, or for which the identifications rest on the assignment of a single band, are listed in Table 1. The band at 3.3 μm has been studied in greatest detail. It is definitely broader than a single emission line and is unresolved at a spectral resolution of $\lambda/\Delta\lambda \sim 3,000$, ruling out the most attractive molecular candidates for the feature⁶. The apparent temperature independence of the feature and its presence in sources with widely differing physical conditions also makes a molecular origin seem doubtful. Here we interpret all of the features in Table 1 as solid resonance bands.

References to the other emission bands are given in Table 1. Less than ten sources containing these features have been observed so correlations among them are not yet firmly established. It seems that the presence of the 3.3- μm band and the 11.3- μm bands is not correlated, but the presence of the 3.4- μm and 11.3- μm bands might be⁶. Emission features at 6.2 μm and 7.7 μm were discovered by Russell *et al.*⁵ in the spectrum of the planetary nebula, NGC 7027. Observations at these wavelengths are just beginning to become available, and the sparsity of sources at this time is merely an observational effect. We have also listed the band at 11.3 μm as 'unidentified' despite previous suggestions of a carbonate origin, since subsequent observations did not reveal expected carbonate features

at 7 μm and between 28 and 35 μm (refs 1, 4). Therefore this identification must still be regarded as provisional.

Strong emission near 3.3 μm is highly suggestive of a substance incorporating carbon–hydrogen bonding since this is the well known wavelength of the fundamental C–H stretching vibration. Absorption bands between 3 and 3.5 μm are observed in carbon stars and some of these have recently been identified as HCN and C_2H_2 by high-resolution spectroscopy⁷. The existence of organic molecules which may participate in particle formation near carbon-rich objects is therefore well established. It is also plausible that in some cases the C–H bond is associated with a solid particle. The C–H could, for example, be attached to a matrix such as the observed silicate particles or the connected graphite grains. It is unlikely, however, that this is the case for all the sources since silicate emission or absorption does not necessarily accompany the unidentified bands. A more likely configuration would be a particle composed largely or entirely of carbonaceous material in which the C–H bonds are an ubiquitous component in an assemblage of organic molecules. Theoretical studies indicate that in a carbon-rich atmosphere, molecules such as C_2H_2 would condense to form a graphite-like substance or carbon particles which would contain at least a small admixture of C–H bonded material^{8,9}.

Grains formed in stellar atmospheres and expelled into a circumstellar environment, planetary nebula, or the interstellar medium would have a complex nucleation and growth history as well as a complex history of interaction with radiation and particles. While the resulting composition and structure of such grains is very uncertain, it seems plausible that they would be composed of a mixture of a large number of compounds reflecting the many chemical and physical processes to which the material has been subjected. For grains forming in a carbon-rich environment, one would then expect an ill-defined substance of high-molecular weight compounds with polymer or tar-like properties. Note, however, that the 3.3- μm band should be present in almost all organic compounds or combinations of them which condense to form solid particles.

The material we propose would resemble the high molecular weight organic compounds found in the carbonaceous chondrites. The major organic component in carbonaceous chondrites is a poorly defined substance which is apparently difficult to study in detail. The material is often called a 'polymer', but it is in fact not made of repeating monomers and seems to be an agglomeration of various units arranged in complicated ways¹⁰. This polymer-type material makes up 90% to 95% of the organic matter in meteorites. The hydrocarbons and amino acids in the chondrites are included in the soluble 5–10% of the organic matter.

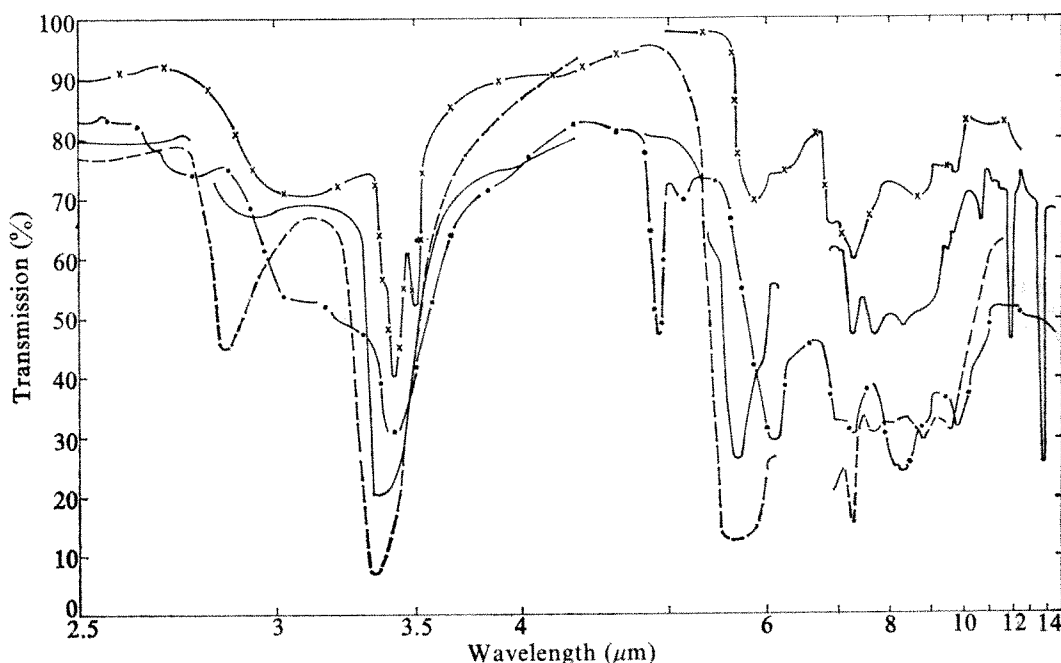
There may be further similarities with organic material synthesised in conditions resembling planetary atmospheres. Methane and ammonia, have been subjected to electrical discharges¹¹, ultraviolet radiation¹², and proton irradiation¹³. In all cases high molecular weight, polymer-like solid material is formed. Khare and Sagan pointed out the similarities between the synthesised material and the organic compounds in carbonaceous chondrites¹². This material may, however, differ in substantial ways from condensation products in stellar atmospheres where conditions do not resemble those in the experiments or in the solar nebula. In this case, the present comparison is quite qualitative and based on the assumption, suggested by the arguments above, that similar carbonaceous material might form in disparate environments. On the other hand, circumstellar shells surrounding protostars are also likely formation sites for interstellar grains¹⁴. Grains formed there might resemble the carbonaceous meteoritic material quite closely.

The infrared spectra of some of these compounds are shown in Fig. 1 which includes spectra typical of the

Table 1 Unidentified infrared emission bands

Wavelength (μm)	Objects	Refs
3.3	Planetary nebulae, HII regions, galaxies	2, 3, 6
3.4	Planetary nebulae, HII regions	2, 6
6.2	Planetary nebula (NGC 7027)	5
7.7	Planetary nebula (NGC 7027)	5
8.7	Planetary nebulae	1, 17
11.3	Planetary nebulae, HII regions, galaxies	1, 4, 17

Fig. 1 Spectra of carbonaceous materials: (—) carbonaceous chondrite, Orgeil (extract), from Meinschein, *et al.*^{1a}; (---) carbonaceous chondrite, Murray (extract), from Meinschein *et al.*^{1a}; (—x—) polymeric material formed by ultraviolet irradiation of gases in reducing atmospheres, from Khare and Sagan¹²; (—●—) polymeric material formed by proton irradiation of gases in reducing atmospheres, from Scattergood, *et al.*¹³.



meteoritic and synthetic materials. This comparison is subject to numerous uncertainties due to differences in sample preparation in the experiments. Particularly significant is the effect of the solvents on the material and that different extracts of the materials were measured. Nevertheless the spectra provide a qualitative comparison of the infrared properties of some of the organic compounds which form in the rather different conditions used in the experiments, and which may have analogues in the interstellar dust.

All of the spectra contain the expected strong absorption near 3.3 μm . The materials tend to be transparent between 3.6 and 5.7 μm and have a strong band located somewhere between 5.8 and 6.1 μm . This is identified as the vibration of C=C (ref. 11). Since this bonding would also be expected to be common in carbonaceous grains, we suggest that the grain feature at 6.2 μm (Table 1) is a C=C vibration. There is a discrepancy in band position between the astronomical spectra and the spectra of Fig. 1 of 0.1–0.5 μm which would have to be explained by a shift between the solid and dissolved material or perhaps by particle size and shape effects. Band displacements of a few tenths of microns are frequently observed in particle spectra¹⁴.

The spectra between 6.5 and 15 μm are different from each other and from the interstellar emitter. There seem to be real differences between the synthetic and chondritic materials. In the spectrum of Russell *et al.*⁵, the 7.7- μm emission seems to extend from 7.5 to 8.5 μm . While there is an overlap with an absorption probably due to C–O vibration between 8 and 9 μm , this is not a very convincing similarity. No strong feature near 11.3 μm occurs in the laboratory spectra. Either the 11.3- μm band is not due to carbonaceous material or is it due to a polymer somewhat different from those shown in Fig. 1. In this picture, however, it has a different molecular origin than the 3.3- μm feature. Unfortunately, the nature of the absorptions in this wavelength region complicates inferences derived from the spectra. Bands near 11.3 μm do occur in some organic compounds, for example the methyl group –CH₃ has an absorption at this wavelength. The 8–14- μm region is characterised by many overlapping bands of organic molecules often not readily assignable to specific fundamentals. Conversely, the patterns are complex enough to characterise molecules as a whole. If the identification

of the fundamentals suggested above proves correct, bands in the 8–14- μm region could provide more detailed information on the particle composition.

The carbonaceous material would probably be strongly absorbing in the visible or ultraviolet. Small particles of this material would be heated efficiently, possibly accounting for the high temperatures required to account for the emission bands in a nebula like NGC7027 (ref. 2). In regions where different solids occur (as distinct particles), temperatures would depend on the individual particle compositions through the emissivities and absorptivities. Small carbonaceous particles with strong ultraviolet absorption would reach elevated temperatures if their infrared emissivity is not too high. This could explain why the unknown features always seem to be in emission even when the silicates are in absorption.

The material proposed here is related in some ways to the graphite-like material which has long been suspected as a possible grain constituent. Unlike some previous suggestions, the carbonaceous particles would not have to meet stringent requirements on size, shape, or purity, at least to give the degree of agreement with the infrared observations discussed above. There seem to be similarities in the grains and in carbonaceous chondrites¹⁶. Further laboratory and observational work, especially in the 5–14- μm wavelength region, may show whether a second meteoritic material, the organic polymer-like material in carbonaceous chondrites, also has an analogue in the interstellar dust.

I thank J. Bregman for pointing out that the methyl group has an 11.3- μm absorption and for useful comments.

R. F. KNACKE

Department of Earth and Space Sciences,
State University of New York,
Stony Brook, New York 11794

Received 3 June; accepted 12 July 1977.

- 1 Gillett, F. C., Forrest, W. J. & Merrill, K. M. *Astrophys. J.* **183**, 87 (1973).
- 2 Grasdalen, G. & Joyce, R. R. *Astrophys. J. Lett.* **205**, L11 (1976).
- 3 Merrill, K. M., Soifer, B. T. & Russell, R. W. *Astrophys. J. Lett.* **200**, L37 (1975).
- 4 Bregman, J. D. & Rank, D. M. *Astrophys. J. Lett.* **195**, L125 (1975).
- 5 Russell, R. W., Soifer, B. T. & Willmer, S. P. *NASA TMX-73*, **190**, 58 (1977).
- 6 Russell, R. W., Soifer, B. T. & Merrill, K. M. *Astrophys. J.* **213**, 66 (1977).
- 7 Ridgway, S. T., Hall, D. N. B., Kleinmann, S. G., Weinberger, D. A. & Wojslaw, R. S. *Nature* **264**, 345 (1976).
- 8 Salpeter, E. E. *Astrophys. J.* **193**, 579 (1974).
- 9 Czyzak, S. J. & Santiago, J. J. *Astrophys. Space Sci.* **23**, 443 (1973).

- ¹⁰ Nagy, B. *Carbonaceous Meteorites* (Elsevier, Amsterdam, 1975).
¹¹ Woeller, F. & Ponnamperna, C. *Icarus* 10, 386 (1969).
¹² Khare B. N. & Sagan C. *Icarus* 20, 311 (1973).
¹³ Scattergood, T., Lesser, P. & Owen, T. *Icarus* 24, 465 (1975).
¹⁴ Herbig, G. H. *Mem. Soc. Roy. Sci. Liège Ser. 5*, 19, 13 (1970).
¹⁵ Knacke, R. F. *Nature* 217, 44 (1968).
¹⁶ Zaikowski, A. & Knacke, R. F. *Astrophys. & Space Sci.* 37, 3 (1975).
¹⁷ Gillett, F. C., Kleinmann, D. E., Wright, E. L. & Capps, R. W. *Astrophys. J. Lett.* 198, L65 (1975).
¹⁸ Meinschein, W. G., Nagy, B. & Henessy, D. J. *Ann. N.Y. Acad. Sci.* 108, 553 (1963).

Lateral variation of phenocryst assemblages in volcanic rocks of the Japanese islands

To understand the origin of calc-alkaline rocks and island arc volcanism, knowledge of the lateral variation of the petrological characters of volcanic rocks is essential. The lateral variation of chemical compositions of volcanic rocks has been investigated by several authors¹⁻⁴ and correlated to the depth of the Benioff zone. The east volcanic zone of Japanese Islands, which contains about 140 discrete Quaternary volcanoes, is the most extensively studied volcanic zone in the world. I have examined the petrological data of these volcanoes, particularly with respect to the phenocryst assemblages in volcanic rocks and their changes during the history of respective volcanoes. The references have been mostly taken from 200 papers compiled by Isshiki *et al.*⁵

The volcanoes are classified into three groups on the basis of the presence or absence of hornblende and biotite phenocrysts in essential materials: (1) those without biotite and hornblende phenocrysts in any of the lavas or pyroclastics; 2) those with hornblende phenocrysts but without biotite phenocryst at least in one lava flow or pyroclastic rock; and (3) those with biotite phenocrysts at least in one lava or pyroclastic rock. The distribution of the volcanoes of these three groups is shown in Fig. 1.

Volcanic rocks containing biotite phenocrysts were erupted only in the western part of the volcanic zone, whereas those which do not contain any hydrous mineral phenocrysts were erupted in the eastern part of the volcanic zone. Volcanoes in a belt situated between the above two belts erupted rocks containing only hornblende as hydrous mineral phenocryst.

Phenocrysts such as pyroxene, hornblende, and biotite are considered to be precipitated in a magma reservoir or

during the ascent of magma. The lateral variation outlined above may be due to the lateral change in physicochemical conditions of magma reservoirs across the volcanic zone. It may also be due to a lateral variation in the chemical composition of the original magmas, for example, their alkali contents—especially the K₂O contents³ and possibly to the variation of H₂O contents in the original magma which may increase westwards across the volcanic zone.

I thank Professor I. Kushiro and Dr R. H. Grapes for their comments and criticisms.

M. SAKUYAMA

*Geological Institute,
University of Tokyo,
Tokyo, Japan*

Received 31 May; accepted 5 July 1977.

- ¹ Kuno, H. *Bull. Volcanol.* 29, 195 (1966).
² Kuno, H. *Bull. Volcanol.* 32, 141 (1968).
³ Hatherton, T. & Dickinson, W. R. *J. geophys. Res.* 74, 5301 (1969).
⁴ Sugimura, A. *Bull. Volcanol. Soc. Jap. 2nd Ser.* 4, 77 (1959).
⁵ Isshiki, N., Matsui, K. & Ono, K. *Geol. Surv. Jap.* (1968).

Rare earth element mobility and geochemical characterisation of spilite rocks

HELLMAN & Henderson¹ have demonstrated the enrichment of the rare earth elements (REE) in the lower spilite portion of the Bhoiwada lava profile relative to the fresh tholeiitic flow top. The remarkable feature of the REE distribution in the spilites was the absolute enrichment of all the elements and not only the light REE, as occasionally seen in oceanic basalts due to submarine weathering². Thus the enhanced spilite REE distribution resembles either alkali basalt or tholeiitic differentiation products rather than tholeiitic basalt. As stated¹, the high degree of REE mobility suggests that these elements must be used with caution in determining the initial magma type or volcanic environment of ancient spilite rocks.

At face value the REE distribution in the spilites is very similar to that exhibited by the more alkaline units of the Deccan basalts^{3,4}. This feature suggests the possibility that the Bhoiwada profile may not be a single flow unit at all and the fresh tholeiitic 'top' may be a different unit to the more 'alkaline' spilite base. Although the section is described as gradational^{5,6}, there are primary textural differences (pillowed/massive; ophitic nature; vesicularity and so on) between tholeiite and spilite, and the contact seems sharp (see photomicrographs and photograph in refs 5 and 6). If the sub-aqueous (flow 'base')-sub-aerial (flow 'top') origin postulated for the flow⁵ is correct, then it lacks some of the features described for such lava flows^{7,8} and does not adequately explain why spilite-silicification should have stopped abruptly at the water-air interface. Also the considerable REE distribution gap (Fig. 2a of ref. 1) between tholeiite and spilite might lend support to the differences in the two zones, especially as there is no apparent gradational increase in normalised REE patterns with progressive spilite-silicification. This is particularly noticeable in the sudden jump of the La/Yb ratio¹ from tholeiite to spilite and also the chemical similarity between the spilite and the (separate) basal pillow lava unit.

Irrespective of whether the profile represents a single unit or not, however, independent criteria are required to characterise the spilite and compare it with the fresh tholeiite. A variety of geochemical (and mineralogical) parameters, which will give the same characterisation, are necessary to determine the nature of ancient altered volcanics. Vallance^{6,9} has advocated the use of relict pyroxenes to determine the magma type of spilite rocks. In the case considered here, the pyroxenes in both tholeiite and spilite are low-Ca augites of tholeiitic character and are similar in composition⁶. Apart from the REE, other relatively 'immobile elements', such as Ti, P, Zr, Y and Nb, have been used to determine magma type^{10,11} and again demonstrates the tholeiitic nature of

Fig. 1 Distribution of Quaternary volcanoes in Hokkaido and northern Honshu. ●, Volcanoes without biotite and hornblende phenocrysts in any of the lavas or pyroclastics; ○ those with hornblende phenocrysts but without biotite phenocryst at least in one lava flow or pyroclastics; open circles represent those with biotite phenocrysts at least in one lava or pyroclastics.

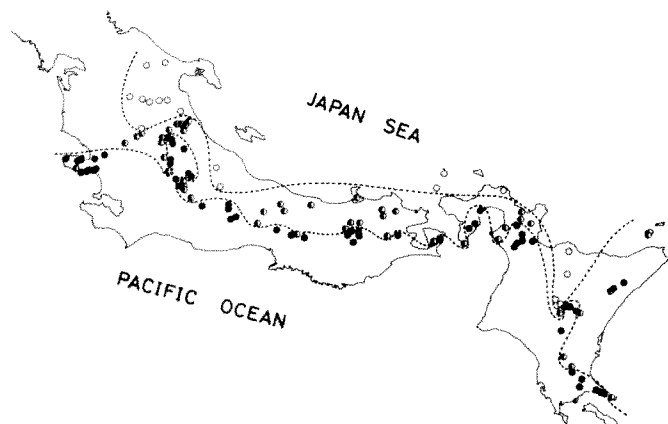


Table 1 Variation in chemical parameters from the Bhoiwada profile^{1,6}

Samples	I	Tholeiite	II	III	Spilite	IV	V (core)	Basal pillow unit V (rim)
Whole rock								
Zr/TiO ₂	0.010		0.015	0.012		0.010	0.013	0.011
Zr/P ₂ O ₅	0.058		0.115	0.120		0.100	0.026	0.025
La/Yb	5.4		5.5	14.0		12.4	13.2	13.1
FeO*/MgO	2.15		1.99	1.78		1.75	2.46	1.55
Fe ₂ O ₃ /FeO	0.27		0.59	0.46		0.65	0.56	0.85
H ₂ O ⁺	0.87		2.46	1.47		1.71	4.61	7.14
Pyroxene fractions (Σ Fe/Mg, at. %)								
Light	0.42		0.38	0.39		0.37		
Medium	—		0.77	—		—		
Heavy	0.65		1.07	0.66		0.62		

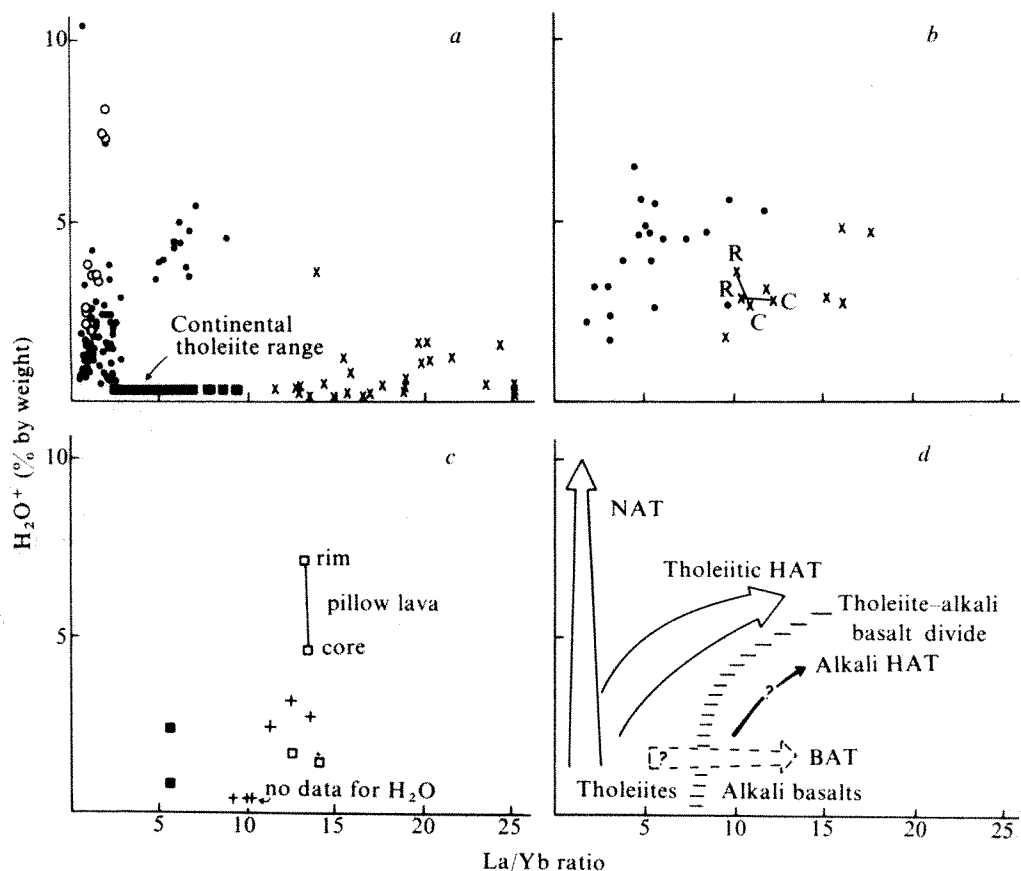
the spilite on the basis of the TiO₂-Zr/P₂O₅ diagram (Fig. 3 of ref. 12). But, there are differences (as well as clear similarities) between the rock units in terms of immobile element ratios (Table 1). Note in particular the more fractionated character of sample II⁶ (sample 2 of ref. 1) relative to sample I (sample 1) as shown by the higher Zr/TiO₂ ratio (see refs 13 and 14 for usage of this ratio). This is substantiated by the higher Σ Fe/Mg ratio of the separated heavy pyroxene fraction from sample II, although not apparently by the lighter fraction (Table 1). The spilites have similar Zr/TiO₂ ratios to the freshest tholeiite (sample I) and thus exhibit a similar degree of basaltic differentiation.

This example from the Bhoiwada profile illustrates the use of variable discriminatory parameters applied to spilitic rocks (compare ref. 13), but is at variance with the REE 'alkaline' designation. But, before any spilite with apparent 'alkali basalt' characteristics is dismissed as an REE-enriched tholeiite, we need first, to check the designation against other geochemical and mineralogical criteria (as above) and second, to test the degree of REE mobility against some alteration parameter, such as H₂O⁺, in each situation. With pillow lavas it is also customary to compare the rim and core portions to determine the zone of possible enrichment.

I now briefly examine the effect of progressive hydration (as H₂O⁺) on the REE in relatively modern basalts and compare the distribution and fractionation (as the La/Yb ratio) with spilites from the Variscan of western Europe. Within this framework the Bhoiwada basalt-spilite fractionation pattern can be compared. It must be emphasised that H₂O⁺ is only one of several possible alteration parameters¹⁵, although in view of the development of hydrous low-grade assemblages in spilites and also hydrated glass in modern sea-floor basalts, it seems the most appropriate (compare ref. 13).

Figure 1a is a plot of relatively recent tholeiitic and alkali basalts mainly from the oceanic environment and shows their separation on the basis of the La/Yb ratio (representing light to heavy REE fractionation). The relative position of continental tholeiites is indicated schematically, due to the general lack of H₂O⁺ determinations and is based on data from various localities^{4,16,17}. The common La/Yb range for continental tholeiites is about 2-7 (with a few values up to ~10), whereas continental and oceanic alkali basalts show a much wider range with La/Yb ~11-40 (Fig. 1a and ref. 17). Associated oceanic island tholeiites and alkali basalts from Hawaii¹⁸ show La/Yb ranges of 3.9-6.8

Fig. 1 Variation of La/Yb ratio with hydration in various basaltic and spilitic rocks. *a*, Fresh and altered tholeiites and alkali basalts, mainly from the modern oceanic environment (data from refs 2, 4, 18-20, 23-33). \times , Alkali basalts; \bullet , tholeiitic basalts; \circ , spilites/greenstones. *b*, Variscan spilites from western Europe (data from refs 21, 22 and unpublished). \bullet , Tholeiitic spilites; \times , alkali basalt spilites. R = rim; C = core. *c*, Bhoiwada profile tholeiites and spilites (data from refs 1, 6) compared with some Deccan basalts (data from refs 3, 4 and 16). \blacksquare , Fresh tholeiite; \square , spilitic rocks; $+$, Deccan basalts. *d*, Summary diagram showing development of various trends for La/Yb. NAT, no alteration trend; HAT, hydrous alteration trend; BAT, Bhoiwada alteration trend.



and 7.8–15.6 respectively and coupled with the above data provide an approximate magma type divide at La/Yb of ~ 7 –8.

The ocean floor/ridge tholeiites in Fig. 1a exhibit two trends with increasing hydration—(1) increasing La/Yb in samples with $H_2O^+ > 3\%$ by weight and (2), no change in La/Yb, irrespective of H_2O^+ content, even up to very high values (10% by weight). Both of these features are seen in basalts that have suffered low-temperature alteration with palagonitisation of glass and developed smectitic clays, carbonate, Fe oxides and rare zeolites. Also higher temperature alteration, exhibited by recent ocean floor rocks and generally termed greenstones or spilites (for example refs 19 and 20), show no significant change in the La/Yb ratio with progressive hydration. Unfortunately there is a lack of analyses of sufficiently altered alkali basalts to show any similar trends, although within any one volcanic province the variation is minimal (up to 2% H_2O^+ by weight) in rocks of similar basaltic composition.

Figure 1b shows the variation of the La/Yb ratio with hydration in various Upper Palaeozoic spilites from western Europe. This includes REE data from north-western Germany^{21,22}, south-western England, Brittany and southern Portugal (unpublished data by PAF). The south-western England data includes both intrusive sills and pillow lavas that have been designated as tholeiites or alkali basalts on the basis of the distribution of other 'immobile' elements (refs 11, 13 and Table 2). Note that the La/Yb ratio increases with progressive hydration

enrichment²⁴, but not to the degree as illustrated by the Bhoiwada spilite. As seen in Table 2, the pillow rims are enriched relative to their cores, with the enrichment in both cases being almost entirely due to Ce and to a lesser extent Sm.

Figure 1c shows the Bhoiwada lava data^{1,6} and various Deccan basalts^{3,4,16}, which characterised by high La/Yb ratios suggest an 'alkaline' character for these particular samples. Compared with the alteration trends derived from Fig. 1a and b, the development of the Bhoiwada spilite is unusual in showing a marked La/Yb enrichment without any attendant H_2O^+ increase. Also, if the basal pillow lava unit was originally tholeiitic, the whole pillow has been REE enriched such that the rim and core have developed similar La/Yb ratios. The pillow lava, due to its high H_2O^+ content, could fall on the end of the altered basalt/spilite trend of Fig. 1a and b. Certainly the form of variation exhibited by the Bhoiwada profile is different from that seen elsewhere and may define another (abnormal?) trend. It is difficult to see what this difference may be due to, as the Bhoiwada alteration products (chlorite, albite, prehnite, laumontite⁵) are not markedly different from the mineralogy of some ocean floor spilites. Adsorption of La (and other REE?) by layer silicates has been suggested¹ as a factor in determining REE distribution patterns, although equally chloritic spilites (or clay-rich altered basalts) do not show such a high degree of enrichment in the marine environment.

In conclusion, it seems that the REE can be fractionated during alteration, although the trends of alteration in such cases can still

Table 2 Variation of immobile element ratios and chemical alteration criteria in associated portions of massive and pillowed spilites from south-western England

Sample no. Portion	Massive sill		Pillow lavas			
	CG12 Central	CG16 Margin	PP7 Core	PP7 Rim	PP10 Core	PP10 Rim
H ₂ O ⁺ (weight %)	3.96	5.53	2.77	2.88	2.67	3.56
Fe ₂ O ₃ /FeO	0.10	0.12	0.37	0.34	0.23	0.63
Σ REE (p.p.m.)	27.19	24.83	100.97	113.07	102.13	120.44
La/Sm	2.2	2.8	5.2	3.6	4.8	3.5
La/Yb	5.3	5.6	12.2	10.4	10.9	10.1
Zr/TiO ₂	0.005	0.007	0.006	0.006	0.006	0.005
Zr/P ₂ O ₅	0.086	0.076	0.053	0.060	0.092	0.055
Nb/Y	0.40	0.40	1.14	1.15	1.00	1.50
Immobile element classification	Tholeiitic basalt		Alkali basalt		Alkali basalt	
Clinopyroxene type	Common augite + rare pigeonite exsolution lamellae		(no pyroxene relicts)			

(especially in samples with $H_2O^+ > 3\%$ by weight) in a similar manner to one of the trends exhibited by the altered oceanic floor tholeiites of Fig. 1a. Although the La/Yb ratio in the altered tholeiites increases towards more alkaline values (~ 10) this only takes place at a relatively high level of alteration (5–6% H_2O^+ by weight) and would only be confused with alkali basalts if their La/Yb ratio remained constant at the same level of alteration. The alkali basalt designated spilites (largely from south-western England) have typical alkaline La/Yb ratios and are separate, in terms of alteration trends, from the tholeiitic spilites. In the alkali basalt pillow lavas the La/Yb ratio marginally decreases from core to rim (Table 2). de Paape *et al.*²³ have also demonstrated the lack of variation in pillow lava segments with core and glassy crust La/Yb ratios being essentially the same, although the H_2O^+ content is considerably different.

Together with variation in the ocean floor basalts, the plotted spilite data suggests variation of the La/Yb ratio with alteration, although the trends of variation seem to still allow spilitic tholeiites to be distinguished from spilitic alkali basalts. The total content of REE in spilites derived from known basaltic parents shows a small

be used to distinguish tholeiites from alkali basalts. To determine if alteration has changed the La/Yb ratio of a basaltic suite it is necessary to use a plot such as Fig. 1, before this ratio is applied to petrogenetic modelling. The designation of spilitic rocks to one magmatic series or another is important in elucidating the nature of ancient volcanism and must be determined using a variety of geochemical 'immobile' parameters. Figure 1d summarises the alteration trends and marks a boundary between altered tholeiites and alkali basalts. This division remains highly tentative, however, until alkali basalts, hydrated to the same level as some tholeiites, have been analysed to check if any change in their La/Yb ratio takes place. If not, then alkali basalts with La/Yb ratios of ~ 10 –12 will overlap with altered tholeiites containing 5–6% H_2O^+ by weight. Both the no-alteration trend (NAT) and the hydrous alteration trend (HAT) are characterised by greenschist facies spilites and very low-grade alteration (submarine weathering) of basalts. What factors determine which of these two main trends develop is unclear (longevity of burial; sedimentation rate; nature of lava units?) and in particular why do modern spilites follow NAT and ancient spilites follow HAT? The Bhoiwada trend

(BAT) is also shown, although further studies are required to substantiate this type of alteration.

I thank Dr J. A. Winchester for helpful comments.

P. A. FLOYD

Department of Geology,
University of Keele,
Keele, Staffordshire, UK

Received 13 June; accepted 5 July 1977.

- ¹ Hellman, P. L. & Henderson, P. *Nature* **267**, 38–40 (1977).
- ² Frey, F. A., Bryan, W. B. & Thompson, G. *J. geophys. Res.* **79**, 5507–5527 (1974).
- ³ Alexander, P. O. & Gibson, I. L. *Lithos* **10**, 143–147 (1977).
- ⁴ Frey, F. A., Haskin, M. A., Poetz, J. A. & Haskin, L. A. *J. geophys. Res.* **73**, 6085–6089 (1968).
- ⁵ Sukheswala, R. N. in *Spilitic and Spilitic rocks* (eds Amstutz, G. C. et al.) 229–250 (Springer, Heidelberg, 1974).
- ⁶ Vallance, T. G. *J. Petrology* **15**, 79–96 (1974).
- ⁷ Jones, J. G. & Nelson, P. H. *Geol. Mag.* **107**, 13–19 (1970).
- ⁸ Furness, H. & Sturt, B. A. *J. Geol.* **84**, 439–453 (1976).
- ⁹ Vallance, T. G. in *Spilitic and Spilitic Rocks* (eds Amstutz, G. C. et al.) 59–68 (Springer, Heidelberg, 1974).
- ¹⁰ Pearce, J. A. & Cann, J. R. *Earth planet. Sci. Lett.* **19**, 290–300 (1973).
- ¹¹ Floyd, P. A. & Winchester, J. A. *Earth planet. Sci. Lett.* **27**, 211–218 (1975).
- ¹² Winchester, J. A. & Floyd, P. A. *Earth planet. Sci. Lett.* **28**, 459–469 (1976).
- ¹³ Floyd, P. A. *J. Petrology* **17**, 522–545 (1976).
- ¹⁴ Winchester, J. A. & Floyd, P. A. *Chem. Geol.* (in the press).
- ¹⁵ Hart, S. R., Erlank, A. J. & Kable, E. J. D. *Contrib. Mineral. Petrol.* **44**, 219–230 (1974).
- ¹⁶ Balashov, Yu. A. & Nesterenko, G. V. *Geochem. Intern.* **3**, 672–679 (1966).
- ¹⁷ Herrmann, A. G. *Contrib. Mineral. Petrol.* **17**, 275–314 (1968).
- ¹⁸ Schilling, J. G. & Winchester, J. W. *Contrib. Mineral. Petrol.* **23**, 27–37 (1969).
- ¹⁹ Melson, W. G., Thompson, G. & van Andel, T. H. *J. geophys. Res.* **73**, 5925–5941 (1968).
- ²⁰ Cann, J. R. *J. Petrology* **10**, 1–19 (1969).
- ²¹ Herrmann, A. G. & Wedepohl, K. A. *Contrib. Mineral. Petrol.* **29**, 255–274 (1970).
- ²² Herrmann, A. G., Potts, M. J. & Knake, D. *Contrib. Mineral. Petrol.* **44**, 1–16 (1974).
- ²³ de Paape, P., Klerkx, J., Hertogen, J. & Plinke, P. *Earth planet. Sci. Lett.* **22**, 347–354 (1974).
- ²⁴ Nicholls, G. D. & Islam, M. R. *Phil. Trans. R. Soc.* **268**, 469–486 (1971).
- ²⁵ Masuda, A. & Nagasawa, S. *Geochem. J.* **9**, 227–233 (1975).
- ²⁶ Blanchard, D. P. et al. *J. geophys. Res.* **81**, 4231–4246 (1976).
- ²⁷ Kay, R., Hubbard, N. J. & Gast, P. W. *J. geophys. Res.* **75**, 1585–1614 (1970).
- ²⁸ Goldich, S. S., Treves, S. B., Suhr, N. H. & Stuckless, J. S. *J. geol.* **83**, 415–435 (1975).
- ²⁹ Flower, M. F. J. *Contrib. Mineral. Petrol.* **31**, 335–346 (1971).
- ³⁰ Price, R. C. & Taylor, S. R. *Contrib. Mineral. Petrol.* **40**, 195–206 (1973).
- ³¹ Tanaka, T. & Sugisaki, R. *J. Petrology* **14**, 489–508 (1973).
- ³² Engel, A. E. J., Engel, C. G. & Havens, R. G. *Bull. geol. Soc. Am.* **76**, 719–734 (1965).
- ³³ Frey, F. A. *Earth planet. Sci. Lett.* **7**, 351–360 (1970).

Transuranic depositional history in South Greenland firn layers

The surface layers of the Greenland ice sheet have preserved a continuous and detailed record of atmospheric fallout of transuranic nuclides from weapons tests over the past 30 yr. The ages of the glacial strata were determined by ^{210}Pb geochronologies. We found that the influence of remobilised soil debris with their sorbed transuranics does not seem to be important, and that fallout maxima occurred in the 1950s and 1960s for $^{239+240}\text{Pu}$ and in 1965–66 for ^{238}Pu . This work may be extended by using alpine glaciers in mid-latitudes for a reconstruction of fallout patterns over the past three decades and for an evaluation of present day dispersions of these nuclides as a consequence of atmospheric fallout and other possible entries through the nuclear fuel cycle.

The observed or measured stratigraphic layers found in glaciers of the polar regions are very attractive for studies of the various species of atmospheric fallout including pollutants. Although glaciers are found at nearly every latitude of the Earth, it is the inland areas of the Greenland and Antarctica ice sheets that provide the most reliable stratigraphical relations¹. This is because summer melting is negligible or non-existent, and orderly sequential buildup is not destroyed by percolation. The snow or firn and the dissolved and particulate substances are essentially immobile following deposition². A number of geochronologies, both radiometric and stratigraphic, allow the introduction of time frames into the layered systems; in many instances, the season of the year, as well as the year itself, can be identified³. The fluxes of some metals from the atmosphere to the glaciers over the past centuries and millenia have identified both the anthropogenic and natural contributions^{4–8}. The fallout of nuclear debris from weapons testing has been observed in both the Arctic and Antarctic glaciers through gross β , ^{90}Sr and ^{137}Cs measurements^{9–13}. With increasing concerns about the dispersion of transuranics in our environment, it was of interest to develop the

pollution history of Pu and Am isotopes from a Greenland ice sheet location.

During the 1975 field operations of the Greenland Ice Sheet Program (GISP) a 30-m deep, 7.6-cm diameter firn core was augered at a location designated 'South Dome' Greenland ($63^{\circ}31.6'\text{N}$, $44^{\circ}34.5'\text{W}$). The June 1975 surface serves as the zero depth horizon. A lightweight mechanical drill was used¹⁴. Both the core and the chips formed during drilling were collected in the same polyethylene bag from each approximate 1-m run of the drill. After being transported frozen to the US, the 4–9-kg samples (dependent on the general increasing density with depth) were transferred to 10-l polyethylene containers, rapidly melted in a microwave oven and acidified to a pH of 1 with HNO_3 .

The chemical analyses were performed on the residues obtained by the evaporations of the 4–9-l of melt water. Pu analyses were carried out¹⁵. Following melting, the waters were acidified with HNO_3 . Later, spikes of Pb carrier and the tracers ^{242}Pu and ^{243}Am were added. The Am contents were determined by a modification of an unpublished method¹⁶. ^{210}Pb assays were made by a routine method developed at the Scripps Institution of Oceanography¹⁷.

The sedimentation rate over the 30-m profile averaged 1 m yr^{-1} for the gross glacial material or 0.6 m yr^{-1} of water equivalent, after density corrections on the basis of ^{210}Pb chronology (Fig. 1; ref. 18). This value compares well with the accumulation rate determined on the basis of firn stratigraphy and oxygen isotopes (Dansgaard et al., personal communication).

Table 1 $^{241}\text{Pu}/^{239+240}\text{Pu}$ ratios, computed at various depths in the Greenland Glacier for the year 1962

Depth (m) water equivalent	Time (yr)	$^{241}/^{239+240}\text{Pu}^*$
0–0.58	0–1.0	31.4
0.58–1.06	1.0–1.7	35.3
1.06–1.45	1.7–2.4	45.0
1.45–1.83	2.4–3.0	21.5
1.65–2.14	3.0–3.5	26.9
2.14–5.28	3.5–4.2	11.1
2.58–3.10	4.2–5.1	15.6
3.10–4.14	5.1–6.7	13.2
4.14–5.16	6.7–8.5	32.0
5.16–5.73	8.5–9.4	14.1
5.73–6.37	9.4–10.4	16.0
6.37–7.03	10.4–11.5	17.1
7.03–7.72	11.5–12.0	18.9
7.72–8.46	12.0–13.0	11.5
8.46–9.02	13.0–14.8	16.9
9.02–9.51	14.8–15.6	11.1
9.51–10.3	15.6–16.9	8.6
10.3–10.77	16.9–17.7	11.8
10.71–11.77	17.7–19.3	20.5
11.77–12.3	19.3–20.2	23.2
12.3–12.9	20.2–21.1	21.6
12.9–13.5	21.1–22.1	26.4
13.5–14.1	22.1–23.0	28.6
14.1–15.4	23.0–25.3	24.7
15.4–16.5	25.3–27.1	39.2
17.8–19.2	29.2–31.5	32.8

*For 0–13.9 yr ago, the $^{241}\text{Pu}/^{239+240}\text{Pu}$ are normalised to a parameter of ^{241}Pu in July 1962 at which time the level of ^{241}Am was zero. For times 13.9–22.1 yr ago, a similar normalisation was made to 1954. For earlier times, the ^{241}Pu is calculated assuming ^{241}Am was zero at this time and decay is taken into account.

The Pu and Am isotopic concentrations as a function of time of deposition or of depth are shown in Fig. 1 with the ^{210}Pb concentrations which were used to establish one of the chronologies. Two distinct maxima are observed for $^{239+240}\text{Pu}$ for the periods 1963–65 and 1955–60. A third maximum may exist in the strata deposited between 1946 and 1948, and may reflect the Nagasaki, Hiroshima and/or Almagordo, New Mexico weapon detonations. The ^{238}Pu profile in the glacial deposits is similar to that of the $^{239+240}\text{Pu}$ up to 1966. The input of this isotope from the burn-up of the SNAP-9A device in 1964, is not recorded in the

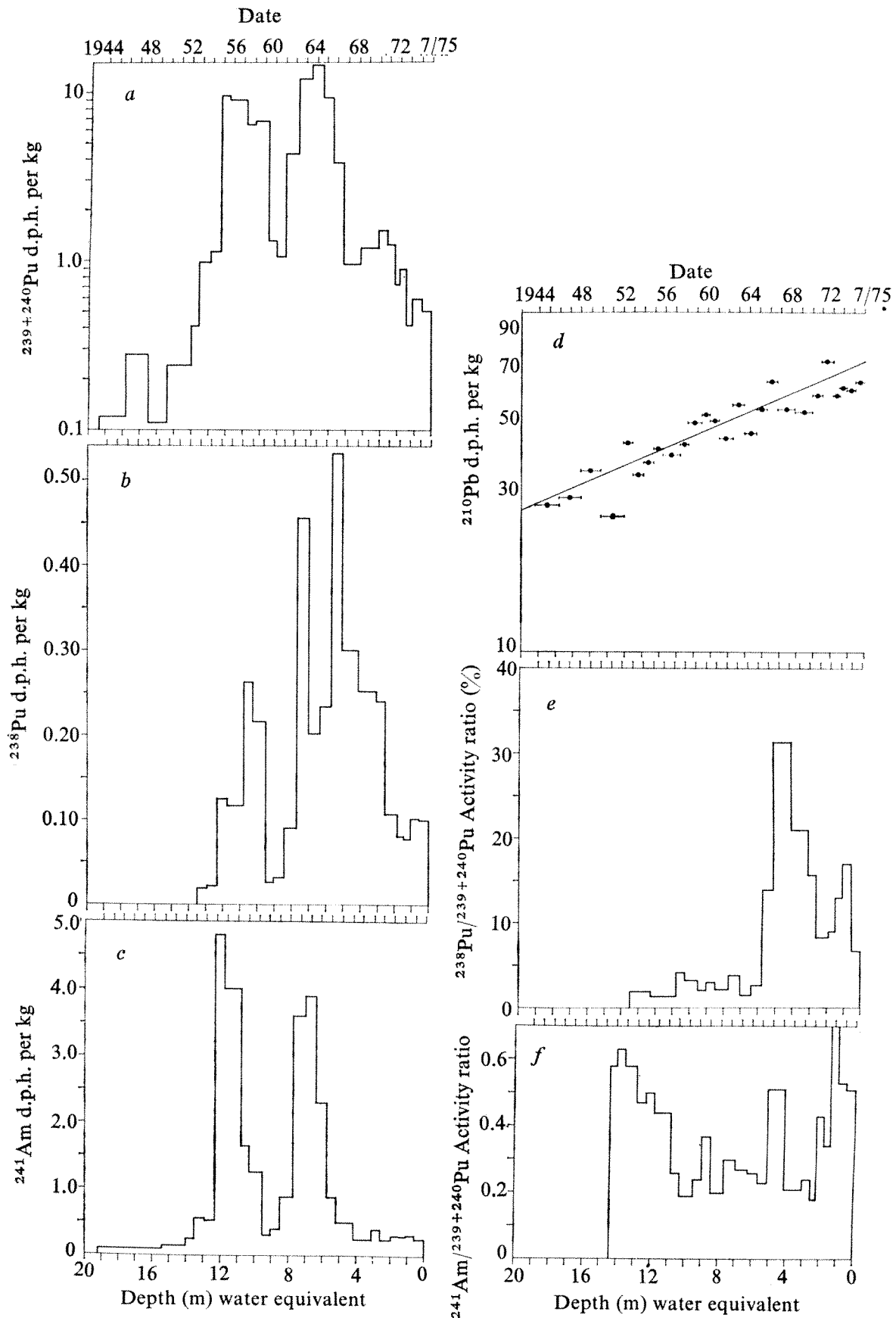


Fig. 1 *a-d*, $^{239} + ^{240}\text{Pu}$, ^{238}Pu , ^{241}Am and ^{210}Pb activities in the Greenland Ice Sheet. *e, f*, The $^{238}\text{Pu}/^{239} + ^{240}\text{Pu}$ and $^{241}\text{Am}/^{239} + ^{240}\text{Pu}$ activity ratios in the Ice Sheet. The timescale is based on the ^{210}Pb geochronology. The counting errors for these nuclides were higher for the surface samples, as a consequence of small sample size, and for the deeper samples, as a consequence of low concentrations of the nuclides. The average counting errors and their ranges (in parentheses) are: $^{239} + ^{240}\text{Pu}$, 7.4% (2.8–15.6); ^{241}Am , 13% (6.2–33); and $^{238}\text{Pu}/^{239} + ^{240}\text{Pu}$, 31% (12.5–53). For ^{210}Pb , the counting errors were 3% or less for all samples.

glacier until 1966. The patterns of ^{241}Am levels are in concord with that of $^{239+240}\text{Pu}$; this nuclide grows in from the decay of its parent ^{241}Pu , produced in weapons testing along with the other Pu isotopes.

The most extensive record of plutonium fallout from the atmosphere as a function of latitude (for samples collected between October 1970 and January 1971) has been carried out by the Health and Safety Laboratory of ERDA in New York¹⁹. Soil samples to a depth of 30 cm, with a surface area of 622 cm² were collected at 65 sites. The assumption was made that the soils were undisturbed and had integrated weapons fallout over the past 25 yr or so. There is, however, strong evidence that soils can be mobilised by winds and waters with a consequential alteration of the fallout record. Coastal sediments often show increasing $^{239+240}\text{Pu}$ fluxes from the 1950s to the present, whereas if stratospheric fallout were the only entry, maxima would be expected in the late 1950s and early 1960s.

The contribution of soil mobilised $^{239+240}\text{Pu}$ to the amount of these isotopes in the Greenland ice sheet can be estimated in the following way. Windom²⁰ has indicated that the fallout rate of particles in a Greenland glacier is 21 mg per cm² per 10³ yr. If we assume a Pu activity of 1.0 d.p.m. per g for atmospheric particles, similar to that observed for northern hemispheric dusts¹⁵, then the remobilised soil debris is contributing 2.1×10^{-5} d.p.m. per cm² per yr. For surface samples of the glacier, there is an accumulation rate of 60 cm, water equivalent per yr, or 0.06 kg per cm² per yr¹. Using a $^{239+240}\text{Pu}$ concentration of 0.01 d.p.m. per kg for the surface strata of the glacier, the total flux of $^{239+240}\text{Pu}$ is of the order of 6×10^{-4} d.p.m. per cm² per yr. Thus, the soil remobilised $^{239+240}\text{Pu}$ may contribute the order of 5% of the total burden of these isotopes in the glacier.

On the basis of the soil record, there has been a cumulative fallout of $^{239+240}\text{Pu}$ for northern latitudes between 60° and 70°N of 1.6 ± 1.0 mCi km⁻² up to 1971. Our integrated value for the ice sheet accumulation through 1975, 0.7 mCi per km², agrees with the previous value.

Through a comparison of transuranic and ^{210}Pb concentrations in a sample collected about 14°N of the one analysed in this work (Camp Century, 77°10'N, 61°08'W; elevation of 1,886 m)⁷, it seems that there may be a uniform fallout of these nuclides over a wide area in Greenland. The Camp Century collection was made in 1965 and the upper layers which were analysed represented snow deposited between the Autumn of 1964 and the Summer of 1965. The concentration of the nuclides in the Camp Century surface layers and 'South Dome' 6 m stratum, corresponding to a time about 1965, are: $^{239+240}\text{Pu}$, 11.3 ± 0.003 and 9.3 d.p.h. kg⁻¹; ^{241}Am , 2.7 ± 0.18 and 2.3 d.p.h. kg⁻¹; $^{238}\text{Pu}/^{239+240}\text{Pu}$, 0.012 ± 0.003 and 0.026 ± 0.005 . The ^{210}Pb of the Camp Century and of a site analysed by Windom²⁰, both decay corrected to the same time, are 96.4 ± 2 and 100 d.p.h. kg⁻¹. These arrangements help dispel the idea that perhaps the results are site specific for any given glacier sample.

Finally, we have examined the ^{241}Am fallout record in relation to that of $^{239+240}\text{Pu}$. It is generally assumed that the ^{241}Am in the environment today arises nearly completely from the decay of ^{241}Pu (half life 14.9 yr) produced in nuclear weapons tests. Although a large number of tests have contributed ^{241}Pu to the environment, we have obtained the ratio $^{241}\text{Pu}/^{239+240}\text{Pu}$ for July 1962 for samples deposited after this time period, for 1954–62 for samples deposited in this interval and for years before 1954. These calculations are described by Livingston *et al.*²¹. These authors indicate that a ratio of 13–14 in fresh debris for the 1961–62 US/USSR tests is representative and clearly our results following the 3.5 most recent years seem to agree with these data. The most recent values of the ratio, ranging from 21.5 to 45, are perhaps explicable by inputs from recent Chinese tests. It should be pointed out that because of small sample sizes, these ratios are less accurate for the near surface and pre-1954 samples.

This research was supported under a Contract with the Energy Research and Development Administration, Environmental Sciences Branch, Division of Biology and Medicine (ERDA E (04-3)-34 P. A. 84) and the National Science Foundation, Division of

Polar Programs (OPP 7609120). Ms Sue Johnson assisted with the transuranic analyses.

MINORU KOIDE

EDWARD D. GOLDBERG

*Scripps Institution of Oceanography,
La Jolla, California 92093*

MICHAEL M. HERRON

CHESTER C. LANGWAY, JR

State University of New York at Buffalo

Received 28 April; accepted 29 June 1977.

- ¹ Langway, C. C., Jr *GSA Special Paper* 125 (1970).
- ² Junge, C. E. *J. geophys. Res.* **65**, 227–237 (1960).
- ³ Dansgaard, W., Johnson, S. J., Clausen, H. B. & Grunderstrup, N. *Meddelelser om Grnland*, Bd. 197, Nr. 2, 53 (1973).
- ⁴ Weiss, H., Bertine, K., Koide, M. & Goldberg, E. D. *Geochim. cosmochim. Acta* **39**, 1–10 (1975).
- ⁵ Weiss, H. V., Koide, M. & Goldberg, E. D. *Science* **174**, 692–694 (1971).
- ⁶ Weiss, H. V., Koide, M. & Goldberg, E. D. *Science* **172**, 261–263 (1971).
- ⁷ Murozumi, M., Chow, T. J. & Patterson, C. *Geochim. cosmochim. Acta* **33**, 1247–1294 (1969).
- ⁸ Koide, M. & Goldberg, E. D. *J. geophys. Res.* **76**, 7689–7696 (1971).
- ⁹ Martell, E. A. *Science* **129**, 1197–1206 (1959).
- ¹⁰ Picciotto, E. & Wilgain, S. *J. geophys. Res.* **68**, 5965–5972 (1963).
- ¹¹ Wilgain, S., Picciotto, E. & DeBreuck, W. *J. geophys. Res.* **70**, 6023–6032 (1965).
- ¹² Crozaz, G. *Earth planet. Sci. Lett.* **6**, 6–8 (1969).
- ¹³ Woodward, R. N. *Nature* **204**, 129 (1964).
- ¹⁴ Ruffli, H., Stauffer, B. & Oeschger, H. *Proc. Ice Core Drilling Symp.* (ed. J. Spleitstoesser) 139–153 (University of Nebraska, Lincoln, 1974).
- ¹⁵ Koide, M., Griffin, J. J. & Goldberg, E. D. *J. geophys. Res.* **80**, 4153–4162 (1975).
- ¹⁶ Wessemann, R. A., Leventhal, L., Lee, K. D. & Major, W. J. paper presented at 18 Conf. Analyt. Chem. Nuclear Technol. (Gatlinburg, Tennessee, 1974).
- ¹⁷ Koide, M. & Bruland, K. W. *Anal. chim. Acta* **75**, 1–19 (1975).
- ¹⁸ Goldberg, E. D. in *Radioactive Dating*, 121–131 (International Atomic Energy, 1963).
- ¹⁹ Hardy, E. P., Krey, P. W. & Volchok, H. L. *Nature* **241**, 444–445 (1973).
- ²⁰ Windom, H. L. *Bull. geol. Soc. Am.* **80**, 761–782 (1969).
- ²¹ Livingston, H. D., Schneider, D. L. & Bowen, V. T. *Earth planet. Sci. Lett.* **25**, 361–367 (1975).

Angiosperm fossils from latitude 70°S

WE have found fossil angiosperm leaves more than 1,000 km further south than any other previously reported. Specimens were obtained *in situ* by R. W. B. from tuffs in a volcanic sequence in the eastern Elgar Uplands of northern Alexander Island (Fig. 1). Hitherto in Antarctica, angiosperm macrofossils have been reported only from the northern Antarctic Peninsula area in Tertiary sediments on Seymour Island¹ and in volcanoclastic sequences on King George Island, South Shetland Islands^{2,3}, although angiosperm microfossils, mainly pollen, have been found in erratics of marine sandstone from the McMurdo Sound area⁴. The source area of these latter specimens however, is not known, and it is well known that some pollens, including that of *Nothofagus*, can be transported in detectable quantities over large distances⁵. No macro plant fossils seem to be associated with the McMurdo Sound pollen.

The flora from the Elgar Uplands is poorly preserved but includes the impressions of at least two kinds of leaf (Fig. 2). Numerous carbonised films and moulds, representing accumulation of logs and branches, also occur in north-western Elgar Uplands. The most common kind of leaf (Fig. 2a–c) reaches in excess of 10 cm long and the venation suggest that it had a broad-based ovate outline, although the margin is not clearly delineated on any of the specimens. There is a straight slender mid-vein from which secondary veins branch at angles of 30–40°. On one possible variant (Fig. 2d) this angle of secondary origin is 50° or more. Small fragments of the leaves indicate that each vein terminates in a serration at the leaf margin (Fig. 2a and b). Similar leaf characteristics are commonly observed on specimens from the South Shetland Islands, some of which have been illustrated as *Nothofagus* or '*Fagus*'⁶. Although the present examples are considerably larger than modern species of *Nothofagus* occurring in Patagonia, they are comparable in size with others from New Guinea, New South Wales and the Queensland rain forest. A second kind of leaf (Fig. 2f) is much broader than the first, has an entire margin and an obtusely pointed apex. The mid-vein is slightly sinuous and gives off thin and curved secondary veins at irregular

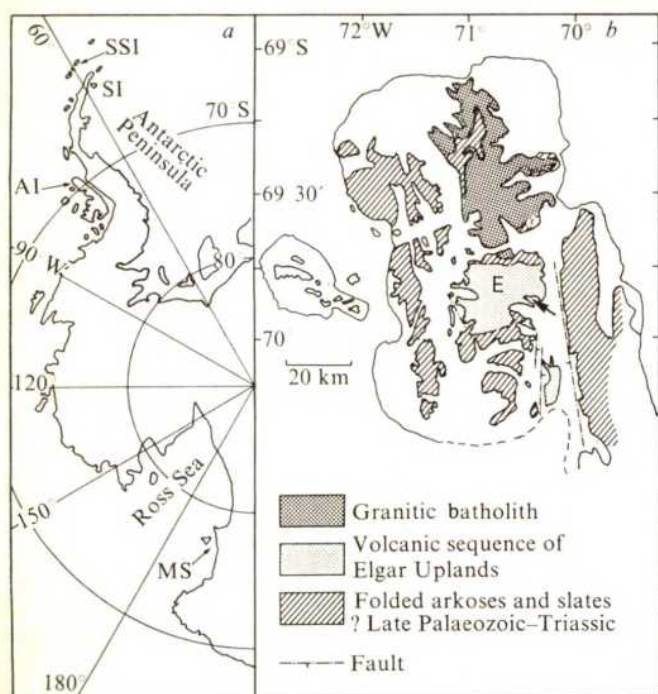


Fig. 1 Maps of Antarctic fossil angiosperm localities. *a*, Lesser Antarctica: AI, Alexander Island; MS, McMurdo Sound; SI, Seymour Island; SSI South Shetland Islands. *b*, Geological map of northern Alexander Island, showing the newly discovered fossil leaf locality (arrow). E, Elgar Uplands.

intervals. The secondary veins seem to have stout triangular bases where they join the mid-vein, but it is possible that this is an accident of preservation rather than an original feature. A fragment of leaf base (Fig. 2e) shows irregular venation which suggests closer affinities to the second kind of leaf than the first.

The plant-bearing tuffs occur in a sequence of tuffs, lavas and agglomerates, more than 500 m thick⁷ which crops out over most of Elgar Uplands. The lavas are dominantly basaltic with phenocrysts of zoned labradorite, augite and hypersthene. Only one andesite has been recorded but alteration may preclude their petrological determination. Hornblende together with labradorite are common constituents in the tuffs. All the rocks are extensively altered with abundant calcite, epidote, chlorite and pyrite, and there is widespread silification. A much later stage of volcanism in the area is probably represented by isolated outcrops of fresh vesicular breccias, palagonite breccia and a vesicular olivine basalt plug. These are closely comparable with late Cainozoic volcanic rocks described from Beethoven Peninsula, Alexander Island⁸.

Beneath the volcanic sequences there is a thick succession of deformed sedimentary rocks, assigned to the late Palaeozoic on the basis of fossil spores⁹ and general similarities to comparable rocks elsewhere in the Antarctic Peninsula¹⁰. A late Triassic marine fauna however, has recently been obtained from the (?) upper part of this sequence¹¹.

Field relationships thus set only broad age limits on the plant-bearing tuffs and their precise age is difficult to assess. A K/Ar date of 70 Myr was obtained from a tuff in the lower part of a supposedly related sequence in the nearby Colbert Mountains¹² and this suggests a post-Cretaceous age for much of the volcanic successions. The leaves are too poorly preserved to be identified with certainty but they show some resemblance to those in the South Shetland Islands. Few published accounts of these are available, although one flora from King George Island was assigned to the Miocene on palaeobotanical evidence³. Associated lavas

have now been dated at about 60 Myr (R. J. Pankhurst, personal communication).

These fossils indicate that at some stage in the Tertiary, Alexander Island was colonised by arborescent angiosperms, and their proven *in situ* occurrence is extended 1,000 km south of the northern Antarctic Peninsula area. It is unlikely that Antarctica has moved much since 40 Myr ago¹³ and therefore they point further to a considerably warmer climate than now at approximately the same latitude. The large size of some leaves (>10 cm) could be interpreted to favour the existence of a warm-temperate to subtropical climate¹⁴, but it is unlikely that the present flora is indicative of more than ordinary to warm-temperate conditions.

Evidence has been cited for early and late Cainozoic glaciation in Antarctica, but a review of the data¹⁵ suggests that full-bodied ice sheets developed first in Greater Antarctica sometime after the Lower Miocene (25 Myr ago) and at a later date in Lesser Antarctica. This is supported by fossil evidence from the Ross Sea¹⁶ where microfloral remains in Oligocene glacial deposits are interpreted as representing cool-temperate vegetation, which was finally eliminated in the Miocene.

Although the fossil angiosperm leaves described in this paper are undoubtedly the southernmost yet known, the occurrence of degraded plant tissue, cuticle and vascular fragments, in the Oligocene deposits in the Ross Sea, is thought to indicate a nearby vegetation source in that area¹⁶. Knowledge of the area suggests that, if the source rocks are still preserved, they are hidden by ice.

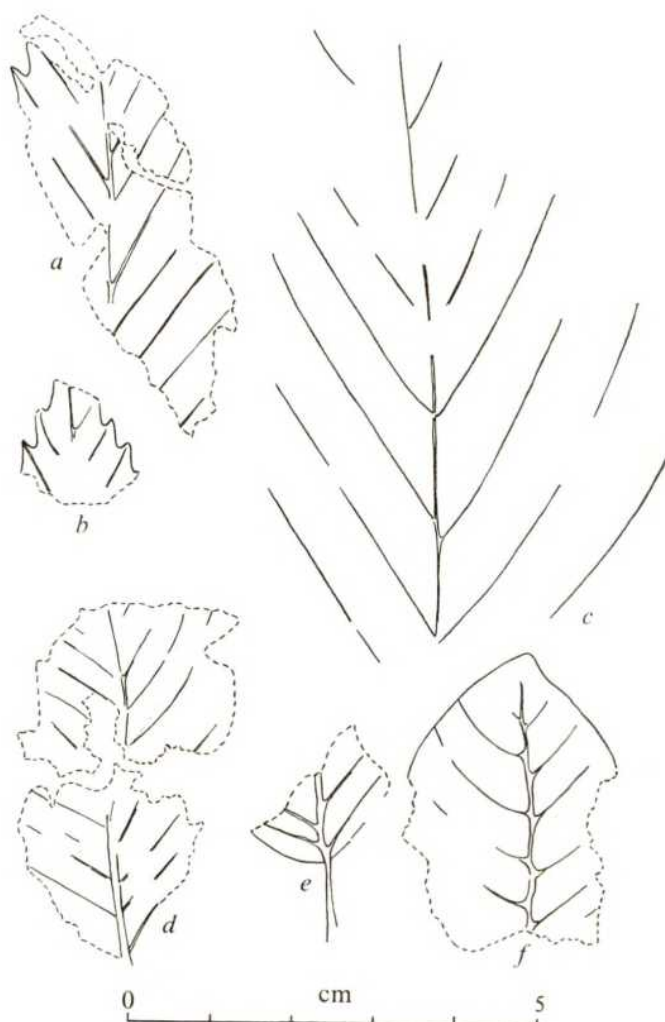


Fig. 2 Camera lucida sketches of fossil angiosperm leaves from Elgar Uplands, Alexander Island.

The fossils are housed in the collections of the British Antarctic Survey at Cambridge. We thank Dr Leo J. Hickey for helpful discussion. The radiometric age date from the South Shetland Islands was determined by Dr R. J. Pankhurst from specimens collected by Dr S. D. Weaver.

M. R. A. THOMSON
R. W. BURN

British Antarctic Survey,
Madingley Road,
Cambridge, UK

Received 26 May; accepted 22 July 1977.

- ¹ Dusen, P. *Wiss. Ergebn. schwed. Südpolarexped.* 3, Lief 3 (1908).
- ² Barton, C. M. in *Antarctic Geology* (ed. Adie, R. J.) 603–8 (North-Holland, Amsterdam, 1964).
- ³ Orlando, H. A. in *Antarctic Geology* (ed. Adie, R. J.) 629–36 (North-Holland, Amsterdam, 1964).
- ⁴ Cranwell, L. M., Harrington, H. J. & Speden, I. G. *Nature* **186**, 700–702 (1960).
- ⁵ Darlington, P. J., Jr *Biogeography of the Southern End of the World* (Harvard University, Cambridge, Massachusetts, 1965).
- ⁶ Adie, R. J. in *Antarctic Research* (ed. Adie, R. J.) 118–62 (Butterworth, London, 1964).
- ⁷ Bell, C. M. *Br. Antarct. Surv. Bull.* No. 39, 36–44 (1974).
- ⁸ Bell, C. M. *Br. Antarct. Surv. Bull.* No. 32, 75–83 (1973).
- ⁹ Grikurov, G. E. & Dibner, A. F. *Dokl. Akad. Nauk. S.S.S.R., Geology* **179**, 410–412 (1968).
- ¹⁰ Grikurov, G. E. in *Antarktika. Mezhdunarodnaya komissiya po izucheniyu Antarkitiki* 13–42 (Izdatel'stvo Nauka, Moskva, 1971).
- ¹¹ Edwards, C. W. *Br. Antarct. Surv. Bull.* (in the press).
- ¹² Grikurov, G. E., Krylov, A. Ya. & Silin, Yu. I. *Dokl. Akad. Nauk. S.S.S.R., Geology* **172**, 168–71 (1967).
- ¹³ Lowrie, W. & Hayes, D. E. *Initial Rep. DSDP* **28**, 869–78 (1975).
- ¹⁴ Bailey, I. W. & Sinnott, E. W. *Science* **41**, 831–834 (1915).
- ¹⁵ Drewry, D. J. *J. geol. Soc., Lond.* **131**, 255–273 (1975).
- ¹⁶ Kemp, E. M. & Barrett, P. J. *Nature* **258**, 507–508 (1975).

Megafaunal biomass in the deep sea

THE conventional notion of the deep-sea ecosystem has been one where a rain of fine organic particles from above provides food for a great diversity of small creatures living in and on the sediments^{1–3}. These, the meiofauna and macrofauna, live as deposit feeders, suspension feeders, and, in lesser proportion, as carnivores preying on other members of the fauna. The macrofauna in turn are thought to provide food for the generally more carnivorous megafauna, the echinoderms, decapods, and fishes. Although no data have been published concerning the abundance of the deep-sea megafauna in terms of biomass, the assumed trophic relationship and the Eltonian concept has led to the common feeling that the biomass of this component must be small in comparison to the meiofaunal and macrofaunal biomass⁴. Our data show that, on the contrary, the megafaunal biomass approaches that measured in the macrofauna. This observation re-focuses attention on the question of food supply to the deep sea, and implies that previously

suggested falls of large dead animals from the pelagial^{5,6} may be important.

As part of a general study of the distribution and ecology of deep benthic communities, we have made 116 trawl collections on the continental slope, rise, and abyss in the western Atlantic south of New England. Our principal gear for deep bottom trawling has been a 41-ft Gulf of Mexico shrimp trawl of 2.5-cm stretch mesh and fished with steel V doors. We have used a smaller 16-ft semiballoon trawl at slope depths. The animals obtained have been identified to species, counted, and weighed wet, usually after preservation in 10% formalin. From these data, we have determined mean wet weight per specimen for every taxon as a function of depth. Absolute abundance estimates were derived from photographic series made with a pair of E.G. & G. cameras mounted on DSRV ALVIN and fired at a rate of one frame per 10 s as the submersible cruised along a measured transect. Over 3,500 frames have been analysed from eight dives (436, 583, 586, 587, 589, 590, 591 and 592) in the same area where the trawling was carried out. Absolute abundance times mean weight within the appropriate depth interval gives the biomass. The data for deep benthic fishes, which comprise as a group about 40% of the total wet weight of megafaunal biomass in our trawl collections, are shown in Table 1.

The only previously available data concerning macrofaunal biomass off southern New England does not differ significantly ($P>0.65$) from the megafaunal (fish) data of Table 1. Most of these data, however, were taken with anchor dredges and seem to underestimate biomass considerably. New data on macrofaunal biomass off southern New England, obtained from 57 DSRV-operated box-core samples sieved through a 0.42-mm screen, were therefore used, and are plotted in Fig. 1. Total megafaunal biomass is obtained by extrapolation from the data of Table 1, using the actual weight percentage comprised by fish in each of the 46 trawls as the conversion factor.

Figure 1 compares macrofaunal and megafaunal biomass in the deep water south of New England. They are essentially of the same order of magnitude. The assumption that the biomass of the larger animals is relatively insignificant⁴ seems to be unfounded. Unless turnover rates in the macrofauna are relatively very high—and all the evidence suggests that this is not the case^{8,9}—the megafaunal biomass cannot be supported by the macrofauna. The megafauna must therefore depend to a considerable extent on food arriving from pelagic regions in the form of large, fast-falling packets, that is, the bodies of fishes, whales, squids and decapods. Based on observations made with a baited deep-sea camera, it has been suggested that this source could form an important fraction of the nutrition for the deep-sea

Table 1 Abundance and biomass of demersal fishes on the continental slope and rise

Mean depth (m)	Area surveyed (m ²)	No. per 1,000 m ²	No. of trawls	No. of specimens	Total weight (g)	Mean weight	Density (g m ⁻²)
497	1,548	56.7*	8	451	21,599	47.9	2.72
996	2,580	27.1*	4	24	2,173	90.5	2.45
1,329	1,517	4.6*	6	158	21,466	135.9	0.63
1,507	2,614	26.2*	4	94	20,757	220.8	5.78
1,768	6,320	12.8	2	315	61,370	194.8	2.49
1,800	2,101	4.2*	3	317	61,830	195.2	0.83
1,814	1,820	11.6*	2	18	3,041	168.9	1.96
1,815	3,372	8.9	1	2	500	250.0	2.23
1,960	12,524	7.9	2	52	26,946	518.2	4.09
2,066	1,656	13.3	2	85	29,797	350.6	4.66
2,332	7,666	3.8	3	118	61,308	519.6	1.97
2,426	24,712	6.1	2	27	8,795	325.7	1.99
2,474	15,948	4.4	3	60	25,676	427.9	1.88
2,786	2,786	4.2	4	127	35,382	278.6	1.17

Columns 1–3 contain data from the submersible; columns 4–7 are from trawls at the same approximate depths. Abundance data marked with an asterisk are from Grassle *et al.*¹⁶

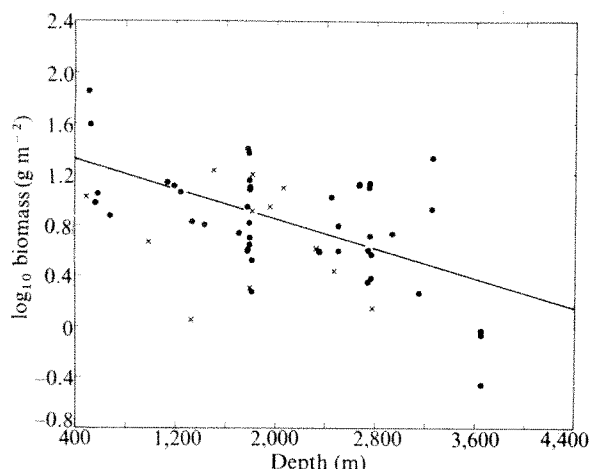


Fig. 1 Biomass against depth in macrofaunal infauna (○) and megafauna (×). The regression line is for the infaunal data ($\log_{10}B \text{ (g m}^{-2}\text{)} = 1.44 - 0.41Z(\text{km})$; $r = -0.58$, $P < 0.001$).

benthic ecosystem⁵. Our data support this idea. Further support is found in studies of the food habits of *Coryphaenoides armatus*, the dominant macrourid fish on the continental rise. This species accounts for as much as 80% of the fish biomass there and depends on large pelagic creatures for its food^{10,11}.

The need to search wide barren areas for a few randomly occurring food items and the fact that the metabolic demands of a large animal are less per unit weight than for a small one¹² should select for large efficient seekers in the deep-sea megafauna. Figure 2 shows the relationship between mean individual fish weight and depth, based on the trawled material of Table 1. There is a dramatic increase at the beginning of the rise near 2,000 m. The 'bigger-deeper' phenomenon, shown here for all fish taxa combined, applies as well within individual species and seems to be quite common in deeper-living marine fishes¹³. (Our data indicate that only a few megafaunal invertebrates are 'bigger-deeper', and the red crab *Geryon* is 'smaller-deeper' (ref. 14.) The efficiency of large deep-benthic fishes is evidenced by the rapidity with which they assemble to baited cameras⁵.

Based primarily on studies of the meiofaunal and macrofaunal elements of the deep-sea benthos, it has been suggested that small forms should dominate food-limited associations¹⁵. Our consideration of the megafaunal fishes implies just the opposite. The point is that both life styles are possible in the deep sea, particularly in the case where

the faunal components are relatively independent of one another. Where mobility is important, large size will be selected for, but where the ability to live in a fine-grained sediment and to feed on a thin organic layer is important, small size will be the rule.

We thank R. Carney, J. F. Grassle, R. R. Hessler, H. L. Sanders, and R. D. Turner for their comments on the manuscript. C. H. Clifford and P. T. Polloni assisted in the photo analyses and at sea. D. M. Cohen was the principal observer on all ALVIN dives, and has provided help and encouragement of this work in all its stages. Support came from the NSF and the National Oceanic and Atmospheric Agency.

RICHARD L. HAEDRICH
GILBERT T. ROWE

Woods Hole Oceanographic Institution,
Woods Hole, Massachusetts 02543

Received 30 May; accepted 13 July 1977.

- 1 Marshall, N. B. *Aspects of Deep-Sea Biology* (Hutchinson, London, 1954).
- 2 Menzies, R. J. *Int. Rev. ges. Hydrobiol.* **47**, 339-345 (1962).
- 3 Hessler, R. R. in *The Biology of the Oceanic Pacific* (ed. Miller, C. B.) (Oregon State University Press, Corvallis, 1974).
- 4 Grassle, J. F. & Sanders, H. L. *Deep-Sea Res.* **20**, 643-659 (1973).
- 5 Dayton, P. K. & Hessler, R. R. *Deep-Sea Res.* **19**, 199-208 (1972).
- 6 Isaacs, J. D. & Schwartzlose, R. A. *Sci. Am.* **233**, 85-91 (1975).
- 7 Rowe, G. T., Polloni, P. T. & Horner, S. G. *Deep-Sea Res.* **21**, 641-650 (1974).
- 8 Smith, K. L. & Teal, J. *Science* **179**, 282-283 (1973).
- 9 Wirsen, C. O. & Jannasch, H. W. *Environ. Sci. Technol.* **10**, 880-886 (1976).
- 10 Haedrich, R. L. & Henderson, N. R. *Deep-Sea Res.* **21**, 739-744 (1974).
- 11 Pearce, W. G. & Ambler, J. W. *Deep-Sea Res.* **21**, 745-759 (1974).
- 12 Hemmingsen, A. M. *Rep. Steno Mem. Hosp. Nordisk Insulin Lab.* **9**, 1-110 (1960).
- 13 Bullis, H. & Struhsaker, P. Q. *J. Florida Acad. Sci.* **33**, 43-76 (1970).
- 14 Wigley, R. L., Theroux, R. B. & Murray, H. E. *Mar. Fish. Rev.* **37**, 1- (1975).
- 15 Thiel, H. *Int. Rev. ges. Hydrobiol.* **60**, 575-606 (1975).
- 16 Grassle, J. F., Sanders, H. L., Hessler, R. R., Rowe, G. T. & McClellan, T. *Deep-Sea Res.* **22**, 457-481 (1975).

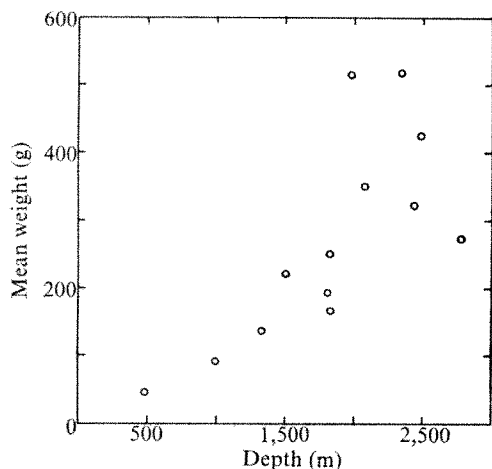
Ultradian rhythms in urine flow in waking humans

THERE is evidence that neural oscillators may regulate the flow of urine, Mandell *et al.*¹ demonstrated ultradian rhythms in urine flow in catheterised patients during sleep, synchronised with the sleep REM-nonREM cycles (REM, rapid eye movement). They attributed these rhythms to rhythmic secretion of antidiuretic hormone (ADH) during sleep. Others have demonstrated episodic secretion of ADH during sleep but no correlation with REM-nonREM cycles². Reports³⁻¹⁰ of ultradian rhythms of 90-100 min analogous to the REM-nonREM cycles in waking subjects in physiological, endocrine and psychological processes, suggested the existence of waking ultradian rhythms in the flow of urine. We report here two experiments which demonstrate these rhythms.

In experiment 1 eight healthy, male college students aged 18-20 yr, paid to participate, and unaware of the hypothesis, avoided alcohol and drugs for 24 h preceding the study. To avoid catheterisation, subjects were required to drink a constant amount of fluid and then to urinate as much as possible every 10 min for the next 10 h. Each began between 10:00 h and 13:00 h. Two subjects drank 30 ml of commercial calorie balanced liquid food, two drank 50 ml of liquid food, and four drank 30 ml of liquid food plus 30 ml of water. Each sample was collected into a graduated cylinder and volume was measured to the nearest ml. After each voiding subjects were given a brief perceptual task lasting approximately 40 s (ref. 10). When not voiding subjects were required to remain seated but could read, write, listen to music or talk to each other (all were tested in pairs).

In the second experiment, 12 healthy, male college students, aged 19-25 yr were tested in a similar way. Subjects drank 25 ml of fruit juice plus 25 ml of water. Part of each 10-min urine sample was frozen immediately for analysis. As before, after each voiding, subjects were

Fig. 2 Mean weight of fish against depth. Data from Table 1. The regression is significant ($W(g) = 0.18Z(m) - 57.3$; $r = 0.73$, $P < 0.002$).



given a brief perceptual task and then remained seated until the next trial.

In spite of the heavy experimental demands 17 out of the 20 subjects provided urine at all 60 attempts. Data for three subjects, two from the first experiment and one from the second, had to be discarded because they had failed to urinate on 10 consecutive attempts. Prominent ultradian rhythms were visible in the flow of urine for most subjects during the 10 h. Figure 1 shows the record of a representative subject in the second experiment. The mean coefficients of variation (s.d./x) for urine volumes were 34.5% and 42% for the first and second study respectively; the overall range varied between 72% and 24%. Na^+ and K^+ concentrations measured by flame photometry for 11 subjects of the second experiment, and urinary osmolality measured with a freezing point osmometer for seven subjects, showed identical rhythms reciprocal in-phase to the rhythms in urine flow.

The statistical significance of the ultradian rhythmicity was determined by time series analysis and inferential statistical procedures. First, the mean for each time series of urine volumes, K^+ and Na^+ concentrations and urine osmolality were subtracted from each data point, and the linear trend was eliminated. Then, autocorrelation functions were computed and the variance spectra were estimated^{11,12}. Spectra were calculated for 10 orthogonal frequencies from 0 to 64.8 cycles per day, in increments of 7.2 cycles per day. For each variable, spectra were hanned (hanning is a smoothing procedure to increase the reliability of the spectral estimates) individually, normalised and then averaged across all subjects. Cross correlation functions, hanned, normalised cross spectra and cross spectral phase angles were also computed for the same 10 spectral frequencies between all pairs of variables for each subject. Average spectra peaked at 14.4 cycles per day (corresponding to 1 cycle every 80–133 min) for all variables (Fig. 2). We had predicted that variance would peak in the spectra at 14.4 cycles per day, so we compared the variance at this frequency with variance at the adjacent spectral frequencies and with the mean variance at the rest of the spectral frequencies, by one-tailed *t* tests. All six comparisons yielded significant *t* ratios in the expected direction ($P < 0.05$, or better).

The ultradian rhythms in urine flow were clearly out of phase with electrolyte concentrations and osmolality rhythms. Pearson product moment correlations between volume time series and electrolyte concentrations and osmolality were negative for all subjects, and differed significantly from zero ($P < 0.001$) for all three variables. Similarly, cross-spectral phase analysis revealed that K^+ , Na^+ and osmolality were in-phase with each other and about 180° out-of-phase with urine volume.

Our results demonstrate prominent ultradian rhythms in urine flow of waking subjects. Although we do not

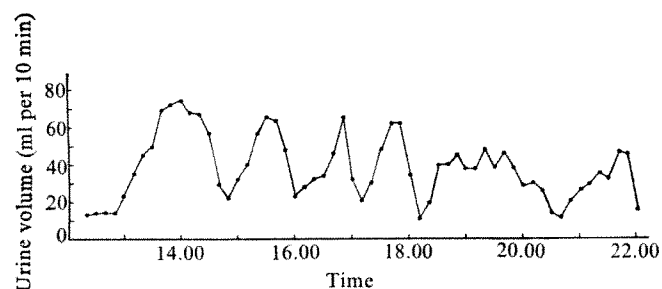


Fig. 1 Time series constructed from raw data of urine volumes sampled every 10 min from 1200 until 2200. Spectral analysis revealed a dominant ultradian periodicity of approximately 80–133 min per cycle.

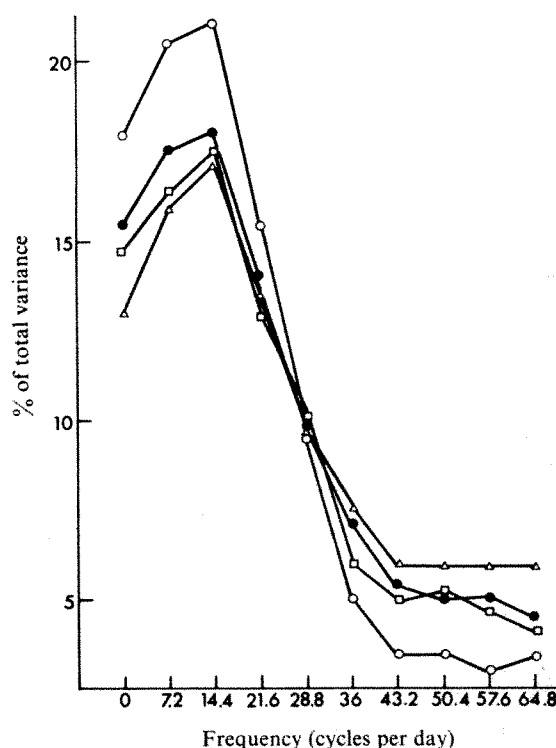


Fig. 2 Average spectra for urine volumes (\square , $n = 17$), osmolality (\circ , $n = 7$), K^+ (\bullet , $n = 11$) and Na^+ (\triangle , $n = 11$) concentrations. All spectra peaked at 14.4 cycles per day (corresponding to 80–133 min per cycle).

know whether these were generated by the oscillatory processes underlying the REM–nonREM cycles and the sleep urinary rhythms, the reciprocal phase relationships between volumes and osmolality indicates that rhythmic secretion of ADH is responsible for the rhythms in urine flow. The reports that all anterior pituitary hormones are secreted episodically during sleep and wakefulness support such an explanation¹³. But to test this endocrinological explanation we are repeating this study, making ADH measurements concomitant with urine sampling.

The accumulation of evidence of ultradian rhythms in multiple organismic processes during both sleep and wakefulness suggest that ultradian rhythms reflect an endogenous 'biological hour' of as yet unknown function and origin, similar to the well documented 20–28-h circadian 'biological day'.

This work was supported by a grant to P.L. from the Israel Center for Psychobiology. We thank Ms N. Adress and F. Krasovec for technical assistance.

P. LAVIE*

Department of Behavioral Biology,
Aba Khoushy School of Medicine,
Gutwirth Building,
Technion City, Haifa, Israel

D. F. KRIPKE

Department of Psychiatry,
University of California,
San Diego, California

Received 7 February; accepted 28 June 1977.

*To whom reprint requests should be addressed.

- ¹ Mandell, A. J. *et al. Science* **151**, 1558–1560 (1966).
- ² Rubin, R. T. *et al. Endocr. Res. Commun.* **2**, 459 (1975).
- ³ Friedman, S. & Fisher, C. J. *Am. Psychoanal. Ass.* **15**, 317–343 (1967).
- ⁴ Kripke, D. F. *Psychosom. Med.* **34**, 221–234 (1972).
- ⁵ Oswald, J., Merrington, J. & Lewis, H. *Nature* **225**, 959–960 (1970).
- ⁶ Lavie, P., Lord, J. W. & Frank, R. A. *Behav. Biol.* **11**, 373–379 (1974).
- ⁷ Lavie, P., Levy, C. M. & Coolidge, L. F. *Physiol. Psychol.* **3**, 144–146 (1975).
- ⁸ Lavie, P. *Chronobiologia* **3**, 214–218 (1976).
- ⁹ Hiatt, J. F. & Kripke, D. F. *Psychosom. Med.* **37**, 320–325 (1975).

- ¹⁰ Lavie, P., Lord, J. W. & Frank, R. A. *Behav. Biol.* **11**, 373-379 (1974).
¹¹ Halberg, F. & Panofsky, H. *Exp. Med. Surg.* **19**, 284-309 323-328 (1961).
¹² Blackman, B. R. & Tukey, J. W. *The Measurement of Power Spectra* (Dover, New York, 1958).
¹³ Weitzman, E. D., Boyar, R. M., Kapen, S. & Hellman, L. in *Rec. Prog. Horm. Res.* **31**, 399-445 (1975).

Natural selection at the α -GDH locus in *Drosophila*

THE molecular basis for the maintenance of a gene-enzyme polymorphism in natural populations of *Drosophila melanogaster* has been reported¹. The locus under analysis codes for the subunit of α -glycerophosphate dehydrogenase, (α -GDH; E.C. 1.1.1.8) a homodimeric enzyme: the primary function of this enzyme in adult muscle is to provide metabolic energy during flight²⁻⁷, and these alleles showed both spatial and temporal variations in frequency. A latitudinal cline was identified, in which the frequency of the α -GDH^S allele decreases southwards along the eastern US coast⁸. The α -GDH^S allele was also found to increase in frequency during cooler months in several New England populations⁶. We evaluated several biochemical parameters using partially purified enzyme from the three common genotypes and uncovered temperature-dependent differences in K_m , specific activity, and reaction rate constancy¹. Based on the population and biochemical data an argument was provided that environmental temperature was operating as a selective agent in demanding the relative frequencies of the two alleles at this metabolically important locus. We present here additional evidence to support this proposal, based on an evolutionary pattern of coincidence.

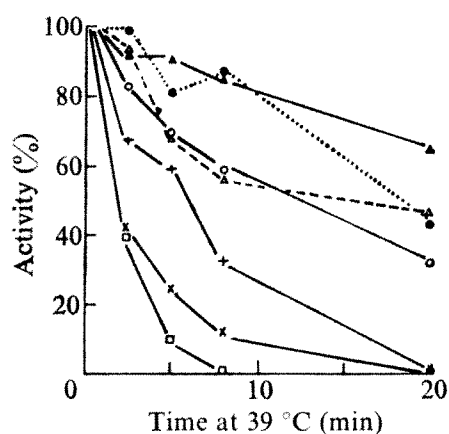


Fig. 1 Thermal stability of α -GDH. Partially purified enzyme (see ref. 1 for procedure) was isolated from 1-week-old *Drosophila* adults raised at 25 °C. Enzyme solutions at 39 °C were chilled, and assayed in a Guilford recording spectrophotometer, at 340 nm, for the reduction of NADH. \circ , *D. melanogaster* α -GDH^S; \blacktriangle , *D. melanogaster* α -GDH^F; \square , *D. virilis*; \times , *D. americana*; $+$, *D. arizonensis*; \bullet , *D. willistoni*; \triangle , *D. equinoxialis*.

The distinctive temporal and spatial patterns of allele frequency distribution, and the biochemical profiles discussed above, led us to refer to α -GDH^S as the cool-climate adapted allele, and α -GDH^F as the warm-climate adapted allele. This is somewhat misleading since α -GDH^F is always the most abundant allele, but as a relative statement the nomenclature is useful. We predicted that if α -GDH function is sensitive to environmental temperature a parallel response should be observed in temperature-dependent kinetic properties if we compared α -GDH of species inhabiting primarily temperate rather than tropical habitats. The observation of such parallel biochemical differentiation, in an evolutionary time frame, should carry considerable weight for the selection model in spite of our inability to experimentally demonstrate the exact *in vivo* significance of differences found in cuvette assays. Since every other *Drosophila* species reported on the essentially monomorphic at the α -GDH locus⁹⁻¹² our analysis would not be confounded by interspecific variation in our additional species samples.

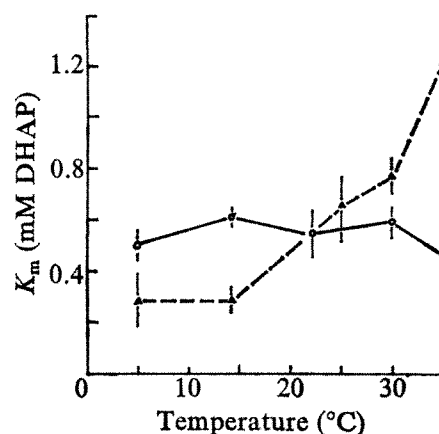


Fig. 2 Apparent K_m values for the substrate, dihydroxyacetone phosphate (DHAP), for *D. melanogaster*, as a function of temperature. The strains S mark and (O) carrying α -GDH^S, and Swedish-C (Δ) carrying α -GDH^F were employed. K_m at each temperature was determined from duplicate measurements of activity at each of 5 substrate concentrations by fitting $1/v$, $1/s$ data into a linear regression computer program. Concentrations of NADH were 120 μ M throughout. Assay temperatures were maintained in the cuvette chamber by a Haake circulating water bath. Bars around representative points indicate standard error values.

We examined three tropical species (*D. willistoni*, *D. arizonensis*, *D. equinoxialis*) and two temperate species (*D. virilis* and *D. americana*) (stocks provided by the Stock Centre, University of Texas, Austin: see ref. 13). Partially purified enzyme was prepared¹ from adult homogenates of each species as well as from strains of *D. melanogaster* homozygous for the two common alleles, and studied in several ways. We found that the electrophoretic mobility of α -GDH from each of the five additional species was indistinguishable from the α -GDH^S allele of *D. melanogaster* in starch gels, in a variety of electrophoretic conditions (pH 6.8 or 8.8, in 9% or 14% starch, in a Tris-citrate buffer system; pH 7.8 or 9.8, in 9% or 14% starch, in a Tris-borate buffer system)¹⁴. This pattern of conservation, with respect to electrophoretic mobility, for α -GDH has been noted before¹⁵. But, we could detect rather large differences among the species¹ enzymes by using thermal denaturation as a probe (Fig. 1). This observation supports a growing number of studies indicating that thermal stability analysis can detect hidden variation within a single electrophoretic mobility class¹⁶⁻¹⁹. We suspect that although

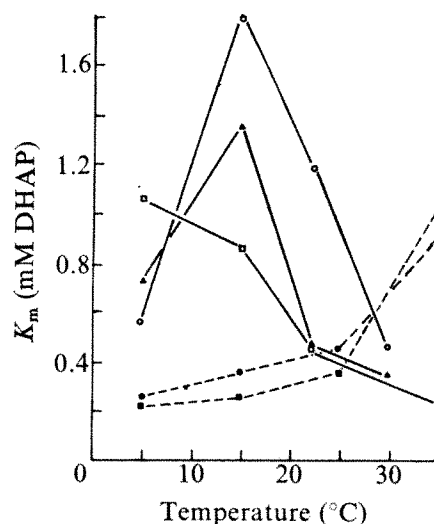


Fig. 3 Apparent K_m for DHAP for α -GDH obtained from: \square , *D. willistoni*; \triangle , *D. equinoxialis*; \circ , *D. arizonensis*; \blacksquare , *D. virilis* and \bullet , *D. americana*. Assay conditions as described in Fig. 2 legend.

closely related species (for example *D. virilis* and *D. americana*, or *D. willistoni* and *D. equinoxialis*) have similar thermostability properties it is unlikely that their primary structures are the same. It is interesting to note that, as a general rule, enzyme from the tropical *Drosophila* species is less heat sensitive than enzyme from the temperate species.

Kinetic studies (Fig. 2) confirmed the distinct K_m against temperature patterns found for the *D. melanogaster* 'fast' and 'slow' homozygotes¹. The α -GDH^F pattern showed temperature independence of affinity for substrate, while the α -GDH^S pattern displayed a temperature dependent pattern in which increased substrate affinity and temperature were inversely related. This latter type of pattern has been referred to as positive thermal modulation¹⁹, a mechanism by which, it is thought, reaction rate constancy is maintained over a range of environmental temperatures¹⁹. Figure 3 summarises the data obtained for the five new species. *D. virilis* and *D. americana*, both temperate species, showed K_m against temperature profiles remarkably like the α -GDH^F pattern. In contrast, the three tropical species had profiles which, while similar within the group, clearly differed from the temperate species¹ (α -GDH^S like) pattern. In contrast to α -GDH^F, in which K_m remained essentially constant, the tropical species had a pattern of negative thermal modulation (K_m decreases with increasing temperature) over most of the temperate range. In any event the tropical species' profile clearly more resembled the α -GDH^F pattern, especially at higher assay temperatures. Activation energies were calculated from the data, and all species and genotypes had quite similar values (7.7 ± 0.8). (Energies of activation determined by Arrhenius plots according to the formula in ref. 20).

The coincidence of K_m against temperature profiles described in the data, as predicted, seem based on species distribution rather than phylogeny. This observation strongly suggests that natural selection is operating at the α -GDH locus within the genus *Drosophila*, with temperature being the mediating agent. Note that this same conclusion has been drawn for α -GDH polymorphisms in two other insect species, *Colias*, a butterfly²¹, and *Cochliomyia*, the screw worm²². We feel that a general pattern may emerge among all flying insects with a carbohydrate based energy metabolism, in which environmental temperature will be shown critical in the adaptation and evolution of flight enzyme kinetics.

This work was supported by US NIH grant GM22866.

STAMATIS ALAHOTIS*

STEVE MILLER†

EDWARD BERGER‡

Biology Department, Dartmouth College
Hanover, New Hampshire 03755

Received 20 May; accepted 27 June 1977.

*Present address: Genetics Department, University of Patras, Greece.

†Present address: Genetics Department, North Carolina State University, Raleigh, North Carolina.

‡To whom correspondence should be addressed.

- ¹ Miller, S., Pearcy, R. W. & Berger, E. *Biochem. Genet.* **13**, 175-188 (1975).
- ² Sacktor, B. & Dick, A. J. *biof. Chem.* **237**, 3259-3262 (1962).
- ³ Sacktor, B. *Adv. Insect Physiol.* **7**, 267-347 (1970).
- ⁴ O'Brien, S. J. & MacIntyre, R. J. *Genetics* **71**, 127-138 (1972).
- ⁵ Grell, E. *Science* **158**, 1319-1320 (1967).
- ⁶ Berger, E. *Genetics* **67**, 121-136 (1971).
- ⁷ O'Brien, S. J. & MacIntyre, R. J. *Am. Naturalist* **102**, 193-205 (1968).
- ⁸ Johnson, F. & Schaffer, H. *Biochem. Genet.* **10**, 149-163 (1973).
- ⁹ Kojima, K., Gillespie, J. & Tobari, Y. N. *Biochem. Genet.* **4**, 627-637 (1970).
- ¹⁰ Zouros, E. *Nature* **254**, 446-448 (1975).
- ¹¹ Barker, J. S. F. & Mulley, J. C. *Evolution* **30**, 213-233 (1976).
- ¹² Powell, J. in *Evolutionary Biology* **8**, 79-119 (eds Dobzhansky, T., Hockett, M. K. & Steere, W. C.) (Plenum, New York, 1976).
- ¹³ Patterson, J. T. & Stone, W. *Evolution in the Genus Drosophila*. (Macmillan, New York, 1952).
- ¹⁴ Shaw, C. R. & Prasad, R. *Biochem. Genet.* **4**, 297-320 (1970).
- ¹⁵ Hubby, J. L. & Throckmorton, L. H. *Am. Nat.* **102**, 193-205 (1968).
- ¹⁶ Bernstein, S., Throckmorton, L. H. & Hubby, J. L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3928-3931 (1973).
- ¹⁷ Singh, R. S., Lewontin, R. C. & Felton, A. A. *Genetics* **84**, 609-629 (1976).
- ¹⁸ Singh, R. S., Hubby, J. L. & Lewontin, R. C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1808-1810 (1974).
- ¹⁹ Hockachka, P. W. & Somero, G. *Strategies of Biochemical Adaptation* (Saunders, Philadelphia, 1973).
- ²⁰ Robert, M. & Gray, I. *Comp. Biochem. Physiol.* **42B**, 389-402 (1972).
- ²¹ Johnson, G. B. *Stadler Genet. Symp.* **7**, 91-111 (1975).
- ²² Bush, G. L., Neck, R. W. & Kitto, G. B. *Science* **193**, 491-493 (1976).

Genetic control of cell division in yeast cultured at different growth rates

HARTWELL *et al.*^{1,2} have isolated a number of temperature-sensitive mutants blocked at various stages of the cell division cycle of *Saccharomyces cerevisiae*. A characteristic feature of any temperature-sensitive cell cycle mutant is its execution point in the cell cycle, that is, the point in the cycle after which cells when shifted to the restrictive temperature undergo one further cell division. If cells are shifted to the restrictive temperature before the execution point, they are unable at the elevated temperature to complete any cell division. The execution point for a particular mutant is taken to be the stage in the cycle that the defective gene completes its function. The execution point of mutant strain *cdc28* is at an earlier stage in the cycle than other cell cycle mutants³. Detailed investigations of a number of cell cycle mutants have suggested that the events in early G₁ that culminate in the execution of the *cdc28*-mediated step represent 'start'. There is evidence that passing 'start' in the cycle represents a point of commitment to division. Before the execution of the *cdc28*-mediated step, 'start', yeast is capable of several developmental programmes but after this step it is committed to the mitotic cell cycle². We report here that at generation times varying from 2.1-6.18 h 'start' occurs approximately 2 h before division. At slower growth rates the length of time from 'start' to division increases somewhat. These results are similar to the finding in the bacterium *Escherichia coli* that the time from the initiation of DNA synthesis to cell division is independent of growth rate except at slow growth rates⁴.

A number of authors⁵⁻⁷ have shown that if one knows the percentage of cells in an asynchronous culture that are past a particular event in the cell cycle, the stage in the cycle that the event occurs can be determined. The execution point of a temperature-sensitive cell cycle mutant can be calculated from the percentage increase in cell number after such a mutant is shifted from the permissive to the restrictive temperature, since this reflects the percentage of cells that are past the execution point at the time of the shift. An allowance has to be made for the fact that the age distribution of cells throughout the cycle is skewed⁸, using an equation which describes the age distribution⁷.

A strain that carries the mutation *cdc28* has been cultured at different growth rates at 24 °C in batch cultures by altering media composition and in chemostat cultures by altering the flow rate of the limiting nutrient, glucose. Cultures at different growth rates were shifted to 38 °C and cell numbers after the shift were monitored. Fig. 1 shows the effect of shifting *cdc28* with a generation time of 6.18 h to 38 °C. Cell numbers continue to increase for a time until cell division stops and a plateau in cell numbers is reached. The percentage increase in cell numbers after the temperature shift is equivalent to the percentage of cells past the *cdc28* execution point and from this data the stage in the cycle of the execution point is determined using the equation of Howell and Nabiloff⁷. In the experiment described in Fig. 1 the execution point for the *cdc28* gene product occurs at 0.42 of the cell cycle.

Table 1 shows the percentage increase in cell numbers after *cdc28* cultured at different growth rates is shifted to 38 °C. The cell number increase is greatest at the fastest growth rates and the percentage increase is least at the slowest growth rates. Clearly, if in an exponential culture many cells at the permissive temperature are past the *cdc28* execution point, this event occurs at an early stage of the cycle. Conversely, if only a few cells

Table 1 Effect of specific growth rate on the stage and time in the cell cycle of the *cdc28* gene product execution point

Specific growth rate (h^{-1})	Generation time (h)	Percentage increase in cell numbers after shift to 38 °C	Stage in the cell cycle of the <i>cdc28</i> execution point	Time before cell division of the <i>cdc28</i> execution point (h)
0.330	2.1	133	-0.12	2.5
0.254	2.73	85.7	0.11	2.4
0.180	3.85	57	0.35	2.5
0.112	6.18	50	0.42	3.58
0.032	21.6	21.4	0.72	6.05

Haploid *Saccharomyces cerevisiae* cells of strain H.185.3.5 carrying the *cdc28* mutation were grown at different steady state specific growth rates in a glucose limited chemostat at 24 °C in the media described in Fig. 1. Samples at different specific growth rates were removed from the chemostat, and diluted in fresh medium at 38 °C. Cell number was monitored at intervals as described in Fig. 1, until cell division at the restrictive temperature ceased. The percentage of cells that divided at the restrictive temperature was used to calculate the stage and time in the cycle of the *cdc28* gene product execution point using the equation described in Fig. 1.

are past this event at the time of the shift then it occurs at a late stage of the cycle. Table 1 shows that stage in the cycle of *cdc28* execution point is dependent on growth rate such that as the growth rate is slowed the execution point occurs later and later in the cycle. The time from the *cdc28* execution point to cell division is constant in cells growing with generation times of 2.1, 2.73 and

execution point for *cdc28* occurs in the previous cycle to the division which it controls, is in keeping with the view that the *cdc28* gene product decays slowly at the restrictive temperature. Others have suggested that the *cdc28* mutation is 'leaky'.

Haploid yeast cells of 'a' mating type produce a small polypeptide ' α factor' of molecular weight 1,400, which

Table 2 Effect of specific growth rate on the stage and time in the cell cycle of the α factor-sensitive step

Specific growth rate (h^{-1})	Generation time (h)	Percentage increase in cell numbers in the presence of α factor	Stage in the cell cycle of the α factor factor-sensitive step	Time before cell division of the α factor-sensitive step
0.256	2.7	56.9	0.13	2.35
0.20	3.46	60	0.33	2.32
0.15	4.62	42.8	0.49	2.36
0.112	6.18	28.5	0.63	2.29
0.076	9.1	26.6	0.65	3.18

Haploid *Saccharomyces cerevisiae* cells of strain H185.3.4 carrying the *cdc28* mutation were grown at different steady-state specific growth rates in a glucose limited chemostat at 24 °C in the media described in Fig. 1. Samples at different specific growth rates were removed from the chemostat, and diluted in fresh medium at 24 °C containing α factor. The α factor was purified as described previously⁸, and a concentration of α factor was chosen that produced greater than 95% unbudded cells and that inhibited budding for at least 8 h. Cell number was monitored at intervals as described in Fig. 1, until cell division in the presence of α factor ceased. The percentage of cells that divided in the presence of α factor was used to calculate the stage and time in the cycle of the α factor sensitive step using the equation described in Fig. 1.

3.85 h but at slower growth rates it expands in time. A possible explanation for this expansion is that the *cdc28*-thermosensitive gene product decays quite slowly at 38 °C. If this is true then the gene product will continue to function for some time at the restrictive temperature, and consequently these cells will continue to divide. The result of this is that the execution point for *cdc28* will seem to be earlier in the cycle than the time at which the gene product normally completes its function. Our observation (Table 1) that at fast growth rates the

contains a number of common amino acids⁸. When the α factor is added to cells of 'a' mating type, the latter are arrested as small unbudded cells before the initiation of DNA synthesis. The point of α factor-mediated cell cycle arrest has been mapped within the sequence of three gene functions before the initiation of DNA synthesis, and it has been shown that the α factor-sensitive step is the same as that mediated by the *cdc28* gene product³.

Because of the uncertainty attached to precise location

Table 3 Effect of growing *Saccharomyces cerevisiae* in different media supporting different growth rates on the stage and time in the cell cycle of the α factor-sensitive step

Culture medium	Specific growth rate (h^{-1})	Generation time (h)	Percentage increase in cell numbers in the presence of α factor	Stage in the cell cycle of the α factor-sensitive step	Time before cell division of the α factor-sensitive step
YEPA	0.247	2.8	57.0	0.35	1.83
Proline	0.208	3.33	50.0	0.42	1.93
YEPG	0.134	5.16	30.5	0.62	1.96
Ethanol	0.084	8.3	22.0	0.71	2.41
Acetate	0.082	8.5	21.5	0.72	2.38

Haploid wild-type *Saccharomyces cerevisiae* strain A364a were grown on different media at 24 °C. The media were: YEPA, containing in 1 l of distilled water, 10 g yeast extract, 20 g bacto-peptone, 20 g glucose; proline, containing in 1 l of distilled water, 2 g proline, 20 g glucose, 1.4 g yeast nitrogen base without amino acids; YEPG, containing in 1 l of distilled water, 10 g yeast extract, 20 g bacto-peptone, 20 ml glycerol; ethanol, containing in 1 l of distilled water, 20 ml ethyl alcohol 95%, 5 g ammonium sulphate, 6.7 g yeast nitrogen base without amino acids; and acetate, containing in 1 l of distilled water, 20 g potassium acetate, 6.7 g yeast nitrogen base without amino acids. All these media, except YEPG and YEPA, also contained 0.04 g tyrosine, 0.04 g lysine, 0.04 g histidine, 0.01 g uracil and 0.1 g adenine per l, trace elements and vitamins. Cultures were grown on rotary shakers at 24 °C. These media support generation times of 2.8, 3.33, 5.16, 8.3 and 8.5 h respectively. Samples at different growth rates were diluted in fresh YEPA medium at 24 °C containing α factor. Cell number was monitored at intervals as described in Fig. 1, until cell division ceased in the presence of α factor. The percentage of cells that divided in the presence of α factor was used to calculate the stage and time in the cell division cycle of the α factor-sensitive step using the equation described in Fig. 1.

in time of the *cdc28* execution point, we have determined the stage and time in the cycle of the α factor-sensitive step for cells cultured at different growth rates in the chemostat and treated with α factor; the percentage of cells that divide in the presence of α factor was used as before to determine the stage in the cycle of the α factor-sensitive step. Table 2 shows that for cells cultured at different growth rates in the chemostat the α factor step was at the beginning of the cycle at fast growth rates, but as the growth rate was slowed it moved to later stages of the cycle. In terms of time, however, it occurred at generation times less than 6.18 h, a constant time before cell division. Similar results were obtained when the time of the α factor-sensitive step was investigated in cells grown at different growth rates using different media in batch culture (Table 3). At longer generation times the time from 'start' to cell division increases; for instance, at a generation time of 9.1 h the time from

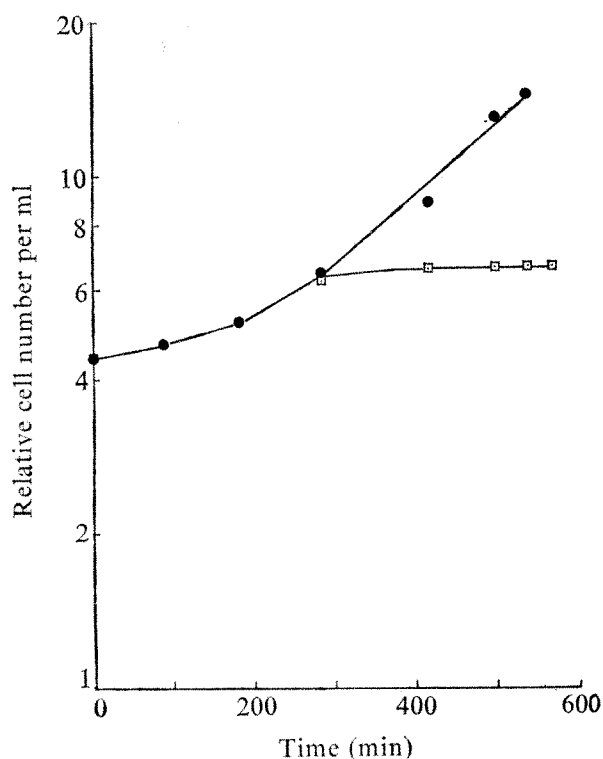


Fig. 1 Effect on cell division of shifting *cdc28* previously grown with a doubling time of 6.18 h at 24 °C in a chemostat to the restrictive temperature 38 °C. Haploid *Saccharomyces cerevisiae* cells of strain H185.3.4 carrying the *cdc28-1* mutation were grown at 24 °C in a chemostat (L.H. Engineering) at a steady-state specific growth rate of 0.112 h⁻¹ (generation time 6.18 h). The chemostat medium contained 10 g yeast extract, 10 g bacto-peptone, 10 g glucose as limiting nutrient, 5 g ammonium sulphate, 2 g ammonium phosphate, 1 g magnesium sulphate and 100 mg adenine in 1 litre of distilled water at pH 5.5. Samples were removed from the chemostat diluted in fresh medium and one sample was incubated at 24 °C (●) and the other at 38 °C (□) in a shaking water bath. Samples were removed from both cultures at intervals, diluted in Isoton (Coulter Electronics) and cell numbers determined in an electronic particle counter (Coulter Electronics) after 90 s treatment with an MSE homogeniser to break up clumps. The percentage increase in cell numbers after the shift to 38 °C (in this case 50%) was used to determine the stage in the cycle of the *cdc28* execution point using the equation

$$X = 1 - \frac{\ln(N/N_0)}{0.693}$$

where X is the stage in the cycle of the execution point, N_0 is equal to the cell number at the time of the temperature shift and N is equal to the final cell number at the restrictive temperature⁶. The stage in the cell cycle of the execution point was calculated to be 0.42.

'start' to cell division was 3.18 h (Table 2). The results presented in Table 1 can also be interpreted to indicate that at very slow growth rates the time from 'start' to cell division lengthens. If the decay time for the *cdc28* gene product is the same at varying growth rates, a constant amount should be subtracted from the time between the apparent execution point and cell division in the last column of Table 1. This would leave this time constant for generation times between 2.1 h and 3.85 h; and if, for example, the decay time took 0.2 h the time from the real execution point to cell division would be nearly the same as the equivalent 'start-division' time in the last column of Table 2. This assumption of a constant decay time, however, would not explain the increased 'start-division' time of for instance 6.05 h found for cells grown with a generation time of 21.6 h (Table 1).

These results indicate that for cells growing with generation times of less than 6.18 h the cell cycle can be divided into an expandable phase before the α factor-sensitive step, the length of which is dependent on growth rate, and a constant phase from the α factor-sensitive step to cell division, the length of which is independent of growth rate. As the α factor step is the same as that mediated by the *cdc28* gene product, which is thought to represent 'start', our results indicate that once cells pass 'start' they proceed to cell division in a constant time regardless of growth rate. Cells take a variable time to reach 'start' at different growth rates. It is interesting to note that when yeast populations were grown at long generation times in a chemostat the budded phase of the cycle was slightly longer than during rapid growth while the unbudded phase was selectively lengthened¹⁰.

At longer generation times the 'start-division' time increased although the pre-start period of the cycle was the main expandable phase. Our results are reminiscent of observations in *Escherichia coli* that the time from the initiation of DNA synthesis to cell division is constant at generation times faster than 60 min but at longer generation times there is a change in cell cycle timing controls.

We thank L. H. Hartwell for advice and yeast strains, the Berkeley Yeast Center for yeast strains, and J. Piggott for a gift of α factor. This work was supported by the Medical Research Council of Ireland and Arthur Guinness, Son and Co. Ltd.

M. N. JAGADISH
B. L. A. CARTER

Department of Genetics,
University of Dublin, Trinity College,
Dublin 2, Ireland

Received 24 May; accepted 19 July 1977.

- Hartwell, L. H. *Bact. Rev.* **38**, 164-198 (1974).
- Hartwell, L. H., Culotti, J., Pringle, J. R. & Reid, B. J. *Science* **183**, 46-51 (1974).
- Hereford, L. M. & Hartwell, L. H. *J. molec. Biol.* **84**, 445-461 (1974).
- Cooper, S. & Helmstetter, C. E. *J. molec. Biol.* **31**, 519-540 (1968).
- Hoffman, J. G. *Bull. Math. Biophys.* **11**, 139-144 (1949).
- Watanabe, I. & Okada, S. *J. Cell Biol.* **32**, 309-323 (1967).
- Howell, S. H. & Nabiloff, J. A. *J. Cell Biol.* **57**, 760-772 (1973).
- Cook, J. R. & James, T. W. in *Synchrony in Cell Division and Growth* (ed. Zeuthen, E.) 485-495 (Wiley-Interscience, New York, 1944).
- Duntze, W. D., Stotzler, E., Bucking-Throm, E. & Kalbitzer, S. *Eur. J. Biochem.* **35**, 357-365 (1973).
- Beck, C. & Meyenburg, H. K. von *J. Bact.* **96**, 479-486 (1968).

Blood flukes have a double outer membrane

THE blood fluke *Schistosoma mansoni* has an unusual tegumental outer membrane which consists of two lipid bilayers^{1,2}. We report here that the double outer membrane is a common feature of the Schistosomatidae (parasites of man which cause schistosomiasis) and also occurs in members of the two other families of blood flukes³, the

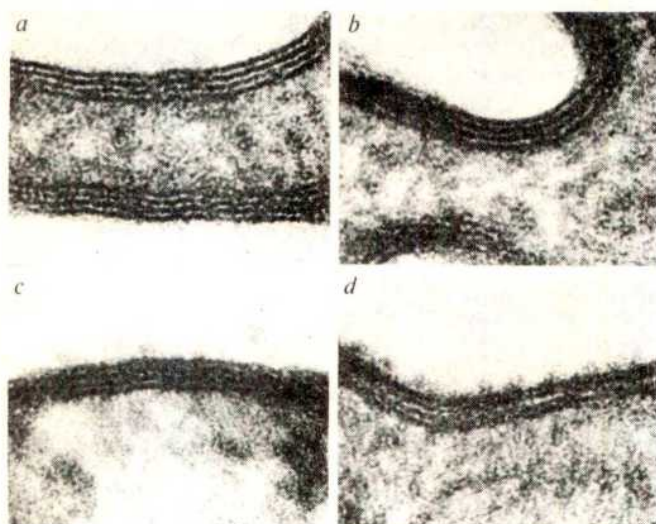


Fig. 1 Electron micrographs showing the double outer membrane of representative specimens from each of the three families of blood flukes. *a*, *Schistosoma japonicum* (Schistosomatidae); *b*, *Schistosoma margrebowiei* (Schistosomatidae); *c*, *Aporocotyle simplex* (Aporocotylidae); *d*, *Spirorchis* sp. (Spirorchiidae). ($\times 240,000$).

Aporocotylidae (parasites of fish) and the Spirorchiidae (parasites of turtles). In contrast, flukes (Digenea) belonging to families which inhabit the gut and associated body cavities of the host are limited by a single lipid bilayer. We suggest that the double outer membrane is an adaptation to the intravascular habitat.

Details of the flukes examined and their hosts and locations are presented in Table 1. Specimens were fixed for electron microscopy in glutaraldehyde, osmium tetroxide and uranyl acetate¹, dehydrated in ethanol and embedded in Araldite. Sections were stained with uranyl acetate and lead citrate.

In the Schistosomatidae the double outer membrane was present over virtually the entire surface of both male and female worms; a few small regions of the membrane

had a trilaminar or multilaminar appearance. In the fish and turtle blood flukes two lipid bilayers were found consistently over the whole surface of the parasite. The double outer membrane had the same appearance and identical dimensions in all the blood flukes examined (Table 1, Fig. 1). Each lipid bilayer was approximately 7 nm in thickness and the double outer membrane was 18 nm thick. Some specimens had a layer of diffuse material on the external surface of the double membrane, but we believe that this material originated from the host and did not represent a true glycocalyx. The flukes recovered from environments other than blood had only a single trilaminar outer membrane (single lipid bilayer) approximately 8 nm in thickness (Table 1, Fig. 2). Each of the non-blood flukes had a glycocalyx which varied in appearance from a thin but dense layer in *Fasciola hepatica* to a wide diffuse layer in *Macrocoelium papilliger*.

The advantage conferred by the presence of an additional membrane at the surface of the blood fluke may be related to the fact that any parasite living in the blood vascular system must be constantly exposed to the host's immune defence mechanisms in the form of leukocytes, complement and antibodies. The ability of adult schistosomes to evade the immune response may be due to the host-like antigenic determinants which are found on the worm's surface, and which may prevent the attachment of antibodies against the parasite^{4,5}. The double outer membrane of *S. mansoni* is formed immediately after host penetration^{1,6}, and before host antigens can be detected on the surface of the worm⁷. It has been suggested that the lipid molecules of the outer bilayer may be orientated for the insertion of host glycoproteins or glycolipids⁵. The idea that the two lipid bilayers may differ with respect to their lipid composition is supported by the fact that the outer bilayer is only visualised after uranyl acetate fixation^{1,8}. Rapid turnover and chemical unreactivity of the outer bilayer have also been suggested as possible mechanisms for countering antibody attack⁹. Turnover has been demonstrated *in vitro*⁹, but freeze-fracture studies have shown that most intercalated proteins occur in the outer lipid bilayer²—suggesting that the outer membrane may be more active than the inner membrane.

Table 1 Flukes examined by electron microscopy

Family	Genus and species	Host	Location
(1) Blood flukes			
Schistosomatidae	<i>Schistosoma haematobium</i>	Hamster	Hepatic portal vein
	<i>S. japonicum</i>	Hamster	Hepatic portal vein
	<i>S. matthei</i>	Hamster	Hepatic portal vein
	<i>S. bovis</i>	Hamster	Hepatic portal vein
	<i>S. leiperi</i>	Hamster	Hepatic portal vein
	<i>S. margrebowiei</i>	Hamster	Hepatic portal vein
	<i>S. intercalatum</i>	Hamster	Hepatic portal vein
	<i>S. haematobium</i> ♂	Hamster	Hepatic portal vein
	<i>S. intercalatum</i> ♀	Hamster	Hepatic portal vein
	♂/♀ hybrid	Hamster	Hepatic portal vein
Aporocotylidae	<i>Aporocotyle simplex</i>	Long rough dab	Gill arteries
	<i>A. spinosicanalis</i>	Hake	Heart
Spirorchiidae	<i>Spirorchis</i> sp.	Painted turtle	Pulmonary venule
(2) Non-blood flukes			
Fellodistomatidae	<i>Steringophorus furciger</i>	Cod	Pylorus
Fasciolidae	<i>Fasciola hepatica</i>	Cow	Bile duct
Echinostomatidae	<i>Echinostoma caproni</i>	Mouse	Ileum
Notocotylidae	<i>Notocotylus tricerialis</i>	Chicken	Caecum
Dicrocoeliidae	<i>Dicrocoelium dendriticum</i>	Rabbit	Bile duct
	<i>Corrigia vitta</i>	Field mouse	Pancreatic duct
Acanthocolpidae	<i>Stephanostomum pristi</i>	Cod	Pylorus
Allocreadiidae	<i>Podocotyle atomon</i>	Scorpion fish	Rectum
	<i>Macrocoelium papilliger</i>	Minnow	Stomach
Lepocreadiidae	<i>Lepidopoda elongatum</i>	Cod	Pylorus
Zoogonidae	<i>Zoogonoides viviparus</i>	Dab	Intestine
Hemiuridae	<i>Hemiurus communis</i>	Flounder	Stomach
	<i>Derogenes varicus</i>	Cod	Stomach

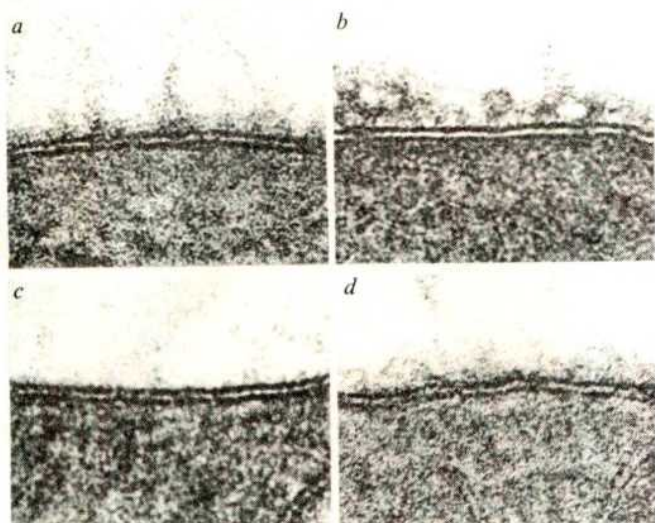


Fig. 2 Electron micrographs showing that the outer membrane of flukes recovered from environments other than the blood, consists of a single lipid bilayer. *a*, *Dicrocoelium dendriticum* (Dicrocoeliidae); *b*, *Derogenes varicus* (Hemiuridae); *c*, *Podocotyle atomon* (Allocreadiidae); *d*, *Echinostoma caproni* (Echinostomatidae). ($\times 240,000$).

We conclude therefore, that the double outer membrane is a characteristic feature of the blood flukes, that it consists of two conventional lipid bilayers with differing properties, and that it assists in protecting the parasite against the immunological response of the host.

We thank S. R. Smithers, V. R. Southgate, R. J. Knowles, C. James, J. Thulin, D. I. Gibson, M. K  ie, M. Matricon, D. D  wel, J. W. Smith, A. R. Hockley, A. W. Pike, R. A. Matthews and M. R. L. Johnston for supplying specimens.

DIANE J. MCLAREN

Division of Parasitology,
National Institute for Medical Research,
Mill Hill, London NW7, UK

DAVID J. HOCKLEY

Division of Viral Products,
National Institute for Biological Standards
and Control,
Hampstead, London NW3, UK

Received 20 May; accepted 21 July 1977.

¹ Hockley, D. J. & McLaren, D. J. *Int. J. Parasit.* 3, 13–25 (1973).

² Hockley, D. J., McLaren, D. J., Ward, B. J. & Nermut, M. V. *Tissue Cell* 7, 485–496 (1975).

³ La Rue, G. R. *Expl. Parasit.* 6, 306–349 (1957).

⁴ Smithers, S. R., Terry, R. J. & Hockley, D. J. *Proc. R. Soc. B* 171, 483–494 (1969).

⁵ Clegg, J. A. *Ciba Fdn Symp.* 25, 161–183 (1974).

⁶ McLaren, D. J., & Hockley, D. J. *Parasitology* 73, 169–187 (1976).

⁷ McLaren, D. J., Clegg, J. A. & Smithers, S. R. *Parasitology* 70, 67–75 (1974).

⁸ Silva, M. T., Guerra, F. C. & Magalhaes, M. M. *Experientia* 24, 1074 (1968).

⁹ Wilson, R. A. & Barnes, P. E. *Parasitology* 74, 61–71 (1977).

Macrophages synthesise and release prostaglandins in response to inflammatory stimuli

WHEN macrophages encounter inflammatory stimuli either *in vivo* or *in vitro*, they respond by releasing a number of products which may account for the central role that this cell has in chronic inflammatory diseases¹. These products include hydrolytic enzymes active at neutral² or acidic pH (ref. 1), components of both the classical³ and alternate pathway⁴ of complement, factors modulating responses of lymphocytes to antigens and mitogens⁵, and factor(s) influencing the proliferation⁶ and synthesis of collagen⁷ by fibroblasts. We now show

that macrophages whose phospholipid components were labelled with ³H-arachidonic acid also synthesise and release ³H-prostaglandins (PGs) in response to inflammatory stimuli. These observations are consistent with the findings that human macrophages on intrauterine devices⁸ and guinea pig macrophages responding to lymphokines⁹ release PGs.

It has been shown that tissues such as spleen¹⁰, heart and kidney¹¹, tumour cells¹² and platelets¹³ incorporate radiolabelled arachidonic acid into phospholipids and release labelled PGs. It is known that macrophage phospholipids contain a high proportion of this fatty acid¹⁴. We have found that unstimulated mouse peritoneal macrophages (Fig. 1) exposed to 1 μ Ci ³H-arachidonic acid incorporate approximately 50% of the label into cellular phospholipids within 4 h. Extraction of either stimulated or unstimulated labelled cells showed that the majority of the isotope was incorporated into lecithin with smaller amounts found in phosphatidylethanolamine and triglycerides.

When cultures of unstimulated macrophages are maintained in a serum-free environment for 24 h, less than 3% of the label is released into the culture medium; only a small proportion of this has mobilities identical with standards of PGF_{2α}, PGE₂,

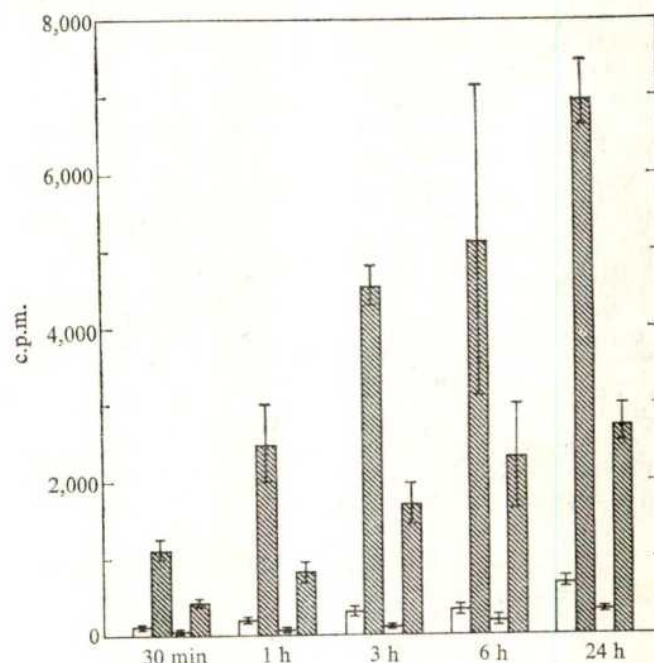


Fig. 1 Zymosan induces a time-dependent synthesis and release of ³H-PGs from unstimulated macrophages. Macrophages were collected from outbred male SW-ICR mice by intraperitoneal lavage with M199 media containing 1% heat-inactivated porcine serum (HIPS) and heparin 20 U ml⁻¹. The cells were plated at 4×10^6 cells per 60-mm dish and incubated for 2 h at 37 °C in 5% CO₂ in air. The non-adherent cells were removed by washing and the adherent cells maintained overnight in M199 plus 10% HIPS. Cellular phospholipids were then labelled by incubating the cells in M199 plus 1% HIPS containing 1 μ Ci ³H-5,6,8,9,11,12,14,15-arachidonic acid (specific activity of 64 Ci mmol⁻¹; New England Nuclear, Boston, Massachusetts) for 4 h. At this time the culture medium was removed, the cells washed twice with 5 ml PBS and incubated in 2 ml M199 devoid of serum with or without sonicated zymosan particles (ICN-K+K Labs, Plainview, New York). The stock zymosan preparations were washed and sterilised by boiling three times in PBS. The medium was removed, acidified to 0.03 M with citric acid, authentic standards added, and extraction carried out using the method of Folch. The combined organic phases were dried and chromatographed on SG81 paper (Whatman) with ethyl acetate-acetone-acetic acid (90:10:1). The standard PGE₂ and PGF_{2α} were visualised using iodine vapour, the corresponding area cut out and the radioactivity corresponding chromatographically with PGE₂ and PGF_{2α}/6KF_{1α} determined. $n = 3 \pm$ s.d. Open columns, control cultures; hatched cultures exposed to zymosan 50 μ g per culture. Left two columns in each set of four, PGE₂; right two, 6KF_{1α}.

PGD₂ and PGA₂. When, however, such cultures are exposed to an inflammatory agent, zymosan, they synthesise and release large amounts of ³H-PGs in a time-dependent manner. Significant increases in synthesis and release are observed as early as 30 min after addition of zymosan, continuing thereafter for at least 24 h (Fig. 1). In the separation system described in Fig. 1, two major peaks of radioactivity were found. The first of these peaks had the same mobility as authentic PGE₂. This identification is supported also by radioimmunoassay and by its conversion to PGB₂ on base hydrolysis (R.J.B. *et al.*, unpublished). The second of these peaks had the same mobility as PGF_{2α} and 6-keto PGF_{1α} (6KF_{1α}) which are not separable in this system. Using a second chromatographic system, ethyl acetate-acetic acid, 99:1, which separates these two components, the second radioactive peak has, however, been tentatively identified as 6KF_{1α}. ³H-thromboxane B₂ (TXB₂) does not seem to be a major product of ³H-arachidonic acid from unstimulated macrophages exposed to zymosan.

Macrophages from mice stimulated with thioglycollate broth—a sterile inflammatory agent—have a greatly diminished capacity for synthesising and releasing ³H-PGs in response to increasing amounts of zymosan, compared with cells from unstimulated mice, although both cell populations incorporated approximately the same amount of ³H-arachidonic acid (Fig. 2). At the lowest dose of zymosan tested (10 µg per plate), unstimulated cells showed a fivefold increase in ³H-PGE₂ and a 2.5-fold increase in ³H-6KF_{1α} release. At 200 µg ml⁻¹ zymosan, unstimulated cells showed a 24-fold increase in ³H-PGE₂ and a 15-fold increase in ³H-6KF_{1α} release. On the other hand, cells from thioglycollate-stimulated mice showed only a fivefold increase in ³H-PGE₂ and a 2.5-fold increase in ³H-6KF_{1α} release in response to 200 µg zymosan per plate. In the absence of zymosan, both unstimulated and stimulated cells release similar small amounts of ³H-PGE₂ and ³H-6KF_{1α}. Cells from stimulated or unstimulated mice cultured in the absence or presence of zymosan remained intact as judged by morphological examination and the retention of the cytoplasmic marker enzyme, lactate dehydrogenase.

The synthesis and release of ³H-PGs by unstimulated macrophages exposed to zymosan can be completely inhibited by indomethacin (Fig. 3). The effect of this cyclo-oxygenase inhibitor on ³H-PGs synthesis in this system is a potent one, dose-response experiments showing an ID₅₀ of 2×10^{-8} M.

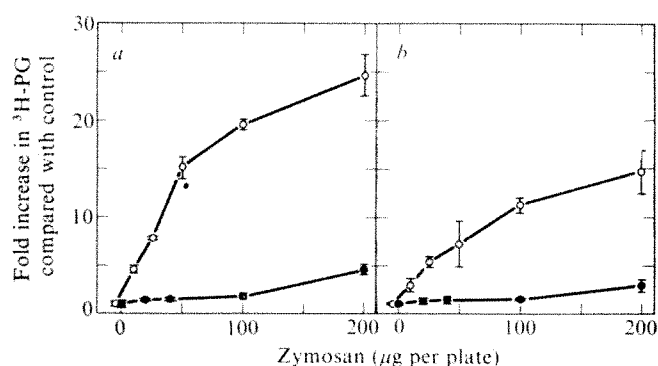


Fig. 2 Thioglycollate-induced macrophages synthesise and release less ³H-PG than unstimulated macrophages in response to zymosan challenge. *a*, PGE₂; *b*, 6KF_{1α}. Unstimulated macrophages and macrophages obtained from mice injected with 2 ml thioglycollate broth (BBL, Cockeysville, Maryland) 4 d previously were cultured and labelled as described in Fig. 1. The radiolabelled cells were then incubated with varying concentrations of zymosan for 4 h. The released radiolabelled PGs were measured as described in Fig. 1. The stimulated cells (●) incorporated 47% of the ³H-arachidonic acid; the unstimulated cells (○) incorporated 57% of the ³H-arachidonic acid. Control cultures of the stimulated cells without zymosan addition contained 365 ± 139 c.p.m. as PGE₂ and 191 ± 12 c.p.m. as 6KF_{1α}; control cultures of the unstimulated cells contained 228 ± 31 c.p.m. as PGE₂ and 102 ± 17 c.p.m. as 6KF_{1α}. $n = 3 \pm$ s.d.

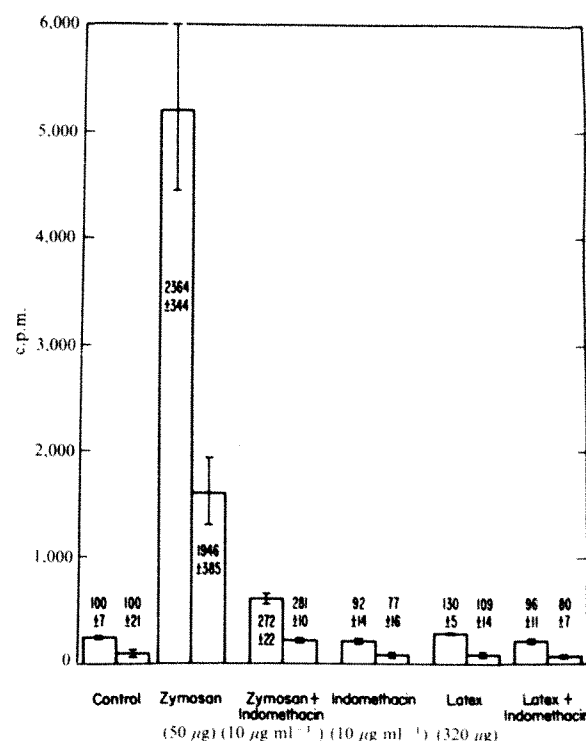


Fig. 3 Zymosan but not latex stimulated ³H-PG synthesis and release from unstimulated macrophages. Unstimulated macrophages were cultured and labelled as described in Fig. 1. The release incubation period was 4 h. Suspensions of latex beads 0.8 µm; (Dow, Indianapolis, Indiana) were sonicated before addition. The numbers in the histogram indicate percentage stimulation over control. $n = 3 \pm$ s.d. Left-hand column in each pair, PGE₂; right-hand, 6KF_{1α}.

PG synthesis does not always accompany phagocytosis by macrophages. Fig. 3 shows that unstimulated macrophages exposed to a large amount of latex particles do not release ³H-PGs above control levels. Examination of cultures by phase contrast microscopy showed that these particles had been phagocytosed. It is noteworthy that latex lacks the ability to induce chronic inflammation¹⁵.

These findings complement those of Glatt and Brune who have used other stimuli to induce release of PGE₂ by mouse peritoneal macrophages¹⁶. In addition, we have unpublished data showing that macrophages release PGs in response to other inflammatory stimuli such as asbestos and antigen-antibody complexes. Clearly, PGs are now established as products of macrophages responding to inflammatory stimuli. The tentative identification 6KF_{1α} as being a major secretory PG by zymosan-induced macrophages remains to be confirmed. Our data, however, suggest that the potent biologically active precursor, PGI₂, which has been shown to be released by cells of the vascular endothelium¹⁷, may also be released by macrophages. The contribution of PGs released by macrophages to the diverse functions of this cell remains to be elucidated.

We thank Dr E. A. Ham for 6KF_{1α} and TXB₂ standards.

J. L. HUMES
R. J. BONNEY
L. PELUS
M. E. DAHLGREN
S. J. SADOWSKI
F. A. KUEHL, JR
P. DAVIES

Merck Institute for Therapeutic Research,
Rahway, New Jersey 07065

Received 8 June; accepted 5 July 1977.

¹ Davies, P. & Allison, A. C. *Immunobiology of the Macrophage* (ed. Nelson, D. S.) 427-461 (Academic, New York, 1976).

- 2 Werb, Z. & Dingle, J. T. *Lysosomes in Biology and Pathology* 5 (eds Dingle, J. T. & Dean, R. T.) 127-156 (North-Holland, Amsterdam, 1976).
- 3 Colten, H. R. & Einstein, L. P., *Transplant. Rev.* 32, 3-11 (1976).
- 4 Bentley, C., Bitter-Suermann, D., Hadding, V. & Brade, V. *Eur. J. Immun.* 6, 393-401 (1976).
- 5 Waksman, B. H. & Namba, Y. *Cell. Immun.* 21, 161-176 (1976).
- 6 Leibovich, S. J. & Ross, R. *Am. J. Path.* 84, 501-513 (1970).
- 7 Lewis, D. M. & Burrell, R. *Br. J. Ind. Med.* 33, 25-28 (1976).
- 8 Myatt, L., Bray, M. A., Gordon, D. A. & Morley, J. *Nature* 257, 227-228 (1975).
- 9 Gordon, D., Bray, M. A. & Morley, J. *Nature* 262, 401-402 (1976).
- 10 Flower, R. J. & Blackwell, J. G. *Biochem. Pharmac.* 25, 285-291 (1976).
- 11 Isakson, P. C., Raz, A. & Needleman, P. *Prostaglandins* 12, 739-748 (1976).
- 12 Hong, S. L. & Levine, L. *Proc. natn. Acad. Sci. U.S.A.* 73, 1730-1734 (1976).
- 13 Bills, T. K., Smith, J. B. & Silver, M. J. *Biochim. biophys. Acta* 424, 303-314 (1976).
- 14 Mason, R. J., Stossel, T. P. & Vaughan, M. *J. clin. Invest.* 51, 2399-2407 (1972).
- 15 Schorlemmer, H.-U., Davies, P., Nylon, W., Gugig, M. & Allison, A. C. *Br. J. exp. Path.* (in the press).
- 16 Glatt, M. & Brune, K. *Proceedings of Future Trends of Inflammation III* (MTP, Lancaster, in the press).
- 17 Moncada, S., Gryglewski, R., Bunting, S. & Vane, J. R. *Nature* 263, 663-665 (1976).

Primary *in vitro* sensitisation of human T cells

METHODS for the *in vitro* development of primary immune responses have been crucial for the understanding of mechanisms of cooperation between T and B cells and between T cells and macrophages¹⁻³. Few efforts to date have been reported for primary *in vitro* sensitisation of human lymphocytes, most of which have involved sensitisation to allo- or xeno-antigens⁴⁻⁶ or plaque-forming responses to sheep red blood cells (SRBC)^{7,8}. Development of killer T cells against HLA-identical⁹, or autochthonous tumour cells^{10,11} have usually required coculture with third party allogeneic cells for development of cytotoxicity. Given the ethical constraints on *in vivo* studies of human T-cell function, and noting the development for the first time of a system for the sensitisation of guinea pig T cells *in vitro* to a variety of antigens¹², we were encouraged to develop an *in vitro* system for the priming and boosting of human T cells to well defined haptenic determinants without the requirement for a third party cell.

We chose trinitrophenol (TNP) as antigen for two reasons: in our hands it elicits the best response; and second, animal studies show that when recognised in conjunction with Ia antigens on macrophages, TNP can elicit helper-type T cells¹², and when recognised simultaneously or as an antigenic complex with H-2 K and D antigen can generate killer T cells¹³. The hapten TNP, therefore, has the potential in the human system to serve as a model for primary *in vitro* sensitisation and recognition of altered self.

Tonsil or peripheral blood cells were divided into two portions (Fig. 1). The responder cell population was purified by nylon wool column fractionation; the stimulator population, unfractionated, was treated with mitomycin C or X-ray irradiation and either conjugated with TNBS (trinitrobenzene sulphonate) or left unconjugated as control. Optimum culture conditions required 5×10^6 responder cells plus $1-5 \times 10^6$ stimulator cells in 2 ml of RPMI-1640 containing 10% AB serum and antibiotics. Cultures were fed twice during the 7-d priming. Boosting was done in microtitre plates on day 7 using autochthonous booster cells prepared exactly as were the stimulator cells. Boosting of peripheral blood cells was done with freshly drawn blood; boosting of tonsil cells was done with cells that had been cryopreserved. Cultures did not require 2-mercaptoethanol or rocking (forward and backing tilting during 37 °C incubation). Responses were measured by incorporation of labelled thymidine (³H-TdR), the virus plaque assay and cytotoxicity.

Figure 2 indicates the kinetics of proliferation in the priming and boosting cultures. Nylon wool purified cells alone showed very little proliferation during culture. The addition of mitomycin-treated TNP-coupled or uncoupled cells caused measurable proliferation between days 3 and 5. Optimum boosting conditions required cells primed for at least six and preferably seven days, when background

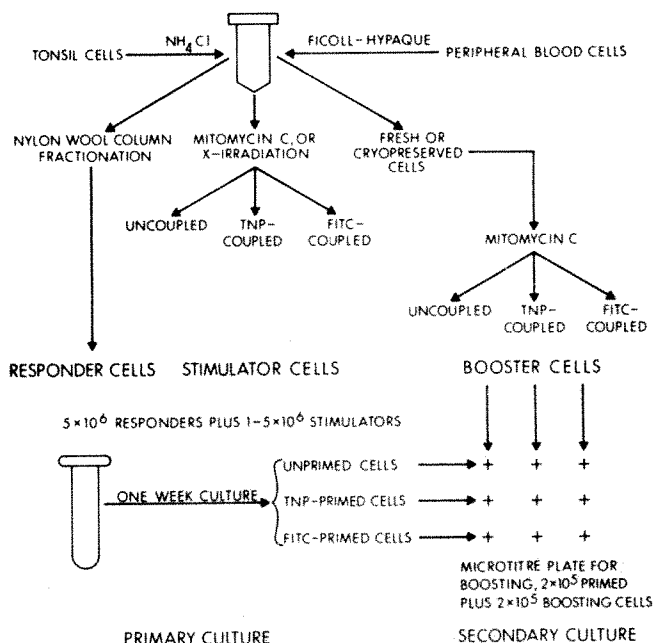


Fig. 1 Schematic representation of the procedure for priming and boosting of T cells to hapten-coupled autochthonous cells. Tonsil cells, after NH_4Cl lysis of red cells or peripheral blood mononuclear cells after Ficoll-Hypaque density centrifugation, were divided into two portions. The first was passed over nylon wool columns for preparation of T cells¹⁴. This gave a population containing fewer than 2%-3% Ig+ cells by immunofluorescence. The second unfractionated population was treated with mitomycin C ($50 \mu\text{g ml}^{-1}$, 37 °C, 30 min) or X-ray irradiation (500 rad), and hapten-conjugated or left unconjugated as control. A portion of tonsil cells was cryopreserved in 10% dimethyl sulfoxide-20% foetal calf serum in RPMI under liquid nitrogen until needed for the secondary (boosting) culture. TNP-coupling with 10-mM TNBS in phosphate-buffered saline (PBS) pH 7.4 was done according to Shearer¹⁵, and FITC-coupling was carried out with $100 \mu\text{g}$ FITC per 10^8 cells per ml in PBS pH 9.0 (ref. 16). Primary cultures were set up in 16×125 -mm tissue culture tubes containing 5×10^6 responder and an equal number of stimulator cells in 2 ml of RPMI 1640 containing 10% AB serum, 8 mg per 100 ml gentamycin, 40 units ml^{-1} mycostatin, $100 \mu\text{g ml}^{-1}$ streptomycin and extra glutamine, 2 mM. Primary cultures were fed twice, on days 2 and 5, and kept in a humidified atmosphere of 5% CO_2 , 95% air. Secondary boosting cultures were set up on day 7 with 2×10^5 viable primed cells plus 2×10^5 boosting cells per 0.2 ml in microtitre plates. Boosting cells were prepared as described for stimulator cells using thawed unfractionated tonsil cells or freshly drawn and prepared peripheral blood mononuclear cells. At 16 h before cell collection $1 \mu\text{Ci}$ ³H-TdR (specific activity 3 Ci mmol^{-1}) was added to each well. Cultures were collected on glass fibre filters, placed in scintillant (NEN 950A) and counted in a Beckman liquid scintillation counter. All determinations were done in triplicate.

proliferation had largely ceased. Boosting of TNP-primed cells with TNP-coupled autochthonous cells resulted in a marked and rapid proliferation of the primed cells between 24 and 96 h after boosting; unprimed cells showed no response (Fig. 2).

The boosted response is antigen specific, as shown in Table 1. T cells from each tonsil were primed to either TNP-cells or FITC-cells. After 1 week both cultures were boosted with both antigens. TNP-primed cells responded to TNP-cells and not to FITC cells, whereas FITC-primed cells responded to FITC cells and not to TNP cells. In 14 experiments, TNP-primed cells gave a mean value above background of $14,765 \pm 3,199$ c.p.m. and a mean stimulation index of 7.0 ± 1.6 when boosted with TNP cells. In five experiments, the response of the FITC-primed cells was always less than the response of TNP-primed cells; the mean incremental ³H-TdR incorporation was $3,743 \pm 2,330$ c.p.m. and the stimulation index was 3.18 ± 1.1 . Ultraviolet light microscopy revealed that the stimulating and boosting cells were coupled with FITC at the

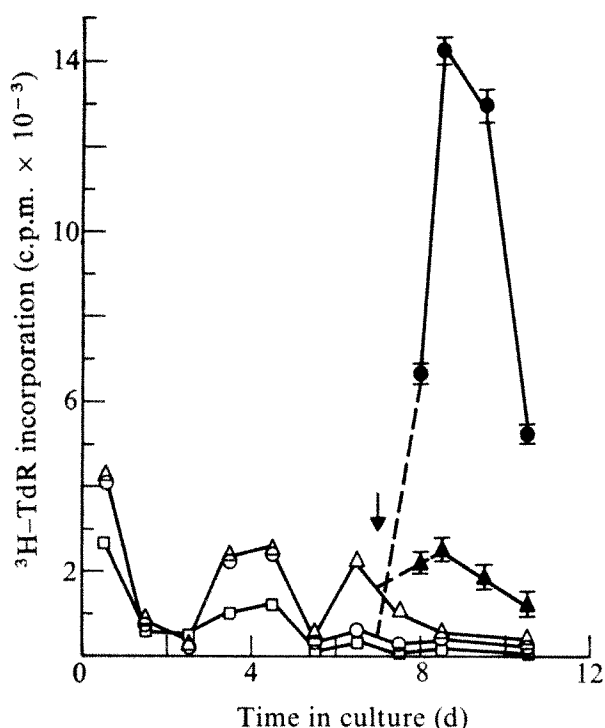


Fig. 2 Time course of incorporation of ^3H -TdR in primary (sensitisation) cultures of 5×10^6 nylon wool cells alone (\square), 5×10^6 nylon wool purified cells plus 5×10^6 uncoupled stimulator cells (control) (\triangle), and after; TNP-boosting (\blacktriangle), and 5×10^6 nylon wool purified cells plus 5×10^6 TNP-coupled autochthonous cells (\circ), and after TNP-boosting (\bullet). The TNP-primed and unprimed cultures were each boosted on day 7 (arrow) with TNP-coupled autochthonous cells, as indicated. S.e.m. is indicated for boosted cultures; for priming cultures, standard errors were always less than 20%.

initiation of culture, and their viability at this time was comparable with that of TNP-coupled cells, that is, greater than 90%.

The response of peripheral blood cells to TNP was usually less than the response given by tonsil T cells (Table 1). In four experiments, three individuals responded (Table 1, and data not shown) while one was completely refractory. Whether this is as a result of individual differences in responder, suppressor or antigen presenting cells types has yet to be determined. To date, we have not been able to achieve a significant *in vitro* response of peripheral blood cells to FITC.

Cultures of T cells plus mitomycin-C treated autochthonous uncoupled cells usually showed a low proliferative response during the priming culture (Fig. 2), as did the T cells cultured together with TNP-coupled cells. When such unprimed control cells were 'boosted'

with uncoupled stimulator cells, the incorporation of ^3H -TdR increased from 2- to 12-fold as compared with their proliferative response when cultured alone (Fig. 2 and Table 1). This seems most consistent with the experiments of Kuntz *et al.*¹⁷, who were able to demonstrate, in humans, a response of purified T cells to autochthonous B and K cells peaking about day 6, though no attempt was made to boost these cells.

The response of primed cells to concanavalin A (con A) was included in all experiments to serve as an internal reference for the relative magnitude of the boost. The response to TNP-cells was in the range of 50% of the con A response. (Table 1).

Viable stimulating cells were required for effective sensitisation. Stimulator cells treated at 56°C for 30 min functioned poorly compared with viable stimulator cells; the response to boosting was only one sixth that of cultures primed with viable cells (data not shown).

We have attempted to estimate the number of antigen-reactive T cells in the boosted cultures using the virus plaque assay¹⁸. Table 2 shows the results of these experiments. The background response was low in all three controls, allowing the detection in specifically boosted cells of approximately one cell per thousand plated. This is a sevenfold increase over primed cells not boosted, and a 50-fold increase over unprimed cells boosted with TNP-cells. These data suggest that the initial number of TNP-reactive cells at day 2 of priming must be relatively low, certainly less than 20 per 10^6 cells. But, one cell in 10^5 in boosted cultures is considerably less than 10–60 per 10^5 cells activated by con A or allogeneic cells^{19,20}, although about 10-fold greater than the number of pre-killer cells activated to TNP in a recently reported murine syngeneic cytotoxicity assay²¹. This may reflect the existence of fewer potentially hapten or self-reactive than allo-reactive clones.

We have attempted to detect a preference on the part of the primed cell for restimulation with TNP coupled to autochthonous cells relative to allogeneic cells. To obviate the problem of allogeneic stimulation, we have taken advantage of the finding that significant stimulation of ^3H -thymidine incorporation can be obtained 24 h after boosting (Fig. 2) when little or no MLR is seen ($\text{SI} < 2.0$). In three experiments, a mean increase of $4,122 \pm 764$ c.p.m. above background was obtained after boosting with TNP-coupled autochthonous (priming) cells, while boosting with TNP-coupled allogeneic cells resulted in a decrease of 27 ± 308 c.p.m. As has been demonstrated in animal models^{22,25}, these results indicate that there is likely to be a restriction on the restimulation of primed T cells to antigen presentation on the priming cell type. Taken together with the requirement for a viable cell for effective antigen presentation, it seems likely that in this system, T cells recognise hapten in conjunction with

Table 1 Response of *in vitro* primed tonsil and peripheral blood T cells to hapten-coupled autochthonous cells

Expt no.	T cells from:	Primed to:	Boosting cell type added, day 3 response (c.p.m.)								Con A
			None	Uncoupled	TNP cells	Δ c.p.m.	SI	FITC cells	Δ c.p.m.	SI	
1	Tonsil	Uncoupled	637 \pm 86*	7,481 \pm 388	9,619 \pm 637	2,138	1.0	12,213 \pm 544	4,732	1.0	19,932 \pm 905
		TNP	868 \pm 174	10,514 \pm 1,094	39,277 \pm 69	28,763	13.5	14,605 \pm 992	4,091	0.9	19,639 \pm 31
		FITC	824 \pm 131	8,424 \pm 585	7,321 \pm 1,552	-103	-0.1	20,924 \pm 1,011	12,500	2.6	17,547 \pm 925
2	Tonsil	Uncoupled	275 \pm 115	246 \pm 38	997 \pm 262	751	1.0	1,165 \pm 245	919	1.0	70,553 \pm 1,856
		TNP	240 \pm 59	262 \pm 50	6,583 \pm 1,472	6,321	8.4	1,251 \pm 249	989	1.1	65,438 \pm 1,717
		FITC	216 \pm 65	243 \pm 49	720 \pm 68	477	0.6	2,907 \pm 907	2,664	2.9	67,096 \pm 2,791
3	Blood	Uncoupled	182 \pm 38	1,334 \pm 204	3,537 \pm 445	2,203	1.0				10,849 \pm 1,269
		TNP	534 \pm 83	2,727 \pm 86	6,187 \pm 953	3,460	1.6				14,809 \pm 1,393
		Uncoupled†	851 \pm 56	2,543 \pm 88	7,024 \pm 265	4,481	1.0				69,296 \pm 1,102
4	Blood	TNP	2,237 \pm 267	8,220 \pm 1,020	33,851 \pm 2,081	25,631	5.7				69,860 \pm 2,100

*Arithmetic means \pm s.e.m.

†Stimulators in this experiment were irradiated with 500 rad.

SI=Stimulation index

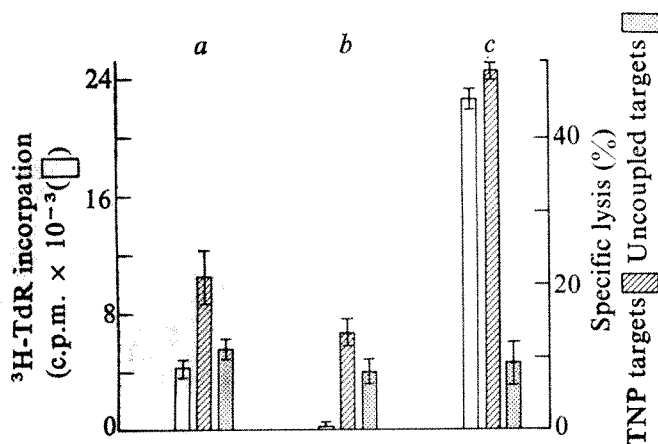


Fig. 3 Virus PFU were determined as for Table 2. *a*, 0.7; *b*, 0.1 and *c*, 1.9 viral plaque-forming cells per 10^5 cells. *a*, TNP-boosted, not primed; *b*, TNP-primed, not boosted; *c*, TNP-primed and boosted. $^3\text{H-TdR}$ incorporation was determined as for Table 1. Cytotoxicity assay was performed in triplicate in round bottom microtitre wells containing 3×10^5 effector cells and 3×10^3 ^{51}Cr labelled targets in 0.2 ml. For preparation of target cells 15 μl of 1:100 diluted phytohaemagglutinin P (DIFCO) was added to 2×10^6 tonsil cells in 2 ml of medium 3 d before assay. On day of assay, targets were labelled with $\text{Na}^{51}\text{CrO}_4$ (Amersham/Searle, 1 mCi ml^{-1}), 250 μCi in 0.4 ml per 15×10^6 cells for 2 h at 37°C . Cells were washed three times and either left uncoupled or coupled with TNBS¹⁵. Effectors plus targets were incubated at 37°C for 16 h after which plates were centrifuged and 0.1 ml was collected and counted in an LKB model 80000 gamma counter. Spontaneous release values for TNP-coupled and uncoupled targets were the same, 47% of total releasable counts as determined by three cycles of freezing and thawing; all assays were performed on the tenth day of culture (day 3 after boosting).

membrane determinants. In the mouse system, Forman has shown that TNP couples directly to H-2 antigens on the cell surface, and that they, most likely, are the site of recognition by prekiller T cells^{26,27}. It will be of obvious interest to determine whether HLA antigens are involved in the recognition of TNP in the present system, and if so, whether there is any consistent restriction like that seen with cytotoxic T cells generated against Y antigen²⁸. On the other hand, the ability of primed T cells to discriminate effectively between TNP and FITC is compatible with the view that some T cells may primarily recognise hapten, a view supported by several studies in experimental animals²⁹⁻³¹.

Table 2 Enumeration of activated T cells primed and boosted *in vitro* by virus plaque assay

Boosting cell	Assay	Tonsil T Cells	
		Unprimed	TNP-primed
Uncoupled cells	$^3\text{H-TdR}$ incorporation	20,624 \pm 1,171	6,503 \pm 875
	V-PFC/ 10^5 Cells	0.045 \pm 0.001	0.15 \pm 0.01
TNP-coupled cells	$^3\text{H-TdR}$ incorporation	12,481 \pm 414	49,153 \pm 1,174
	V-PFC/ 10^5 Cells	0.02 \pm 0.002	1.02 \pm 0.23

Assay for virus plaques was performed 40 h after boosting. Values are arithmetic means \pm s.e.m. V-PFC, virus plaque-forming cells.

Attempts were made to ascertain whether specific cytotoxic T lymphocytes (CTL) could be generated. The data presented in Fig. 3 indicate that CTL capable of killing TNP-coupled targets are generated from naive human T cells after *in vitro* priming and boosting with TNP-coupled autochthonous cells. No CTL were detectable in cultures that were primed but not boosted, or not primed and

'boosted'. In contrast with results obtained in the mouse system¹⁵, CTL were not found in primary cultures, but only in restimulated cultures. It is unclear whether CTL in man are developed on first exposure to antigen but at undetectable levels, or whether a further differentiative step requiring restimulation is necessary. It will also be of interest to clarify the nature of the antigen-presenting cell(s) in this system.

We thank Ethan Shevach for helpful discussions. This work was supported by USPHS grants AI 09807, AI 10702 and a grant from The Jane Coffin Childs Memorial Fund for Medical Research. W. F. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

WALTER NEWMAN
GERALD L. STONER*
BARRY R. BLOOM

Department of Microbiology and Immunology,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received 2 May; accepted 18 July 1977.

*Present address: Armauer Hansen Research Institute, PO Box 1005, Addis Ababa, Ethiopia.

- Waldron, J. A., Horn, R. J. & Rosenthal, A. S. *J. Immun.* **111**, 58-64 (1973).
- Shortman, K. & Palmer, J. *Cell. Immun.* **2**, 399-410 (1971).
- Cheers, C., Breitner, J., Little, M. & Miller, J. F. A. P. *Nature new Biol.* **232**, 248-250 (1971).
- Sheehy, M. J., Sondel, P. M., Bach, M. L., Wank, R. & Bach, F. H. *Science* **188**, 1308-1310 (1975).
- Lindahl, K. F. & Bach, F. H. *J. exp. Med.* **144**, 305-318 (1976).
- Long, M. A., Handwerker, B. S., Amos, B. & Yunis, E. J. *J. Immun.* **117**, 2092-2099 (1976).
- Luzzati, A. L., Taussig, M. J., Meo, T. & Pernis, B. *J. exp. Med.* **144**, 573-585 (1976).
- Dosch, H. & Gelfand, E. W. *J. Immun.* **118**, 302-308 (1977).
- Sondel, P. M., O'Brien, C., Porter, L., Schlossman, S. & Chess, L. *J. Immun.* **117**, 2197-2203 (1976).
- Zarling, J. M., Raich, P. C., McKeough, M. & Bach, F. H. *Nature* **262**, 691-693 (1976).
- Golub, S. H. *Cell. Immun.* **28**, 379-389 (1977).
- Thomas, D. W. & Shevach, E. M. *J. exp. Med.* **144**, 1263-1273 (1976).
- Shearer, G. M., Rehn, T. G. & Garbarino, C. A. *J. exp. Med.* **141**, 1348-1364 (1975).
- Greaves, M. F., Janossy, G. & Curtis, P. in *In Vitro Methods in Cell-Mediated and Tumour Immunity* (eds Bloom, B. R. & David, J. R.) 217-227 (Academic, New York, 1976).
- Shearer, G. M. *Eur. J. Immun.* **4**, 527-533 (1974).
- Starzinski-Powitz, A., Pfizenmaier, K., Röllinghoff, M. & Wagner, H. *Eur. J. Immun.* **6**, 799-805 (1976).
- Kuntz, M. M., Innes, J. B. & Weksler, M. E. *J. exp. Med.* **143**, 1042-1054 (1976).
- Sutcliffe, S., Kadish, A., Stoner, G. & Bloom, B. R. in *In Vitro Methods in Cell-Mediated and Tumour Immunity* (eds Bloom, B. R. & David, J. R.) 319-331 (Academic, New York, 1976).
- Jimenez, L., Bloom, B. R., Blume, M. R. & Oettgen, H. F. *J. exp. Med.* **133**, 740-751 (1971).
- Bloom, B. R. *et al.* *Cold Spring Harb. Symp. quant. Biol.* **41**, 73-83 (1976).
- Lindahl, K. F. & Wilson, D. B. *J. exp. Med.* **145**, 508-522 (1977).
- Pierce, C. W., Kapp, J. A. & Benacerraf, B. *J. exp. Med.* **144**, 371-381 (1976).
- Zinkernagel, R. *Nature* **261**, 139-141 (1976).
- von Boehmer, H. & Haas, W. *Nature* **261**, 141-142 (1976).
- Pfizenmaier, K., Starzinski-Powitz, A., Rodt, H., Röllinghoff, M. & Wagner, H. *J. exp. Med.* **143**, 999-1004 (1976).
- Forman, J., Vitetta, E. S., Hart, D. & Klein, J. *J. Immun.* **118**, 797-802 (1977).
- Forman, J., Vitetta, E. S. & Hart, D. *J. Immun.* **118**, 803-808 (1977).
- Goulmy, E., Termijtelen, A., Bradley, B. A. & van Rood, J. J. *Nature* **266**, 544-545 (1977).
- Alkan, S. S., Williams, E. B., Nitecki, D. E. & Goodman, J. W. *J. exp. Med.* **135**, 1228-1246 (1972).
- Janeway, C. A., Cohen, B. E., Ben-Sasson, S. Z. & Paul, W. E. *J. exp. Med.* **141**, 42-55 (1975).
- Cosenza, H., Julius, M. H. & Augustin, A. A. *Immun. Rev.* **34**, 3-33 (1977).

Genetic non-responsiveness of murine fibroblasts to bacterial endotoxin

LETHAL infections with Gram-negative bacteria are thought to be due in large part to the toxicity of cell wall lipopolysaccharide (endotoxin). The mechanisms of action of lipopolysaccharide (LPS) have recently been clarified by *in vitro* studies which have helped explain many of the pathophysiological sequelae of endotoxin exposure^{1,2}. C3H/HeJ mice, which are genetically resistant to many of the effects of endotoxin, have proved a valuable model for the systematic investigation of endotoxin-mediated phenomena³⁻⁵. Bacterial endotoxins are able to interact

in vitro with a variety of cells and serum factors^{1,2,6} and it is known that LPS acts on murine B lymphocytes as a polyclonal activator for both mitogenesis and immunoglobulin synthesis⁷. This function has been found to be deficient in the C3H/HeJ mouse^{3,4}. Also, endotoxins activate macrophages⁸⁻¹⁰, and this function also seems to be deficient in C3H/HeJ mice⁵ (Ryan, J. L., Glode, L. M. and Nathan, C. F., unpublished observations). To determine if the C3H/HeJ genetic defect in responsiveness to endotoxin is more general, we have investigated the ability of bacterial endotoxin to stimulate C3H/HeJ embryonic fibroblasts. Fibroblasts were chosen because it has recently been shown that they are endotoxin sensitive cells¹¹.

To measure responsiveness of fibroblasts to LPS, we have assayed their rate of glucose utilisation in culture. This has previously been shown to be a sensitive assay for LPS-induced activation of spleen cells and peritoneal cells (Ryan, J. L. *et al.*, unpublished observations). Fibroblasts were isolated from 12-14 d C3H/HeJ and C3H/HeN embryos by removing the head, extremities, and liver, and mincing the remaining tissue with forceps before incubating with BSS and trypsin-EDTA for 10 min each. The tissue was further incubated with trypsin-EDTA for 20 min and the resulting cell suspension filtered through nylon gauze. These filtered cells were cultured at a density of 10^6 per 10 ml in 100-mm plastic tissue culture dishes using Dulbecco's modified MEM with 10% FCS. Cultures were collected with trypsin and transferred to new plates every 5-7 d.

To measure glucose utilisation, 5×10^4 fibroblasts which had been passed at least once were plated in flat-bottomed microtitre plates. This number of embryonic fibroblasts forms almost a complete monolayer in the wells once the cells have spread. Two types of LPS were used to stimulate the cultures. Both were derived from *Escherichia coli* K235, one by phenol extraction¹² and the other by butanol extraction¹³. It has previously been shown that butanol-extracted K235 LPS contains protein tightly bound to the lipid A moiety which activates C3H/HeJ spleen cells. This protein is not present in phenol-extracted K235 LPS which is thus unable to activate C3H/HeJ lymphocytes while having the ability to activate C3H/HeN lymphocytes normally^{14,15}.

The rate of glucose utilisation by C3H/HeJ and C3H/HeN fibroblast cultures stimulated by K235 LPS is shown in Table 1. These data show that glucose utilisation can be used to measure responsiveness of murine fibroblasts to LPS and that C3H/HeJ fibroblasts are not

Table 1 Rate of glucose utilisation by C3H/HeJ and C3H/HeN fibroblast cultures stimulated by K235 LPS

LPS	C3H/HeN (nmol glucose used per 24 h)	C3H/HeJ (nmol glucose used per 24 h)
None	384 ± 8.0	148 ± 2.0
Phenol K235	502 ± 18	144 ± 4.0
Butanol K235	574 ± 32	264 ± 2.0

The glucose content of 10- μ l aliquots of culture supernatant was determined enzymatically during a 2-3-d culture period. Each value represents the total glucose used over an average 24-h period by a culture of 5×10^4 fibroblasts. Values are means \pm s.e.m. of triplicate cultures. The amount of glucose in each aliquot was determined by adding 0.9 ml of the enzyme mixture to each 10- μ l aliquot, mixing vigorously and reading the A_{340} after a 2-min equilibration time. The enzyme mixture was composed of 0.7 ml buffer (0.1 M Tris, 0.064 M NaCl, 3.5 mM MgCl₂, 0.15 mM CaCl₂, pH 7.5) (ref. 21), 0.1 ml 20 mM ATP, 0.1 ml 5 mM NADP and 5 μ l of a mixture of hexokinase and glucose 6-phosphate dehydrogenase (250 U ml⁻¹ hexokinase). The mixed enzymes were obtained from Sigma Chemical (St Louis, Missouri). Both types of LPS were added to a final concentration of 10 μ g ml⁻¹. Cultures were carried out in flat-bottomed microtitre plates using 200 μ l Eagles MEM supplemented with glutamine 1 mM, streptomycin 10 μ g ml⁻¹, penicillin 100 U ml⁻¹, and gentamycin 25 μ g ml⁻¹. LPS was the gift of Dr D. C. Morrison, Scripps Clinic, La Jolla, California.

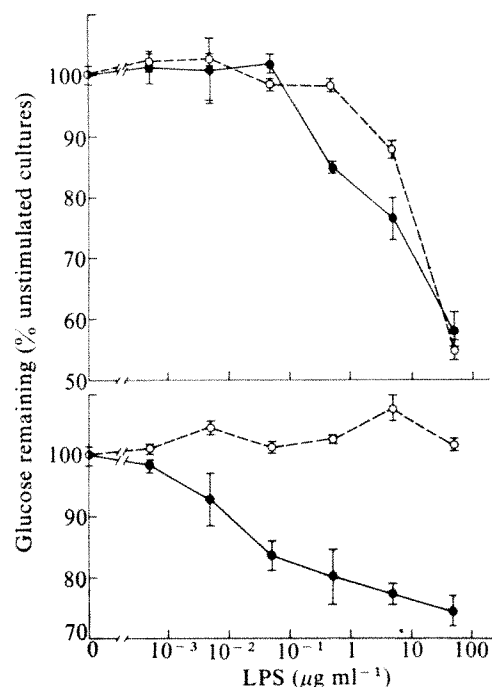


Fig. 1 The upper graph shows the amount of glucose remaining in cultures of C3H/HeN (continuous line) and C3H/HeJ embryonic fibroblasts (dotted line) after stimulation by different concentrations of butanol K235 LPS compared with unstimulated cultures. The lower graph shows similar data for cultures stimulated by phenol K235 LPS. Each point represents the mean of glucose assays from triplicate cultures \pm s.e.m. Culture conditions and glucose assays were as described previously. Measurements on C3H/HeN fibroblasts were made after 32 h of culture, whereas those on C3H/HeJ fibroblasts were made after 66 h of culture, to equate the total glucose utilisation by both cultures at the 50% level. ---, C3H/HeJ; —, C3H/HeN.

responsive to phenol K235 LPS, whereas they are responsive to butanol K235 LPS ($P < 0.01$). In addition, it seems that approximately equal numbers of the C3H/HeJ fibroblasts use glucose at a lower rate than C3H/HeN fibroblasts in both the presence and absence of LPS. The significance of the lower baseline rate of glucose utilisation in C3H/HeJ fibroblasts is not explained, but a similar phenomenon has been observed for C3H/HeJ macrophages (Ryan, J. L. *et al.*, unpublished observations).

A dose-response curve for both types of LPS in C3H/HeJ and C3H/HeN fibroblast cultures is shown in Fig. 1. As seen in the upper graph, both strains respond well to butanol LPS. The lowest dose at which a significant response can be measured is 0.5–5 μ g ml⁻¹ ($P < 0.01$) LPS for the butanol-extracted material. Activation by phenol K235 LPS is shown in the lower graph. In this case the C3H/HeJ fibroblasts are not stimulated at any of the doses tested ($P < 0.001$), whereas the C3H/HeN fibroblasts are stimulated at a dose of 50 μ g ml⁻¹ LPS ($P < 0.01$), which is at least 10-fold lower than the concentration of butanol LPS required for activation. This suggests that the protein present in the butanol LPS may in fact be blocking the activity of the lipid A moiety. Modulation of the biological activities of lipid A by protein associated with lipid A has been suggested¹⁶ and these data seem to corroborate those conclusions.

C3H/HeJ non-responsiveness to LPS has been demonstrated in B lymphocytes^{3,4}, macrophages⁵, and now in embryonic fibroblasts. This finding suggests that there is a global cellular defect in the ability of the C3H/HeJ mouse to respond to highly purified LPS. It has also been shown in many laboratories that C3H/HeJ mice are very resistant to the lethal effects of intravenous LPS having an LD₅₀ of 3,000 μ g, whereas normal mice usually have an LD₅₀ of 200–500 μ g LPS (ref. 17).

We may conclude that activation of LPS-sensitive cells may have a critical role in mediating the lethal effect of intravenous LPS (ref. 17). It must be recognised, however, that this non-responsiveness of C3H/HeJ mice to LPS does not seem to protect them from death due to infection with Gram-negative bacteria¹⁷⁻¹⁸. Thus, it is evident that there are factors other than LPS which contribute to the toxicity of Gram-negative bacteria. The role of cell wall lipoprotein in mediating toxicity is only beginning to be defined²⁰. Studies using LPS non-responder mice should permit a better understanding of the function of non-LPS cell wall material in producing the pathophysiological effects of endotoxin.

JOHN L. RYAN
KEITH P. W. J. MCADAM

Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 29 May; accepted 11 July 1977.

- ¹ Morrison, D. C. & Kline, L. F. *J. Immun.* **118**, 362-368 (1977).
- ² Morrison, D. C. & Cochrane, C. J. *exp. Med.* **140**, 797-811 (1974).
- ³ Sultz, B. M. & Nilsson, B. S. *Nature new Biol.* **240**, 198-200 (1974).
- ⁴ Watson, J. & Riblet, R. M. *J. exp. Med.* **140**, 1147-1161 (1974).
- ⁵ Chedid, L. *et al. Infect. Immun.* **13**, 722-727 (1976).
- ⁶ Kabir, S. & Rosenstreich, D. L. *Infect. Immun.* **15**, 156-164 (1977).
- ⁷ Moller, G., Andersson, J. & Sjöberg, O. *Cell. Immun.* **4**, 416-424 (1972).
- ⁸ Wahl, L. M., Wahl, S. M., Mergenhagen, S. E. & Morton, G. R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3598-3601 (1974).
- ⁹ Weiner, E. & Levanon, D. *Lab. Invest.* **19**, 584-590 (1968).
- ¹⁰ Shilo, M. A. *Rev. Microbiol.* **45**, 255-278 (1959).
- ¹¹ Vaheri, A., Ruoslahti, E., Sarvas, M. & Nurminen, M. *J. exp. Med.* **138**, 1356-1364 (1973).
- ¹² McIntire, F., Sievert, H., Barlow, G., Finley, R. & Lee, A. *Biochemistry* **6**, 2363-2372 (1967).
- ¹³ Morrison, D. C. & Leive, L. *J. biol. Chem.* **250**, 2911-2919 (1975).
- ¹⁴ Morrison, D. C., Betz, S. J. & Jacobs, D. M. *J. exp. Med.* **144**, 840-846 (1976).
- ¹⁵ Sultz, B. M. & Goodman, G. W. *J. exp. Med.* **144**, 821-827 (1976).
- ¹⁶ Betz, S. J. & Morrison, D. C. *Fedn Proc.* **36**, 1234 (1977).
- ¹⁷ Glode, L. M., Mergenhagen, S. E. & Rosenstreich, D. L. *Infect. Immun.* **14**, 626-630 (1976).
- ¹⁸ von Jeney, N., Günther, E. & Jann, K. *Infect. Immun.* **15**, 26-33 (1977).
- ¹⁹ Rowley, D. J. *Infect. Dis.* **123**, 317-327 (1971).
- ²⁰ Wober, W. & Alaupovic, P. *Eur. J. Biochem.* **19**, 357-367 (1971).
- ²¹ Kinsky, S. C., Haxby, J. A., Zopf, P. A., Alving, C. R. & Kinsky, C. B. *Biochemistry* **8**, 4149-4158 (1969).

Density-dependent inhibition of fibroblast growth is overcome by pure mitogenic factors

CELL density is of interest because it may have a critical role in the regulation of growth, development, and in malignant transformation. In cell culture, crowding slows and eventually stops cell proliferation of a variety of cells maintained on a solid surface¹⁻⁵. This property is usually lost after malignant transformation¹⁻⁵. Two major hypotheses have evolved to explain this phenomenon. The contact inhibition hypothesis holds that specific surface receptors are activated by cell-cell contact and generate a negative signal which halts further growth¹⁻³. The humoral hypothesis proposes that high cell density limits the availability of medium components, particularly of growth factors present in serum^{4,5}. Although much experimental evidence has been adduced in support of the humoral hypothesis, most experiments in which the effect of cell density on growth has been thoroughly investigated were performed in the presence of whole serum as the source of growth factors. The chemical complexity of serum leaves open the possibility that some of its effects may be exerted by molecules that interfere with inhibitory cell-cell contacts. Thus, definitive evidence for a humoral hypothesis in growth regulation in culture is still lacking. To circumvent this objection we have examined the effect of cell density in a model system in which chemically defined molecules rather than whole serum stimulate DNA synthesis and cell division in sparse or crowded cells arrested in G₀/G₁. We report here that density dependent inhibition of fibroblast growth is overcome by pure mitogenic factors.

Epidermal growth factor (EGF) and insulin, added at low concentrations dramatically stimulate DNA synthesis in 3T6 cells maintained in serum-free Dulbecco's modified Eagle's medium (DEM) supplemented with vitamin B₁₂ (ref. 6). To establish whether the response of the cells to constant concentrations of EGF and insulin is dependent on the cell density of the culture, cell suspensions were plated at different densities, arrested in G₀ by limitation of serum and then exposed to factors and ³H-thymidine for 26 h (Fig. 1). The labelling index is optimal at a density of approximately 2 × 10⁴ cells cm⁻² but strongly decreases at higher concentrations where extensive cell contacts take place. Figure 1 also shows that EGF and insulin stimulate DNA synthesis at low densities (0.5 × 10⁴ cells cm⁻²) where only 5% of the available surface is occupied by 3T6 cells and very few cells in close contact are observable microscopically. This renders unlikely the possibility that the pure peptides act by diminishing inhibitory cell-cell contacts. Thus the striking dependence of stimulation on cell density raises the possibility of investigating this phenomenon in chemically defined conditions rather than in the presence of serum.

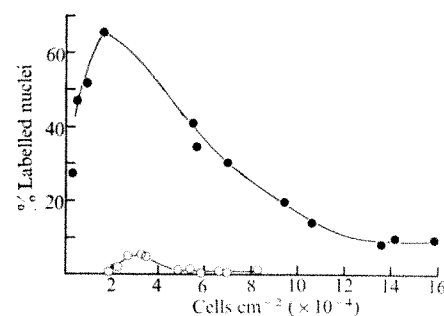


Fig. 1 Effect of cell density on the stimulation of cultures of 3T6 cells into the S-phase by EGF and insulin. Cultures were exposed to 1 ng ml⁻¹ EGF, 100 ng ml⁻¹ insulin, 400 ng ml⁻¹ vitamin B₁₂ and ³H-thymidine (4 µCi ml⁻¹ 0.2 µM) in DEM for 26 h. They were then fixed for autoradiography. Each solid circle represents the mean of two separate determinations (deviation not greater than 5%). Control dishes (B₁₂ containing DEM alone) pooled from a number of experiments are shown by open circles. Stock 3T6 cells were passaged at 3-d intervals in DEM containing 10% foetal calf serum (FCS), 100 U ml⁻¹ penicillin and 100 ng ml⁻¹ streptomycin in a 10% CO₂ atmosphere at 37 °C. In all experiments cell suspensions were plated in Nunc dishes at the appropriate density in 0.5% FCS-DEM, and kept for at least 5 d; they were then washed twice in DEM and left in serum-free medium for 2 d before use. The cell density (abscissa) represents the density at the end of the experiment.

A critical prediction of the humoral hypothesis is that raising the concentrations of the same pure factors that support growth in sparse cultures should overcome the inhibition of growth characteristic of high-density cultures. To test this prediction we determined the dose response for stimulation of DNA synthesis by EGF and insulin in cultures prepared at two different cell densities, sparse and dense (Fig. 2). EGF is more mitogenic in sparse than in dense cultures. Thus EGF, in the absence of insulin stimulates 60% of the cells blocked in G₀ at low density to enter into the S-phase of the cell cycle (Fig. 2a), while it only stimulates 30% of the cells of the denser culture (Fig. 2b). In turn, insulin stimulates DNA synthesis in sparse cultures, even in the absence of EGF and markedly enhances the response to EGF in both sparse and dense cultures. The salient feature of Fig. 2 is that a high concentration of factors is capable of maximally stimulating DNA synthesis in the dense cultures. Essentially similar results were obtained when incubation time was 24 instead of 48 h and also when the total ³H-thymidine incorporated, rather than the labelling index, was measured. The data indicate

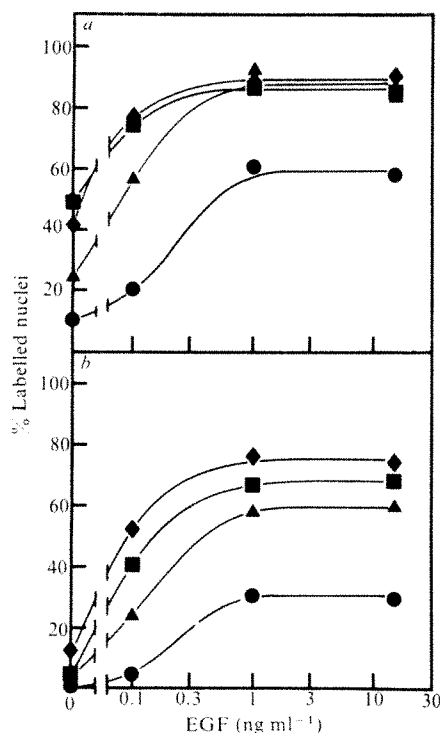


Fig. 2 Dose response to EGF at various insulin concentrations for two different cell densities: *a*, 3.0×10^4 cells cm^{-2} ; *b*, 6.5×10^4 cells cm^{-2} . Insulin concentrations: ●, zero; ▲, $0.1 \mu\text{g ml}^{-1}$; ■, $1 \mu\text{g ml}^{-1}$; ◆, $10 \mu\text{g ml}^{-1}$. Cultures were exposed to mitogens and ^3H -thymidine for 48 h and fixed for autoradiography.

that high levels of factors overcome the density dependent inhibition of growth.

To further substantiate this conclusion we investigated the effect of cell density on the ability of stimulated cells to complete the cell cycle and divide. Cultures were prepared at two different densities (3.3×10^4 and 17.4×10^4 cells cm^{-2}), arrested in G_0 and then exposed to factors at low and high concentrations for 72 h. At the end of the incubation the number of cells per culture was determined. Table 1 shows that both concentrations of factors stimulate an increase in cell number (70%) in sparse cultures. In contrast, only the combination of factors at high concentrations is able to produce a comparable increase in the crowded cultures. This result, together with the autoradiographic data clearly demonstrates that the growth block imposed by high cell density can be overcome by using appropriate levels of pure factors.

We considered the possibility that the ability of high

Table 1 Effect of cell density on growth

EGF (ng ml^{-1})	Insulin ($\mu\text{g ml}^{-1}$)	Cell density (cells $\text{cm}^{-2} \times 10^{-4}$)	
		Sparse	Dense
—	—	2.5	13.3
1	0.2	5.6	14.8
10	5.0	5.4	24.1

Cells were plated at two densities in 50-mm Nunc dishes and arrested in G_0/G_1 before exposure to factors. Cell densities before exposure were 3.3×10^4 and 14.7×10^4 cells cm^{-2} for sparse and dense cells respectively. Two levels of factors were used. After 72 h incubation, cells were removed by trypsinisation and counted with a Coulter counter. In sparse cultures for each determination six dishes were divided into two groups of three pooled dishes. In dense cultures nine dishes were divided into three groups of three pooled dishes. Three aliquots of each group were counted. Data are mean group values. Group duplicates and triplicates did not vary by more than 15% and in general were below 7.5%.

levels of insulin to potentiate the effect of EGF in crowded cultures may be due to a mechanism of action unrelated to its hormonal effect, such as the mild proteolytic activity reported to be associated with the pure hormone and with the B chain of the molecule⁷. We found several lines of evidence that exclude this possibility in our system: (1) The purified B chain of the insulin molecule does not replace the hormone in stimulating the entry of sparse or dense cultures into S. (2) The B chain, added with sufficient amounts of insulin and EGF to stimulate low density cultures, does not overcome the density block in crowded cells. (3) Ovomucoid ($100 \mu\text{g ml}^{-1}$) and soybean ($50 \mu\text{g ml}^{-1}$) trypsin inhibitors which were reported to suppress the proteolytic activity of insulin⁷, do not prevent the enhancing effect of high concentrations of insulin. Furthermore, we verified that the preparation of insulin used in our studies yields a single band (molecular weight 6,400) when analysed in sodium dodecyl sulphate-polyacrylamide gel electrophoresis. In addition zinc, which is present in the insulin preparation and has been shown to promote growth⁸ is completely inactive when tested at 6×10^{-8} M and 6×10^{-7} M in the presence and absence of factors. All these experiments indicate that in crowded cultures as in sparse ones, the action of insulin is related to its metabolic effects mediated by specific surface receptors.

Our results emphasise that chemically defined growth promoting factors used in *in vitro* systems can overcome the restriction of growth imposed by high cell density. These findings have two interesting implications. First, the use of two pure peptides instead of a complex substance like serum for overcoming the block imposed by high cell density removes a major objection in accepting a humoral rather than a contact hypothesis for regulation of growth. The possibility remains that the pure peptides act by diminishing contact inhibition but this seems unlikely because the factors are effective at low cell densities (Fig. 1). Second, the fact that cells transformed by some RNA tumour viruses have an alteration in the EGF growth regulatory mechanisms⁹, that cultures of BHK cells transformed by SV40 viruses produce growth promoting polypeptides¹⁰ and our finding that defined peptides can release cells from density dependent inhibition of growth suggest that growth factors may have a critical role in modulating normal and abnormal cell proliferation.

KARL MIERZEJEWSKI
ENRIQUE ROZENGURT

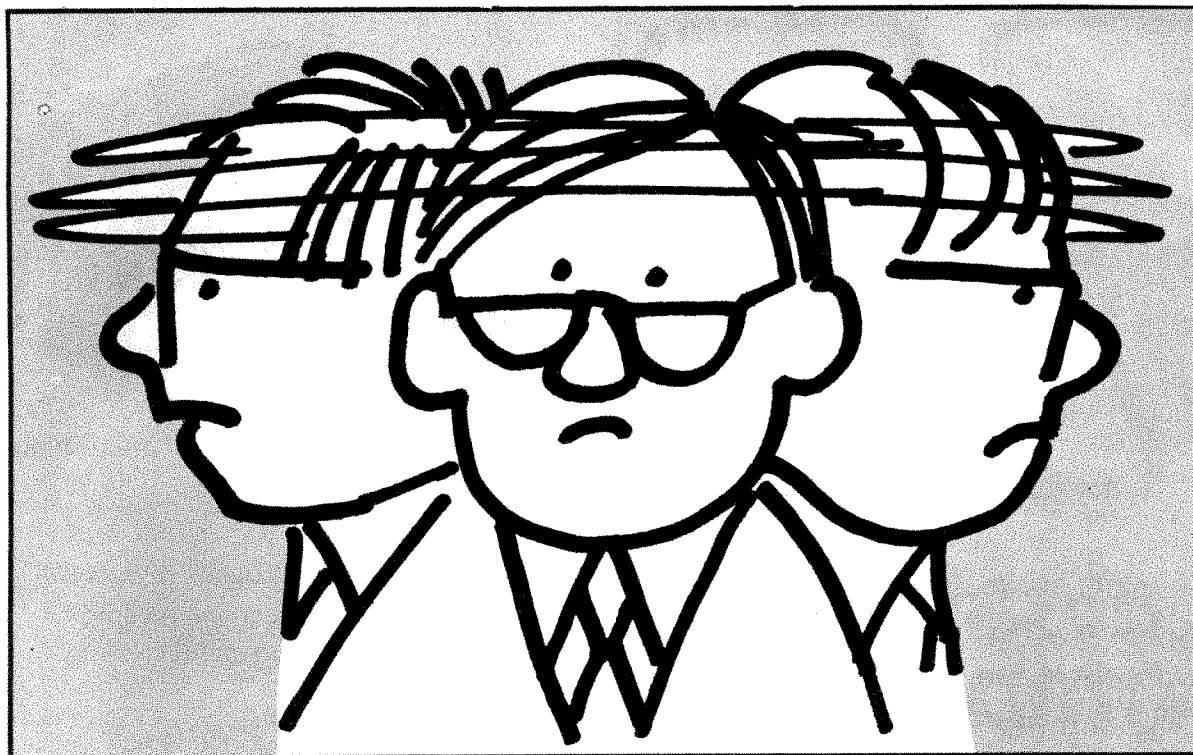
Imperial Cancer Research Fund Laboratories,
Lincoln's Inn Fields,
London, UK

Received 26 April; accepted 12 July 1977.

- ¹ Dulbecco, R. in *Growth Control in Cell Cultures* (eds Wolstenholme, G. E. W. & Knight, J.) 71 (Churchill Livingstone, Edinburgh and London, 1971).
- ² Noonan, K. D. & Burger, M. M. *Prog. Surf. Membr. Sci.* **8**, 245 (1974).
- ³ Westermarck, B. *Biochem. biophys. Res. Commun.* **69**, 304 (1976).
- ⁴ Holley, R. W. *Nature* **258**, 487 (1975).
- ⁵ Rozengurt, E. in *Eukaryotic Cell Function and Growth* (eds Dumont, J. E., Brown, B. L. & Marshall, N. J.) 711 (Plenum, New York, 1976).
- ⁶ Mierzejewski, K. & Rozengurt, E. *Biochem. biophys. Res. Commun.* **73**, 271 (1976).
- ⁷ Huang, D. & Cuatrecasas, P. *J. cell. Biol.* **250**, 8251 (1975).
- ⁸ Rubin, H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 712 (1972).
- ⁹ Todaro, G. J. & De Larco, J. E. *Nature* **264**, 26 (1976).
- ¹⁰ Bourne, H. R. & Rozengurt, E. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4555 (1976).

Cultured human breast cancer cells lose selectivity in direct intercellular communication

VARIOUS small molecules, up to 1,600 molecular weight¹, can pass directly between certain animal cells in contact, probably through gap junctions^{2,3}. Such communication



When you're studying cyclic nucleotides and you don't know which way to turn

We understand that when you are working in the cyclic nucleotide area, the subject is so diverse and so complex that you don't want to have to worry about the problems of accurate measurement.

So we would like to take a little weight off your mind and offer you our two kits in this field.

Cyclic AMP and Cyclic GMP.

Both of the tests are simple, accurate and will take you about four hours from start to finish.

So it's not surprising that you can carry on with your research and leave the problems of accurate measurement to us.

You know where you are with our Cyclic AMP & GMP kits

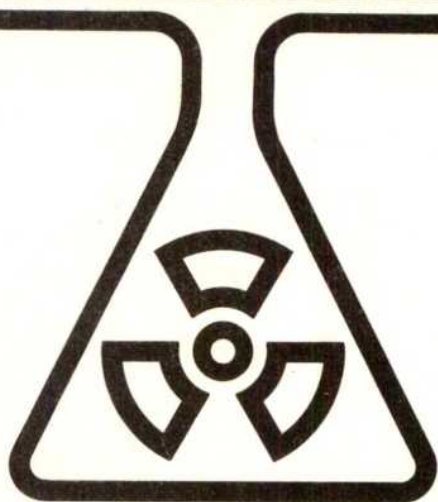


**The Radiochemical Centre
Amersham**

Circle No. 03 on Reader Enquiry Card.

Full information is available on request.
The Radiochemical Centre Limited, Amersham, England. Telephone: 024-04-4444
In the Americas: Amersham Searle Corp. Illinois 60005. Telephone: 312-593-6300
In W. Germany: Amersham Buchler GmbH & Co KG, Braunschweig. Telephone: 05307-4693-97

0880



Latest new compounds

D-Ala ² -[tyrosyl]-3,5- ³ H] enkephalin (5-L-methioninamide) TRK.564	15-30Ci/mmol
[G- ³ H] Kainic acid TRK.566	2-10Ci/mmol
[methyl-1', 2'] Thymidine TRK.565	70-100Ci/mmol
[11, 12(n)- ³ H] Chenodeoxycholic acid TRK.562	25-50Ci/mmol
[11, 12(n)- ³ H] Lithocholic acid TRK.560	25-50Ci/mmol
17 α -Hydroxy-11-deoxy [1, 2- ³ H] corticosterone TRK.557	40-60Ci/mmol

Please enquire for further details



The Radiochemical Centre Amersham

The Radiochemical Centre Limited, Amersham, England.
Tel: Little Chalfont (024 04) 4444.

In the Americas: Amersham Corporation, Illinois 60005.
Tel: 312-593-6300.

In W. Germany: Amersham Buchler GmbH & Co, KG,
Braunschweig. Tel: 05307-4693-97.

1393/23

Now Available DIAZEPAM [³H] SPIROPERIDOL [³H] High specific activity

Ligand for neurochemical brain studies:

Diazepam, [methyl-³H]-

30-60Ci/mmol

Ethanol.

NET-564 \$100/250 μ Ci \$250/1mCi

Ligand for the dopamine receptor:

Spiroperidol, [1-phenyl-³H(N)]-

20-40Ci/mmol

Ethanol, in dry ice.

NET-565 \$100/250 μ Ci \$250/1mCi

Not for use in humans or clinical diagnosis

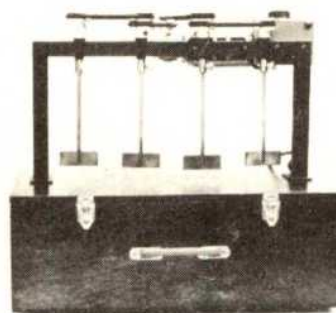
NEN New England Nuclear

549 Albany Street, Boston, Mass. 02118
Customer Service 617-482-9595

NEN Chemicals GmbH, Dreieich, W. Germany; NEN Canada Ltd., Lachine, Quebec

Circle No. 06 on Reader Enquiry Card.

NEW VOSS PORTABLE FLOCCULATOR



Type: FLO/POR 4-bank in portable case	£125
FLO/1 6-bank with illuminated base	£115
FLO/1/NL not illuminated	£97
excl. Vat, ex Works	

VOSS INSTRUMENTS LTD
FARADAY WORKS ★ Maldon ★ Essex CM9 7EY
Phone (0621) 53517

Circle No. 05 on Reader Enquiry Card.

may partly mediate growth control in multicellular organisms and cultured cells, so that disturbances in the normal pattern of communication could be related to malignant growth^{4,5}. The ³H-nucleotide transfer method⁶, based on metabolic cooperation⁷ has shown that communication is not always an all-or-none phenomenon, but may be selective^{8,9}. For example, human mammary epithelial cells from benign tumours or milk do not communicate with human breast fibroblasts (HumF), although either type can communicate well with homologous cells. We have examined the communication between cancer cells from several human breast cancer sources and found that, unlike normal

mammary epithelium, they do not show selectivity in communication. The cancer cells fall into one or other of the two functional groups we have defined as non-selective communicators (communicate with all cells capable of communication) or non-communicators (unable to communicate with homologous or heterologous cells) suggesting that the change to malignancy involves some change in the pattern of cell communication.

The breast cancer cells were either established lines derived from malignant pleural effusions, or primary cultures obtained by the 'spillage' technique¹⁰, from surgical specimens¹¹. Two established lines, MCF-7 (ref. 12) and MDA-157 (ref. 13), and three primary cultures, T162, T231 and T235, were examined. The putative cancer cells which are obtained from primary and metastatic carcinomas by the spillage method, we have called HumE¹ (ref. 11). These cells have a low labelling index (less than 2% in 48 h), but metabolise and can incorporate 5-³H-uridine into their nucleotide pool, and hence may be used as donors.

The transfer of 5-³H-uridine nucleotides from prelabelled donor cells was tested using as recipients representatives of each functional group, that is, non-communicators (L929), non-selective communicators (calf lens), and selective communicators (HumF) unable to communicate with normal HumE cells. Transfer of nucleotide was quantitated by grain counts from autoradiographs as described in Fig. 1, which summarises the results and presents data obtained previously using HumE cells as donors. The cell line MCF-7 and cells from the primary carcinoma T231 show a complete loss of ability to communicate with any of the recipients tested. Cell line MDA-157 and cells from T162 and T235, however, show a loss of selectivity in communication and donate nucleotide not only to calf lens but also to human mammary fibroblasts. Further experiments (data not shown) with the two established lines confirmed that MCF-7 did not communicate with itself whereas MDA-157 retained this ability.

The main problem in interpreting this data is that HumE¹ cells from primary carcinomas have not been fully characterised as the actual cancer cells. They have, however, been shown to be epithelial in ultrastructure¹², and are the only cells other than human mammary epithelium (HumE), and human mammary fibroblasts (HumF), that have been obtained from primary or secondary breast cancers in this laboratory. HumE¹ cells have never been derived from non-malignant breast tissue or breast secretions, and it is therefore most likely that they do represent the malignant cells of the carcinoma.

This being so, the data presented here show that malignant change may be associated not only with a loss of ability to communicate, but also with a loss of specificity in communication. Other workers have shown that whereas some cancer cells show a reduced ability to communicate directly with other cells^{4,5}, some are good communicators¹⁴. Our results suggest that in both cases a change in the pattern of communication might have occurred. Such changes could be selectively advantageous for the metastasis of cancer cells, enabling them as non-communicators to ignore inhibitory signals from the other epithelial cells, or, as non-selective communicators to receive stimulatory messages from abnormal sources such as fibroblasts.

We thank Dr M. G. P. Stoker for his valuable comments.

IAN S. FENTIMAN
JOYCE TAYLOR-PAPADIMITRIOU

Imperial Cancer Research Fund,
Lincoln's Inn Fields,
London, UK

Received 27 May; accepted 19 July 1977.

¹ Simpson, I., Rose, B. & Loewenstein, W. R. *Science* **195**, 294 (1977).

⁴ Gilula, N. B., Reeves, O. R. & Steinbach, A. *Nature* **235**, 262 (1972).

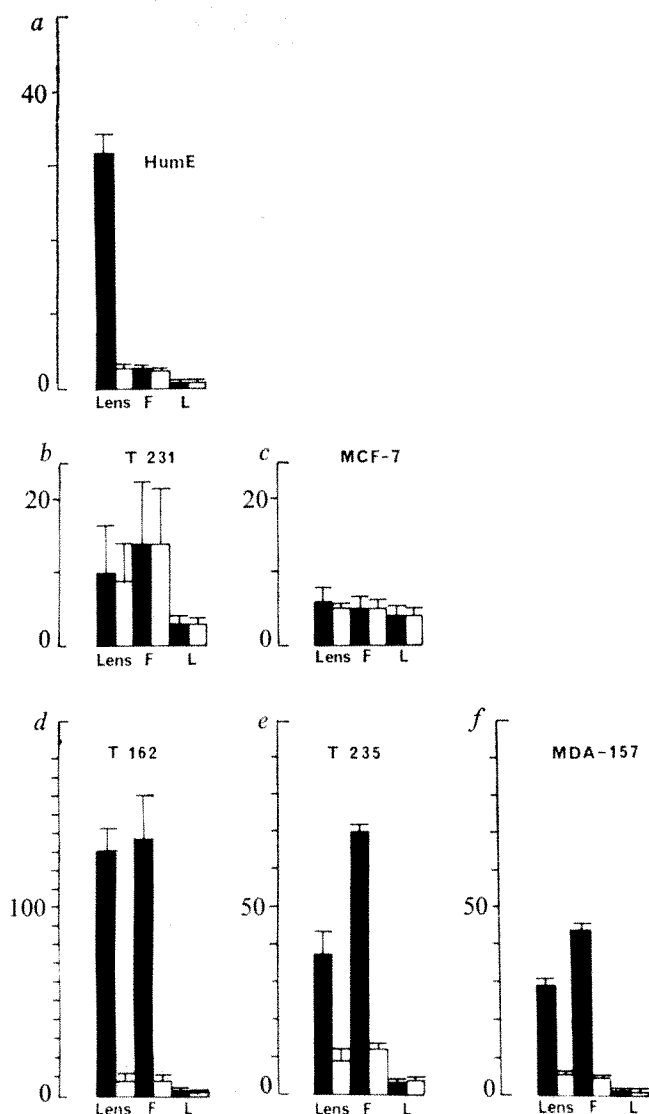


Fig. 1 Grain counts from autoradiographs, showing mean \pm s.e. Closed blocks, recipients touching donors. Open blocks, non-touching recipients. Donor cells were seeded at approximately 6×10^3 cells per 35-mm dish and recipients at 2.5×10^3 per 50-mm dish in the relevant medium (L cells: Dulbecco's modified Eagle's medium with 10% foetal calf serum (FCS); MDA-157 and calf lens: medium 199 with 10% FCS; HumE (ref. 1) and HumF: medium 199 with 20% human serum, 15% FCS, insulin $10 \mu\text{g ml}^{-1}$, hydrocortisone $5 \mu\text{g ml}^{-1}$). After overnight incubation at 37°C , $2 \mu\text{Ci ml}^{-1}$ 5-³H-uridine was added to the donors for 3 h. The cells were then washed three times with medium, and recipient cells, which had just been detached by trypsinisation, were added in medium (199 with 20% human serum, 15% FCS, insulin and hydrocortisone). The donors and recipients were cocultured for 3 h at 37°C , fixed and processed for autoradiography⁸. In each experiment five donor dishes were used and tested with calf lens (twice), HumF (twice) and L cells (once). Experiments were repeated three times and on each dish, grain counts were made over ten recipients touching donors, and over ten recipients touching neither donors, nor other recipients. a, HumE; b, T231; c, MCF-7; d, T162; e, T235; f, MDA-157.

- ³ Azarnia, R., Larsen, W. J. & Loewenstein, W. R. *Proc. natn. Acad. Sci. U.S.A.* 71, 880 (1974).
⁴ Loewenstein, W. R. & Kanno, Y. *J. Cell Biol.* 22, 565 (1964).
⁵ Borek, C., Higashino, S. & Loewenstein, W. R. *J. membr. Biol.* 1, 274 (1969).
⁶ Pitts, J. D. & Simms, J. W. *Expl Cell Res.* 104, 153 (1977).
⁷ Subak-Sharpe, J., Burk, R. R. & Pitts, J. D. *J. cell. Sci.* 4, 353 (1969).
⁸ Fentiman, I. S., Taylor-Papadimitriou, J. & Stoker, M. *Nature* 264, 760 (1976).
⁹ Pitts, J. D. & Burk, R. R. *Nature* 264, 762 (1976).
¹⁰ Lasfargues, E. Y. & Ozzello, L. *J. natn. Cancer Inst.* 21, 1131 (1958).
¹¹ Hallows, R. C., Millis, R., Stoker, M. & Taylor-Papadimitriou, J. *clin. Oncol.* 3, 81 (1977).
¹² Rose, H. N. & McGrath, C. M. *Science* 190, 673 (1975).
¹³ Young, R. K., Cailleau, R. M., Mackay, B. & Reeves, W. J. *In vitro* 9, 239 (1974).
¹⁴ Sheridan, J. D. *J. Cell Biol.* 45, 91 (1970).

Growing central axons deprived of normal target neurones by neonatal X-ray irradiation still terminate in a precisely laminated fashion

THE development of the central nervous system is characterised by the formation of specific synaptic connections. Highly selective connections form not only between classes of neurones, but also between specific neurones within classes. An additional degree of specificity is observed when the presynaptic axons contact only a limited portion of the target neurone. This kind of specificity is conspicuous in the dentate gyrus (fascia dentata) of the mammalian cerebral cortex. We describe here a situation in which the dentate gyrus offers a unique opportunity to test some of the hypotheses that may explain the selective termination of different afferents on restricted portions of the surface of a neurone.

The most numerous neurones in the dentate gyrus are the granule cells, whose cell bodies are gathered in a dense layer from which the dendrites radiate towards the surface of the brain through the molecular layer (Fig. 1a). The predominant afferent fibre systems that converge on the granule cells are spatially segregated, with little or no overlap, in three distinct zones across the dendritic trees. The outer zone receives fibres from the lateral entorhinal cortex of the temporal lobe, the middle zone is innervated by the medial entorhinal cortex, and the inner zone receives afferents from the hilus fasciae dentatae of both hemispheres¹⁻³ (Fig. 1a).

The majority of granule cells develop postnatally, whereas the sources of their major afferents are generated before birth⁴. Proliferating granule cell precursors are, unlike differentiating or mature neurones, extremely radiosensitive. This allows selective reduction of the granule cell population, while the afferent supplies would be expected to remain intact⁵. The granule cells arise in a temporal sequence from lateral to medial along the curvature of their layer⁴⁻⁶ and irradiation that reduces the number of cells in the lateral limb of the U-shaped granule cell layer totally eliminates the greater part of the medial limb⁵.

We have studied the fate of the axons that normally terminate in a highly selective laminar fashion in the molecular layer of the medial limb of the dentate gyrus when this target is removed by neonatal X-ray irradiation. Rat pups were shielded with lead, except for a field above the hippocampus and the dentate gyrus, and exposed to X rays from a 250-kV source at a distance of 34 cm. At a rate of 400 min⁻¹, 200 R was delivered on postnatal days 2, 3, 5 and 7. A comparable schedule has been shown to irreversibly reduce the number of granule cells to about 20%⁵. The animals were allowed to live for 2-4.5 months when selective lesions were made in the medial or lateral entorhinal cortex or the commissural and association pathways to the dentate gyrus. After a 3-d survival period the animals were killed and histological sections through the brains made and impregnated with silver to show degenerating axon terminals⁷ or treated according to a histochemical method for heavy metals⁸ which in normal brains precisely displays the terminal

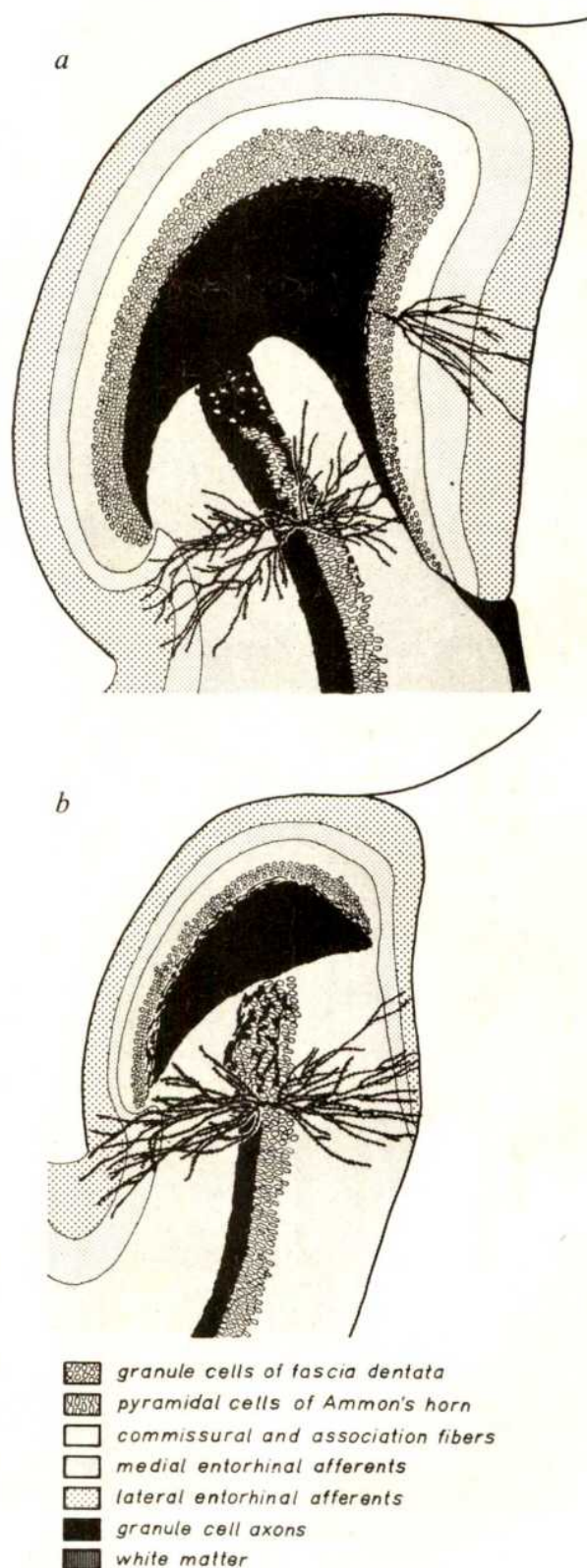


Fig. 1 Schematic drawings of sections through the dentate gyrus and adjacent part of Ammon's horn from a, a normal and b, a neonatally X-ray irradiated rat. The terminal zones of the converging fibre pathways are shown by different shadings. Three cells drawn from Golgi preparations are plotted on the diagrams. Directions: medial—right, anterior—bottom. $\times 50$.

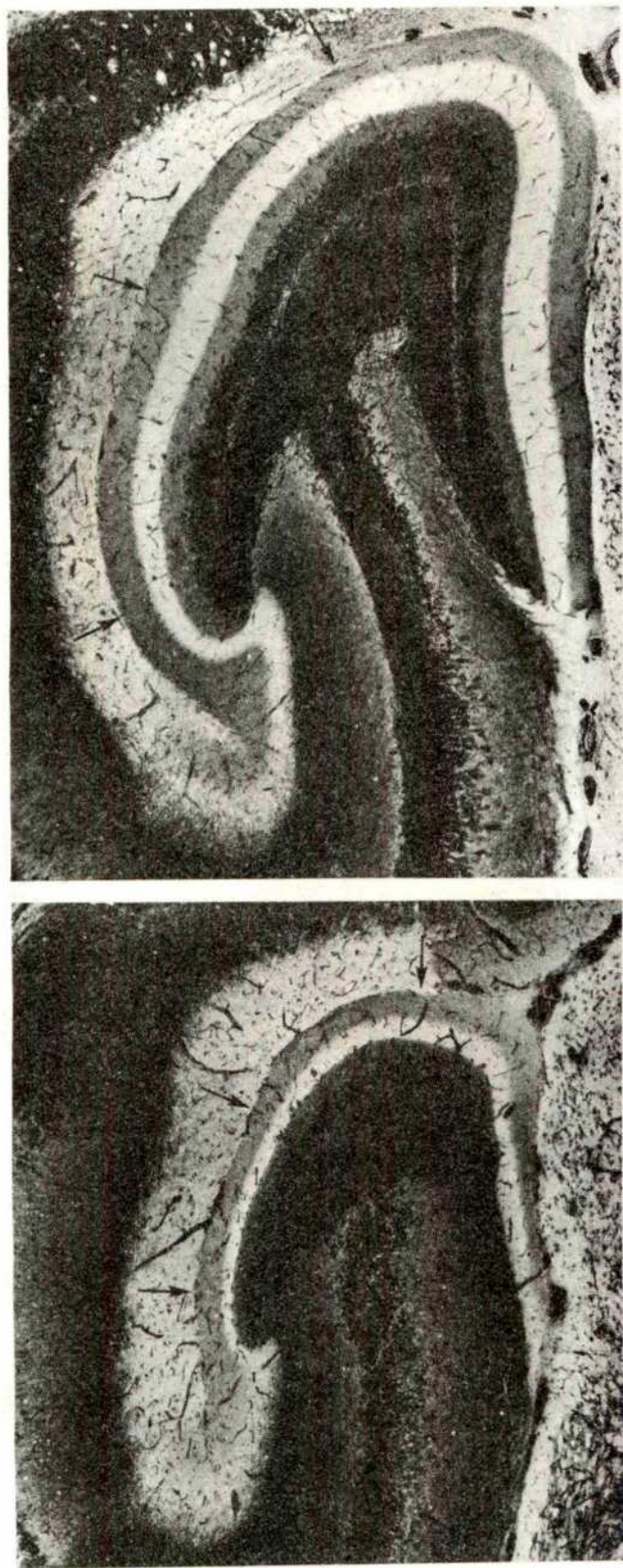


Fig. 2 Section through the dentate gyrus of a normal (top) and a neonatally X-ray irradiated (bottom) rat brain. The sections were stained with a histochemical method for heavy metals⁸ and with thionine, they display in different shades the terminal zones of the afferent pathways also shown in Fig. 1. Arrows point to the hippocampal fissure. $\times 45$.

zones of the three major systems of afferents to the dentate gyrus (Fig. 2).

In the irradiated animals, a histochemically distinct stratification is found not only in the molecular layer above the surviving granule cells but also medially where granule cells are absent (Figs 1, 2). The deep zone in this part is normally delimited by the granule cell layer; however, in the irradiated animals it merges with the stratum oriens beneath the pyramidal cell layer of the Ammon's horn. The degeneration material shows that each histochemically defined zone corresponds to an equally distinct terminal zone of one of the three major systems of afferents to the dentate gyrus. Degeneration in the inner zone is illustrated as an example (Fig. 3). Sections prepared according to the Golgi method, which impregnates single neurones, show that the medially located terminal zones of the entorhinal projections in the absence of granule cells are penetrated by basal dendrites of pyramidal cells of the adjacent Ammon's horn (Fig. 1b). These clearly elongated dendrites normally do not penetrate the terminal zones of the entorhinal fibres (Fig. 1a).

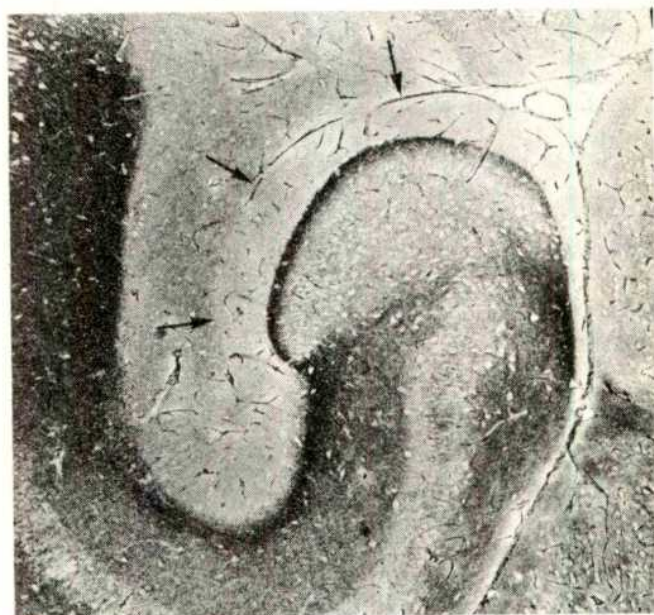


Fig. 3 Section through the dentate gyrus of a neonatally X-ray irradiated rat brain showing terminal degeneration of commissural and association fibres (compare Figs 1, 2). Fink-Heimer silver impregnation. Arrows point to the hippocampal fissure. $\times 45$.

These findings indicate that the laminar distribution of the terminal fields of the pathways converging on the dentate gyrus can exist without the developing axons selectively recognising particular coded portions of the normal target cell surface. This principle is similar to that based on observations made on the laminar distribution of afferents to the piriform cortex and to the dentate gyrus in the reeler mutant mouse. In both locations in the reeler, the termination is relatively independent of the location of the abnormally located target neurones^{9,10,17}.

It has been suggested that the complementary ordering of entorhinal afferents and commissural and ipsilateral association fibres to the molecular layer might be accounted for by a sequential arrival of the two groups of fibres¹¹. The earliest stage examined so far is that of the third postnatal day. At this time, the afferents from the medial and lateral entorhinal cortices and the ipsilateral association fibres have already arrived at the molecular layer and attained the same topographic relationship to each other as they do in mature brains. But, the

commissural projection to the deep zone arrives several days later and terminates in the same field as the ipsilateral association fibres. This illustrates that a sequential arrival of different classes of afferents does not necessarily result in a spatial segregation of their terminal ramification¹².

The abnormal growth of the basal dendrites of pyramidal cells close to the dentate gyrus is probably not a direct effect of radiation, since it has been shown that low-level X-ray irradiation of other parts of the cerebral cortex of immature rats results in dendrites that are shorter than normal¹³. The remarkable extension of the dendrites seen in this study is not understood. It may occur because the dentate gyrus normally hinders further growth and has been removed or, more likely, because the afferents deprived of normal recipients induce the abnormal growth. There are several examples suggesting that dendritic growth is influenced by afferent fibres¹⁴. The preparations described here suggest that neurones may adapt the size of their receptive surface in response to the amount of available afferents. While a reduction in dendritic size, on removal of afferents, has been described^{15,16}, this preparation indicates that the adaptation may also be in the nature of an increase in size.

This work was supported by the Danish MRC, the Danish Cancer Society and Den laegevidenskabelige Forskningsfond i Nordjyllands Amtskommune. We thank I. M. Jensen, K. Kirkholt, B. Krunderup, A. Meier, T. Nielsen and M. Sørensen for valuable technical assistance, and M. Hjelm-Hansen for assistance with the radiation procedure and dosimetry.

SØREN LAURBERG
ANDERS HJORTH-SIMONSEN

Institute of Anatomy, University of Aarhus and
Institute of Cancer Research, Radiumstationen,
DK-8000 Aarhus C, Denmark

Received 29 April; accepted 14 July 1977.

- 1 Steward, O. J. *comp. Neurol.* **167**, 285-314 (1976).
- 2 Hjorth-Simonsen, A. *J. comp. Neurol.* **146**, 219-232 (1972).
- 3 Hjorth-Simonsen, A. & Laurberg, S. *J. comp. Neurol.* **174**, 591-606 (1977).
- 4 Angevine, J. B., Jr *Expl. Neurol. suppl.* **2**, 1-70 (1965).
- 5 Bayer, S. A. & Altman, J. *J. comp. Neurol.* **163**, 1-20 (1975).
- 6 Schlessinger, A. R., Cowan, W. M. & Gottlieb, D. I. *J. comp. Neurol.* **159**, 149-176 (1975).
- 7 Hjorth-Simonsen, A. *Stain Technol.* **45**, 199-204 (1970).
- 8 Haug, F.-M. S. *Adv. Anat. Embryol. Cell Biol.* **47**, 1-71 (1973).
- 9 Devor, M., Caviness, V. S., Jr & Derer, P. J. *comp. Neurol.* **164**, 471-482 (1975).
- 10 Stanfield, B. B., Caviness, V. S., Jr & Cowan, W. M. *Neurosci. Abst.* **1**, 1190 (1975).
- 11 Loy, R., Lynch, G. & Cotman, C. W. *Brain Res.* **121**, 229-243 (1977).
- 12 Fricke, R. & Cowan, W. M. *J. comp. Neurol.* **173**, 231-250 (1977).
- 13 Hicks, S. P. & D'Amato, J. in *Advances in Teratology* (ed. Woollam, D. H. M.) 195-250 (Logos, London, 1966).
- 14 Cotman, C. W. & Banker, G. A. in *Reviews of Neuroscience* (eds Ehrenpreis, S. & Kopin, I. J.) 1-62 (Raven, New York, 1974).
- 15 Bradley, P. & Berry, M. *Brain Res.* **109**, 133-151 (1976).
- 16 Jones, W. H. & Thomas, D. B. *J. Anat.* **96**, 375-381 (1962).
- 17 Stirling, V. R. & Bliss, T. V. P. *Brain Res.* (in the press).

Corollary discharge to cockroach giant interneurons

A PROBLEM faced by animals as they move about is to distinguish between sensory input which is generated as a direct consequence of the animals' own movements (called refference¹), and that generated by independent external events. One way an animal might handle refference would be to use an approximate copy of motor output (corollary discharge²) to repress or otherwise compensate for it. Cockroaches usually react to gentle puffs of air by rapidly running away, the so-called 'escape response'. Since they do not respond similarly to air currents set up by their own walking (even though the receptors involved are very sensitive to slight air movements from any direction³), they must have some mechanism for recognising and ignoring refferent input. The escape response had originally been thought to be initiated by the activity of the large interneurons known as

giant fibres⁴. This view has been disputed^{5,6}, however, and the function of the giants is at present unclear. I report here evidence that some of these giants are directly excited during walking by neural input which may represent corollary discharge from motor centres in the thorax, and which may aid the insect in handling refferent input.

Neural activity in the ventral nerve cord may be monitored during walking in a cockroach by suspending and eviscerating the insect, and recording with suction electrodes while the animal walks on a lightweight styrofoam ball, rotating this ball beneath itself⁷. In these conditions, several units become active (Fig. 1a). These units, identified as giant fibres on the basis of their large amplitudes and high conduction velocities^{4,7}, are considerably more active during walking (or in response to stimuli such as air puffs), than when the animal is quiet and inactive (Fig. 1b). That the activity of these neurones is not an artefact of the experimental arrangement is suggested by the finding that a similar pattern, that is, activity of giants during walking, near-silence during rest, may also be seen in animals which can move about freely (Fig. 1c, d).

The GFs receive input in the last abdominal ganglion from primary afferents arising at the bases of wind-sensitive hairs on the cerci, a pair of small, peg-like appendages at the end of the abdomen. Since I have observed that all action potentials in the giants during walking travel toward the head, the most obvious explanation for the observed activity is refference through the cerci; that is, excitation of the cercal afferents by air currents set up by the animal's own movements. Removal of the cerci seemed to support this idea, because it caused a marked reduction of neural activity

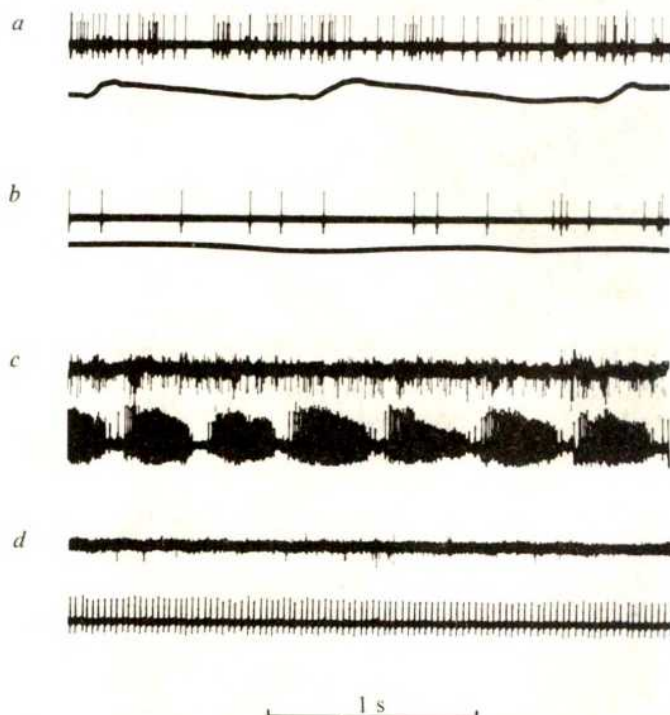


Fig. 1 Records of neural activity (top traces) in the ventral nerve cord of a single cockroach during walking (a, c) and at rest (b, d). a, b, Giant fibre activity in a suspended animal holding a styrofoam ball. The lower trace shows a monitor of the movement of one rear leg. Downward deflection represents extension. Spontaneous non-giant neural activity present in the nerve cord is hidden in the baseline and not visible in these records due to the low level of amplification used to record from the giant fibres. c, d, Activity in a free-walking animal. The lower trace shows the activity of the main femoral extensor muscle of one leg. Bursts of activity in this muscle indicate that the animal is walking (c). The muscle has a steady, low rate of firing when the cockroach is standing still (d). Note that very much higher levels of firing occur in the ventral cord during walking than at rest.

in the ventral nerve cord during walking. (It does not have any noticeable effect on the walking itself.) I have observed, however, that in intact animals the cercal nerves are never silent, even in a quiet, still room. It seems likely, therefore, that some of this continuous barrage of input from the cerci may provide subthreshold depolarisation to the recipient giant fibres such that any additional input would be enough to fire them. If the background input were removed, the level of depolarisation of the giants caused by other inputs might then be insufficient for the giant fibres to show much activation during walking.

I tested experimentally the hypothesis that there are non-cercal inputs driving the giants. First, I set up an eviscerated, suspended animal with a styrofoam ball as described above⁷. I then arranged a rubber hose over the abdomen of the cockroach so that a continuous stream of air could be blown posteriorly over the cerci. If the giant fibre activity which could be recorded during walking were only, or largely, the consequence of air currents set up by the animal's own movements, then recordings of ventral nerve cord activity during walking in the air stream should show no difference from similar recordings from quiet animals in the air stream. On the other hand, if refference from the cerci due to stimulation by air currents were not the main source of the activity, one would expect to see an increase in activity during walking even in the presence of the air stream. The latter turned out to be the case.

show no difference from similar recordings from quiet cockroach there is an initial burst of activity in the giant fibres in the ventral nerve cord which settles in to fairly high-frequency firing for a minute or more (Fig. 2*a*). If the velocity of the air stream is high enough this activity will soon die away (even though the air is still on) (Fig. 2*b*). In the stream of air, even vigorous puffs of air directed at the cerci from only a few centimetres away elicit no responses from the giant fibres (Fig. 2*c*), suggesting that in this situation the animal cannot generate air currents capable of eliciting any giant fibre response. Nevertheless, when the animal walks, one still observes the strong activity of the GFs also seen in animals not in an air stream (Fig. 2*d*). This activity disappears when the animal is again quiet (Fig. 2*e*).

While these results show that the giant fibre activity observed during walking is not brought about by stimulation of hair receptors by air currents, they do not eliminate the possibility that other receptors on the cerci or elsewhere could be stimulated by walking and thereby excite the giants. Cricket giant fibres, for example, are known to be very responsive to vibrational stimuli⁸. To test the possibility that refference from vibration-sensitive receptors in the posterior of the abdomen was responsible for giant fibre activity during walking, I arranged an animal so that the posterior third of its abdomen was supported by a small platform fastened to a wooden framework resting on the floor. The rest of the animal was supported at the thorax by a holder resting on the bench. By completely severing the abdomen, except for the nerve cord, between the platform and the other holder, it was possible to isolate mechanically the posterior part of the abdomen from the rest of the animal. Tests showed that vibrational stimuli which before the operation easily caused some of the giants to fire failed to do so after the operation. Nevertheless, giant fibre activity concomitant with walking was still apparent. To eliminate the effects of air currents in this situation, I also placed animals treated like this in an air stream, or coated their cerci with petroleum jelly. In no case was the giant fibre activity elicited by walking eliminated.

I conclude from these experiments that refference from cercal or other receptors during walking is not sufficient to account for the giant fibre activity which can be observed. Instead, it seems that the animal actively turns on some of the giants, by sending excitatory signals through small fibres down to the last abdominal ganglion from the thorax or

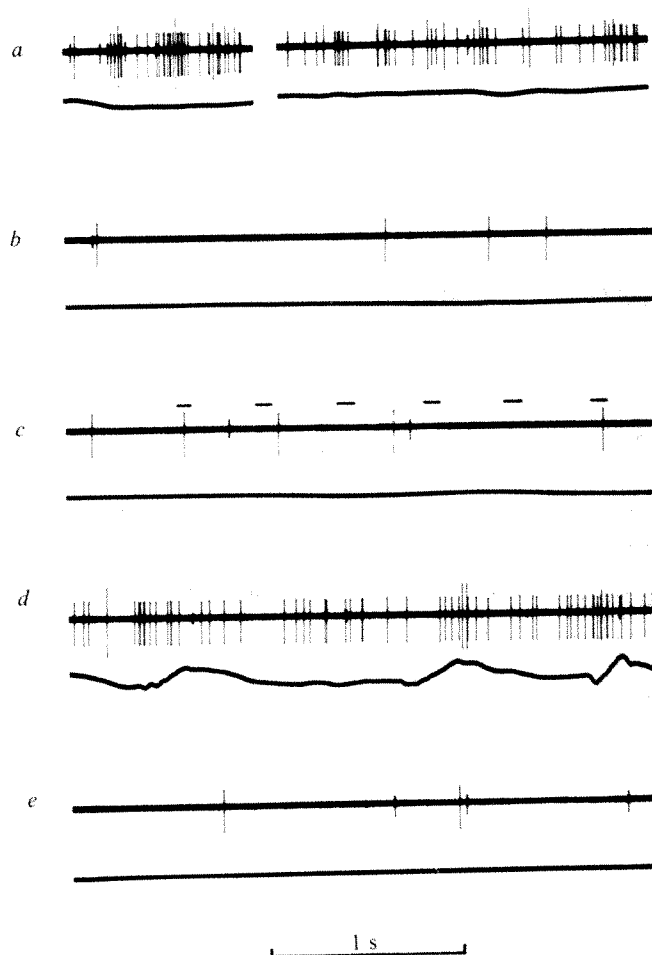


Fig 2. Records of neural activity in a suspended cockroach in the presence of a continuous, tailward stream of air. Upper traces, ventral nerve cord record; lower traces, leg movement monitor (down is extension). *a*, Onset of the air stream (left) and activity 1 min later (right). *b*, Activity after several minutes. *c*, Activity during delivery of puffs of air (bars above the record) at the cerci. The lack of response to the puffs indicates that in the presence of the air stream, additional air currents are ineffective in eliciting giant fibre activity. *d*, Activity during walking, in the air stream. *e*, Activity during rest, still in the presence of the air stream.

higher centres. It should be possible to test this hypothesis by cutting the ventral nerve cord in the abdomen. Receptors posterior to the lesion would continue to provide their stimulatory input during walking, but the descending excitation would be eliminated, thereby eliminating or drastically reducing giant fibre activity during walking.

This experiment was performed. A cockroach was prepared as before and recordings taken from two positions on the same side of the ventral nerve cord, one just anterior to the last abdominal ganglion, and one at about the middle of the abdomen. After recording the neural activity at both sites during walking (Fig. 3*a*), the entire nerve cord was severed between the two recording electrodes. The effect of this procedure was a dramatic reduction in giant fibre activity recorded at the posterior electrode during walking (Fig. 3*b*). Cutting the nerve cord did not seem to change the responsiveness of the giants to cercal stimulation since gentle puffs of air still elicited vigorous firing in the intact segments of the giants posterior to the cut. The result therefore clearly supports the hypothesis that descending excitation to the giants, which accompanies motor activity in the legs, is the main factor in bringing about the activity during walking.

Compensating for refference can be a special problem for an animal which has sensitive sensory systems which can



Fig. 3 Records of neural activity in a suspended cockroach before (a) and after (b) cutting the ventral nerve cord between the two recording electrodes. Top trace, recording from anterior-most electrode; bottom trace, recording from posterior-most electrode; middle trace, leg movement monitor (down is extension). Note the complete abolition of giant fibre activity in the anterior record (because the headward-moving spikes in the giant fibres cannot pass the cut) and the drastic reduction of activity in the posterior record after the ventral cord was cut.

be stimulated by the animal's own movements and whose activity can trigger evasive behaviour. Some fish and amphibians with lateral line systems handle the problem by suppressing the activity of the sensory systems, either centrally or peripherally^{9,10}. Insects may use this mechanism as well. The activity of some of the giant fibres can be inhibited during walking in the cricket¹¹ and in the cockroach (D. L. Daley, personal communication).

The results I report here show, however, that in cockroaches there is also a population of giants which is excited during walking. Since the excitation seems to derive from thoracic or higher centres and parallels movements of the legs, it can be considered to represent corollary discharge from the thoracic locomotor control centres. Excitation of some of the giant fibres by this corollary discharge presumably would have a role in allowing the animal to respond more quickly to escape-producing stimuli while it is walking, but at present even this possibility is only speculation, and the mechanism by which it would function is completely unknown.

D. L. Daley and B. J. Mendius made useful comments on the manuscript. The work was supported by a Bio-Medical Science Support Grant and USNIH grant NS12142.

FRED DELCOMYN

Department of Entomology and
Program in Neural and Behavioral Biology,
University of Illinois,
Urbana, Illinois 61801

Received 9 May; accepted 11 July 1977.

- ¹ von Holst, E. & Mittlestaedt, H. *Naturwissenschaften* 37, 464–476 (1950).
- ² Sperry, R. W. *J. comp. physiol. Psychol.* 43, 482–489 (1950).
- ³ Nicklaus, R. Z. *vergl. Physiol.* 50, 331–362 (1965).
- ⁴ Roeder, K. D. *J. exp. Zool.* 108, 243–261 (1948).
- ⁵ Dagan, D. & Parnas, I. *J. exp. Biol.* 52, 313–324 (1970).
- ⁶ Harris, C. L. *Comp. Biochem. Physiol.* 56A, 333–335 (1977).
- ⁷ Delcomyn, F. J. *Insect Physiol.* 22, 1223–1227 (1976).
- ⁸ Edwards, J. S. & Palka, J. *Proc. R. Soc. B* 185, 83–103 (1974).
- ⁹ Russell, I. J. *J. exp. Biol.* 54, 621–641 (1971).
- ¹⁰ Russell, I. J. *J. comp. Physiol.* 111, 334–358 (1976).
- ¹¹ Murphey, R. K. & Palka, J. *Nature* 248, 249–251 (1974).

Regulation of adenylate cyclase activity in glial–adrenal hybrid cells

SOMATIC cell hybrids, formed by fusion of hormone-responsive and insensitive cells, have been used to evaluate the structure and genetic regulation of the hormone-sensitive adenylate cyclase system^{1–6}. In the cell hybrids studied so far, catecholamine-sensitive adenylate cyclase activity was lost when catecholamine-responsive and insensitive cells were fused. Prostaglandin E₁

(PGE₁)-sensitive adenylate cyclase activity, on the other hand, was retained in hybrids between PGE₁-sensitive and insensitive cells. Where examined, the presence or the absence of hormone sensitivity in the hybrids closely correlated with hormone receptor activity as measured by ligand-binding assays^{1–3}. These observations suggest that genetic mechanisms may control the expression of hormone responsiveness at the level of the hormone receptor. To examine these patterns of regulation further, we have formed cell hybrids between rat glial tumour cells with adenylate cyclase activity responsive to β -adrenoceptor agonists⁷ and mouse adrenocortical tumour cells with adrenocorticotrophic hormone (ACTH)-sensitive adenylate cyclase activity⁸. Besides having distinct functional hormone receptors, the adenylate cyclases of parental cells exhibit marked quantitative differences in response to fluoride ion^{7,8}. We find that the glial–adrenal hybrids retain the adrenaline-sensitive adenylate cyclase activity characteristic of the glial parent, and lose ACTH sensitivity. The level of the fluoride response, however, is characteristic of the adrenal parent. These data indicate that hormone receptor and catalytic unit activities in the adenylate cyclase system are independently regulated.

The clones used in the cell hybridisation experiments presented here are listed and described in Table 1. C6 cells were from a rat glial tumour cell line¹⁰; Y1 and OS3 adrenocortical cells were from mouse adrenocortical tumour cell lines^{11,12}. The isolation and characterisation of C6 cells deficient in thymidine kinase activity (TK[−]) has been described previously¹³. The isolation of Y1, OS3 and C6 clones deficient in hypoxanthine–guanosine phosphoribosyl transferase activity (HGPRT[−]) was accomplished by mutagenesis with ethyl methanesulphonate 300 μ g ml^{−1} and selection with 6-thioguanine 5 μ g ml^{−1}. The HGPRT[−] parents had less than 5% of the HGPRT activity¹⁴ of controls and did not form colonies when 10⁷ cells were plated in the presence of amethopterin¹⁵. Cell hybrids were prepared by fusing 10⁶ HGPRT[−] adrenal cells in monolayers with 10⁴ TK[−] glial cells, using chemically inactivated Sendai virus (Connaught Laboratories) as described by Watkins¹⁶. After fusion, hybrids were selectively grown in medium containing hypoxanthine, thymidine and amethopterin^{13,17}. The hybrid nature of the cells isolated in selective growth conditions was confirmed by analysis of karyotypes¹⁸. Cells isolated after fusing Y1_{HGPRT[−]} or OS3_{HGPRT[−]} cells with C6_{TK[−]} cells were polyploid with slightly fewer than 14 metacentric chromosomes denoting the contribution from one glial and one adrenal parent (Table 1).

The adenylate cyclase systems from glial and adrenal parental cells were readily distinguished by their responses to hormones and to fluoride. In the C6 cell lines, adenylate cyclase activity was increased by adrenaline, whereas in the Y1 cell line, the enzyme was stimulated by ACTH (Table 1). High levels of fluoride-stimulated enzyme activity (approximately 200 pmol cyclic AMP formed per min per mg protein) seemed to be a characteristic feature of the mouse adrenocortical tumour cells^{8,9,19}, and also was observed in the OS3 adrenal line, an ACTH-insensitive, Y1 cell variant (for example, Table 1). These levels of fluoride-stimulated adenylate cyclase activity were \geq threefold those seen in C6 cells (ref. 7), in TK[−] and HGPRT[−] subclones (Table 1), or in C6_{TK[−]} \times C6_{HGPRT[−]} hybrids (Table 1).

Hormone sensitivity in C6_{TK[−]} \times Y1_{HGPRT[−]} hybrids resembled that of the C6_{TK[−]} glial cell parent. Adenylate cyclase activity was stimulated by adrenaline, but not by ACTH (Table 1). In the presence of fluoride, adenylate cyclase activity was \geq 200 pmol cyclic AMP formed per min per mg protein, characteristic of the Y1_{HGPRT[−]} adrenal parent (Table 1). These patterns of expression were observed consistently in 11 different C6_{TK[−]} \times Y1_{HGPRT[−]} hybrid populations, in four C6_{TK[−]} \times OS3_{HGPRT[−]} hybrids, and also in clonal isolates from each (Table 1).

The retention of adrenaline sensitivity (β^+) and loss of ACTH sensitivity (ACTH[−]) in the adenylate cyclase system of glial (β^+ , ACTH[−])–adrenal (ACTH^{+, β^-) hybrids, suggest the presence of regulatory influences distinct from those reported previously^{1–4}. The high level of fluoride-stimulated adenylate cyclase activity observed in the glial–adrenal hybrids but not in glial–glial hybrids (Table 1) indicate the contributions of adrenal components}

Table 1 Properties of parental clones and hybrids

	Cell line	Chromosome number		Adenylate cyclase activity				n
		Total	Metacentric	Basal	Adrenaline	ACTH	NaF	
Parents	C6 _{TK} ⁻	35	14	7 ± 2	94 ± 1	ND	66 ± 6	4
	C6 _{HGPRT} ⁻	42	14	10 ± 1	81 ± 5	ND	61 ± 7	4
	Y1 _{HGPRT} ⁻	40	0	6 ± 1	ND	40 ± 3	217 ± 23	5
	OS3 _{HGPRT} ⁻	42	0	2	2	2	190	1
Hybrids								
	C6 _{TK} ⁻ × Y1 _{HGPRT} ⁻	78 (72-89)	12	13 ± 3	66 ± 16	14 ± 3	263 ± 31	11
	C6 _{TK} ⁻ cloned	83	ND	26	199	24	424	1
	C6 _{TK} ⁻ × OS3 _{HGPRT} ⁻	82 (73-88)	11	5 ± 3	60 ± 8	4 ± 2	231 ± 55	4
	C6 _{TK} ⁻ cloned	88 (79-95)	9	6	79	6	437	1
	C6 _{TK} ⁻ × C6 _{HGPRT} ⁻	72	ND	6 ± 1	43 ± 8	6 ± 1	59 ± 7	5

Chromosome numbers for parental lines are modal numbers based on scores from at least 50 spreads. Values for hybrids are either modal numbers or averages with the range given in parenthesis.

Adenylate cyclase activity is given as pmol cyclic AMP formed per min per mg protein ± s.e.m. The number of determinations is given under *n*. Adrenaline 33×10^{-5} M; ACTH $1-24 \times 10^{-5}$ M; and NaF 15×10^{-3} M were added where indicated. C6 cells are insensitive to ACTH and Y1 cells are insensitive to adrenaline^{7,9}. Activity was assayed in cell homogenates by conversion of labelled ATP to labelled cyclic AMP^{7,8}.

ND, Not determined.

The total hybrid population surviving each selection was analysed. Data were accumulated from the *n* hybrid preparations indicated in the last column.

determining the catalytic activity of the adenylate cyclase system. Glial components of the fluoride response may be present but masked. Membrane fusion experiments of Orly and Schramm²⁰ suggest that a receptor from one cell should be able to couple functionally with the adenylate cyclase from another cell. These observations raise the interesting possibility that the adenylate cyclase system in the glial-adrenal hybrids is a mixture of components from each parent.

We thank Drs J. Logothetopoulos and C. J. Ingles for discussions, Mrs Deborah Brymer for assistance and Dr W. Rittel (Ciba-Geigy) and Dr H. Strade (Organon) for gifts of ACTH₁₋₂₄. This work was supported by the MRC of Canada.

BERNARD P. SCHIMMER
JENNIVINE TSAO
NGAI HOI CHEUNG

Banting and Best Department of
Medical Research,
University of Toronto,
112 College Street,
Toronto, Ontario M5G 1L6, Canada

Received 23 May; accepted 27 June 1977.

¹ Gilman, A. G. & Minna, J. D. *J. Biol. Chem.* **248**, 6610-6617 (1973).

² Minna, J. D. & Gilman, A. G. *J. Biol. Chem.* **248**, 6618-6625 (1973).

³ Brunton, L. L., Maguire, M. E., Anderson, H. J. & Gilman, A. G. *J. Biol. Chem.* **252**, 1293-1302 (1977).

⁴ Hamprecht, B. & Schultz, J. *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1633-1641 (1973).

⁵ Ayad, S. R. & Foster, S. J. *Cell* **3**, 135-140 (1974).

⁶ Sharma, S. K., Nirenberg, M. & Klee, W. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 590-594 (1975).

⁷ Schimmer, B. P. *Biochim. biophys. Acta* **252**, 567-573 (1971).

⁸ Schimmer, B. P. *J. Biol. Chem.* **247**, 3134-3138 (1972).

⁹ Taunton, O. D., Roth, J. & Pastan, I. *J. Biol. Chem.* **244**, 247-253 (1969).

¹⁰ Benda, P., Lightbody, J., Sato, G., Levine, L. & Sweet, W. *Science* **161**, 370-371 (1968).

¹¹ Yasumura, Y., Buonassisi, V. & Sato, G. H. *Cancer Res.* **26**, 529-535 (1966).

¹² Schimmer, B. P. *J. Cell Physiol.* **74**, 115-122 (1969).

¹³ Schimmer, B. P., Stevenson, L. F., ter Hofstede, C., Cheung, N. H. & Marks, A. *Exptl Cell Res.* **86**, 425-428 (1974).

¹⁴ Harris, H. & Cook, P. R. *J. Cell Sci.* **5**, 121-134 (1969).

¹⁵ Littlefield, J. W. *Proc. natn. Acad. Sci. U.S.A.* **50**, 568-576 (1963).

¹⁶ Watkins, J. in *Methods in Virology* 5 (eds Maramorosch, K. & Koprowski, H.) 1-32 (Academic, New York, 1971).

¹⁷ Littlefield, J. W. *Science* **145**, 709-710 (1964).

¹⁸ Rothfels, K. H. & Siminovich, L. *Stain Tech.* **33**, 73-77 (1958).

¹⁹ Schimmer, B. P. *Nature* **259**, 482-483 (1976).

²⁰ Orly, J. & Schramm, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4410-4414 (1976).

tricyclic antidepressant drugs may also have this activity. To test this hypothesis, four tricyclic antidepressants—representing four different structural types—were tested on the H₂ receptor linked to adenylate cyclase in homogenates of the guinea pig hippocampus and cortex. Both dimaprit and histamine were used as agonists. Dimaprit has potent activity at the H₂ receptor with negligible activity at the H₁ receptor, that is, less than 0.0001% of that of histamine².

Each experiment was done on fresh preparations of hippocampus and cortex from adult guinea pigs. The tissues were homogenised in a Potter-Elvehjem glass-Teflon vessel in 50 volumes of a medium containing 0.32 M sucrose, 5 mM Tris-chloride, 1 mM EGTA, and 1 mM dithiothreitol, pH 7.4. The method for measuring adenylate cyclase activity has been described³. The assay system contained 75 mM Tris-chloride (pH 7.4), 1 mM ATP, 2 mM MgCl₂, 1 mM cyclic AMP, 5 mM phosphocreatine, 0.5 mg ml⁻¹ creatine kinase (ATP creatine phosphotransferase, EC 2.7.3.2) 4 mM theophylline, 10⁻⁵ M GTP, 0.1 M sucrose, 0.3 mM EGTA, 0.3 mM dithiothreitol, and enzyme protein (150-190 μg) in a final volume of 225 μl. All assays were performed in triplicate. All additions were made to the assay tubes on ice. They were then transferred to a 30 °C shaking incubator and preincubated for 5 min to allow the enzymatic activity to reach a steady rate and to eliminate the influence of any lag periods in hormone activation. After the preincubation period, 25 μl α-³²P-ATP (1-2 Ci) were added and the reaction was allowed to proceed for 13 min when it was stopped by adding 100 μl of 1% sodium dodecylsulphate. After addition of 650 μl of ³H-cyclic AMP (5,000-10,000 c.m.p.) to monitor recovery, the labelled cyclic AMP was isolated with alumina and Dowex AG-50W (ref. 4). The reaction was linear with protein concentration⁵ in the range used and for at least 15 min after the addition of the α-³²P-ATP. Curve fitting techniques⁶ with the PROPHET (ref. 7) computer system were used to estimate the apparent ED₅₀ (50% effective dose) values, maximum stimulation by agonists, and parallelism of the dose-response curves. Antagonism was analysed by means of Schild plots^{8,9}.

All four tricyclic antidepressants shifted the dose-response curve of histamine- and dimaprit-stimulated adenylate cyclase in a parallel manner. Figure 1 shows histamine-stimulated adenylate cyclase activity in the absence and presence of amitriptyline. The competitive nature of antagonism by amitriptyline and imipramine was further revealed by Schild plots. As shown for amitriptyline (Fig. 2) the slope of the Schild plot, with either histamine or dimaprit as agonist, is 1.02, which is not significantly

Tricyclic antidepressant drugs block histamine H₂ receptor in brain

THE observation that cyproheptadine is a competitive antagonist of the histamine H₂ receptor linked to adenylate cyclase in brain¹ suggested that the chemically similar

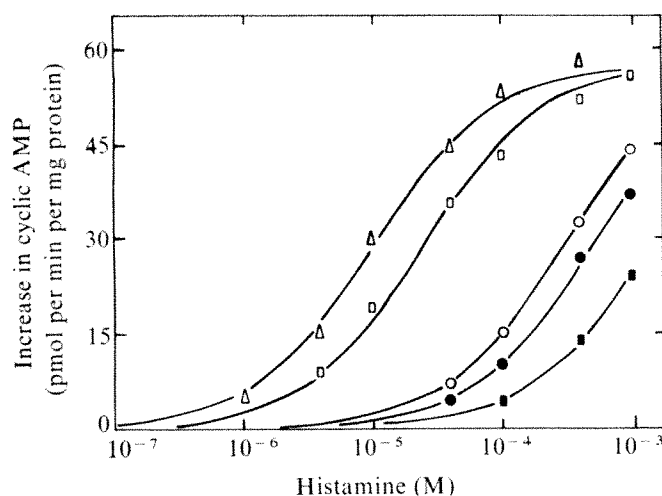


Fig. 1 Increase in adenylate cyclase activity of the guinea pig hippocampus in response to varying concentrations of histamine in the absence and presence of different concentrations of amitriptyline. Each point is the mean of triplicate determinations on a single enzyme preparation. Amitriptyline alone did not affect basal activity. Control, Δ ; amitriptyline at 4×10^{-7} M, \square ; 10^{-6} M, \circ ; 4×10^{-6} M, \bullet ; and 10^{-5} M, \blacksquare .

different from unity. Table 1 summarises the pA_2 values of the four antidepressants. Amitriptyline is the most active drug, about as potent as cyproheptadine ($pA_2=7.43$, ref. 1). Dibenzepin and iprindole have about 100-fold less affinity for the cyclase. As the histamine-sensitive adenylate cyclase in broken cell preparations of guinea pig cortex and hippocampus respond identically to histamine, dimaprit and antagonists¹, it was appropriate to compare the sensitivities

Table 1 pA_2 values of tricyclic antidepressant drugs in inhibiting histamine- and dimaprit-stimulated adenylate cyclase activity in homogenates of guinea pig hippocampus

Drug	Histamine pA_2 values	Dimaprit
Amitriptyline	7.23 ± 0.27 (7)	7.47 ± 0.55 (7)
Imipramine	6.39 ± 0.16 (7)	6.61 ± 0.19 (6)
Dibenzepin	5.49 ± 0.11 (3)	5.81 ± 0.11 (3)
Iprindole	5.63 ± 0.11 (3)	5.49 ± 0.27 (3)

Values are means \pm s.d. The numbers of different hippocampi that the drugs were tested on are given in parentheses.

of the cyclases in the cortex and hippocampus to tricyclic antidepressants. Figure 2 shows that the values obtained on homogenates from cortex are described by the same Schild plot as derived from observations on homogenates of the hippocampus.

It should be noted that these drugs also blocked dimaprit-activated adenylate cyclase activity in a competitive manner. Since dimaprit has negligible H_1 -antagonist activity² the inhibition of dimaprit-sensitive adenylate cyclase by tricyclic antidepressants permits their classification as histamine H_2 antagonists. The pA_2 values of H_2 antagonists on histamine- or dimaprit-activated adenylate cyclase activity in homogenates of guinea pig hippocampus^{1,10}, and cortex¹, and cardiac ventricles³ were very nearly identical to the pA_2 values on pharmacological preparations thus showing that blockade of this adenylate cyclase in these tissues reflect pharmacological effects on the H_2 receptor in other tissues.

Brain concentration of imipramine after a single dose of 15 mg per kg body weight intramuscularly was found¹¹ in

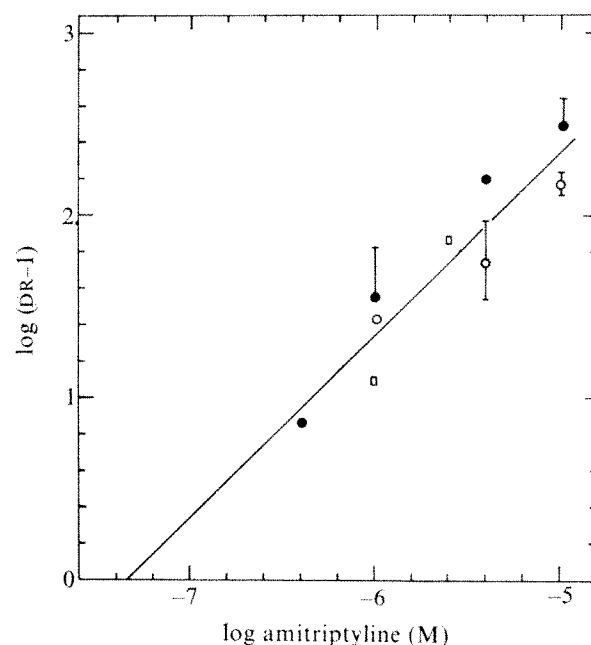


Fig. 2 Schild plot for inhibition by amitriptyline of dimaprit- and histamine-stimulated adenylate cyclase activity in the guinea pig hippocampus and cortex. The dose ratios (DR) were calculated as the ratio of the ED_{50} of histamine in the presence and absence of different concentrations of amitriptyline (B). Simple competitive antagonism results in a straight line of slope 1 when $\log(DR-1)$ is plotted against $\log B$; and the intercept with the abscissa is $-\log K_B$, where K_B is the apparent dissociation constant for the antagonist-receptor interaction; $-\log K_B$ is referred to as pA_2 . The slope of the line is 1.02, not significantly different from unity, and the pA_2 value is 7.35. Each point is the mean of experiments on tissue from at least two animals. Dimaprit stimulated activity in \bullet , hippocampus and \square , cortex. \circ , Histamine stimulated activity in hippocampus.

Table 2 Potencies of some tricyclic antidepressant drugs in blockade of the histamine H_2 receptor, the muscarinic receptor, and in inhibiting uptake of 5-hydroxytryptamine (5-HT) and noradrenaline (NA)

Drug	Anti- H_2 activity		Antimuscarinic activity		IC ₅₀ (M) for inhibition of uptake:	
	$K_B^*(M)$	IC ₅₀ [†] (M)	$K_B^{\ddagger}(M)$	$K_B^{\S}(M)$	5-HT (ref. 14)	NA (ref. 15)
Amitriptyline	3.4×10^{-8}	6.8×10^{-8}	3.3×10^{-8}	8.3×10^{-9}	4.9×10^{-7}	5.5×10^{-8}
Imipramine	2.4×10^{-7}	4.8×10^{-7}	2.0×10^{-7}	6.5×10^{-8}	2.8×10^{-7}	1.0×10^{-6}
Dibenzepin	1.5×10^{-6}	3.0×10^{-6}	1.8×10^{-6}	—	—	—
Iprindole	3.2×10^{-6}	6.4×10^{-6}	—	—	1.0×10^{-6}	—

*Dimaprit as agonist, from Table 1.

[†]Calculated according to: $IC_{50} = K_B(1 + [A]/K_A)$, where K_B = the apparent dissociation constant for the drug-receptor interaction; $[A]$, agonist concentration, taken arbitrarily as $[A] = 2 \times K_A$; and K_A is the apparent dissociation constant for the dimaprit-receptor interaction which equals the ED_{50} value.

[‡]From ref. 12, where 3H -N-methyl-4-piperidylbenzilate was used as the labelled ligand ($K_B = 5 \times 10^{-10}$ M), at a concentration of 5×10^{-10} M.

[§]Calculated from ref. 13, where 3H -3-quinuclidinylbenzilate was used as the labelled ligand ($K_B = 3 \times 10^{-10}$ M), at a concentration of 6×10^{-11} M.

rat to be $1,500\mu\text{g l}^{-1}$, which corresponds to $5.4 \times 10^{-6}\text{ M}$. This concentration is higher than the apparent K_B value (Table 2), suggesting that the brain H_2 receptors could be occupied *in vivo* after administration of pharmacologically active doses. Among the mechanisms proposed to account for the pharmacological effects of the tricyclic antidepressants are blockade of re-uptake of noradrenaline and of 5-hydroxytryptamine and blockade of muscarinic receptors. Table 2 shows the potency of these drugs at these sites and at the H_2 receptor. Their K_B values at the H_2 receptor and muscarinic receptor (column 3 of Table 2) are surprisingly similar. The IC_{50} values (concentrations for 50% inhibition) for uptake cannot be directly compared with the IC_{50} values for blocking the H_2 receptor because the affinities of 5-hydroxytryptamine and noradrenaline for the uptake system are not known; but the estimates (Table 2) suggest that potency for H_2 blockade is at least comparable with potency in blocking uptake of the amines.

Nevertheless, the information available cannot support an assertion that blockade of the H_2 receptor (or any other single action of these drugs) contributes to their therapeutic effect. But some of the pharmacological effects of the antidepressant drugs may rest on blockade of H_2 receptors. For example, histamine increases fluid consumption in rats by a central mechanism¹⁶⁻¹⁸, and tricyclic antidepressant drugs diminish fluid consumption in rats¹⁹. One of the rare adverse effects of these drugs is hallucinations²⁰; perhaps pertinent is the finding that the hallucinogen, *d*-lysergic acid diethylamide, also blocks the H_2 receptor¹. Of special and practical interest is that amitriptyline has greater affinity for the H_2 receptor (Table 1) than does cimetidine ($pA_2=6.22$; ref. 1), the H_2 antagonist that is so effective in treating peptic ulcer^{21,22}. The antimuscarinic activity of some of the tricyclic antidepressants should further enhance their usefulness in treating this disease. Like cimetidine, antidepressant drugs have been shown to decrease gastric acidity in man²³ and gastric acid secretion in laboratory animals²⁴⁻²⁷ and to prevent gastric ulcer formation in animals^{24,27-30}—effects that were also noted with an antidepressant drug lacking antimuscarinic activity²⁷.

This research was supported by a grant from the US National Institute of Mental Health.

J. P. GREEN
S. MAAYANI

Department of Pharmacology,
Mount Sinai School of Medicine of
the City University of New York,
New York, New York 10029

Received 20 June; accepted 11 July 1977.

- 1 Green, J. P., Johnson, C. L. & Weinstein, H. in *Histamine Receptors* (ed. Yellin, T. O.) (Spectrum, New York, in the press).
- 2 Parsons, M. E., Owen, D. A. A., Ganellin, C. R. & Durant, G. J. *Ag. Actions* 7, 31-38 (1977).
- 3 Johnson, C. L. & Mizoguchi, H. *J. Pharmac. exp. Ther.* 200, 174-186 (1977).
- 4 Salomon, Y., Londos, C. & Rodbell, M. *Analyt. Biochem.* 58, 541-548 (1974).
- 5 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* 193, 265-275 (1951).
- 6 Waud, D. R. & Parker, R. B. *J. Pharmac. exp. Ther.* 177, 13-24 (1971).
- 7 Raub, W. F. *Fedn Proc.* 33, 2390-2392 (1974).
- 8 Schild, H. O. *Pharmac. Rev.* 9, 242-246 (1957).
- 9 Arunlakshana, O. & Schild, H. O. *Br. J. Pharmac.* 14, 48-58 (1959).
- 10 Hegstrand, L. R., Kanof, P. D. & Greengard, P. *Nature* 260, 163-165 (1976).
- 11 Nagy, A. J. *J. Pharm. Pharmac.* 29, 104-107 (1977).
- 12 Rehavi, M., Maayani, S. & Sokolovsky, M. *Biochem. Pharmac.* (in the press).
- 13 Snyder, S. H. & Yamamura, H. I. *Archs gen. Psych.* 34, 236-239 (1977).
- 14 Horn, A. S. & Trace, S. H. *Br. J. Pharmac.* 51, 399-403 (1974).
- 15 Horn, A. S., Coyle, J. T. & Snyder, S. H. *Molec. Pharmac.* 7, 66-80 (1971).
- 16 Gerald, M. C. & Maickel, R. P. *Br. J. Pharmac.* 44, 462-471 (1972).
- 17 Gutman, Y. & Krausz, M. *Eur. J. Pharmac.* 23, 256-263 (1973).
- 18 Liebowitz, S. F. *Brain Res.* 63, 440-444 (1973).
- 19 Zabik, J. E., Levine, R. M., Spaulding, J. H. & Maickel, R. P. *Neuropharmacology* 16, 267-271 (1971).
- 20 Klerman, G. L. & Cole, J. O. *Pharmac. Rev.* 17, 101-141 (1965).
- 21 Editorial *Br. med. J.* 1, 1275-1278 (1976).
- 22 Editorial *J. Am. med. Ass.* 237, 2224 (1977).
- 23 Varay, A., Berthelot, J., Billiotet, J., Vetterbo, G. & Graf, B. *Bull. Mém. Soc. méd. Hôp. Paris* 76, 228-239 (1960).
- 24 Bonfils, S., Dubrasquet, M. & Lamblin, A. *J. appl. Physiol.* 17, 299-300 (1962).
- 25 Bass, P. & Patterson, M. A. *J. Pharmac. exp. Ther.* 156, 142-149 (1967).
- 26 Lippmann, W. *Biochem. Pharmac.* 18, 2517-2529 (1969).
- 27 Lippmann, W. & Seethaler, K. *Life Sci.* 20, 1393-1400 (1977).
- 28 Garattini, S., Giachetti, A., Jori, A., Pieri, L. & Valzelli, L. *J. Pharm. Pharmac.* 14, 509-514 (1962).
- 29 Lippmann, W. *J. Pharm. Pharmac.* 22, 568-573 (1970).
- 30 Lippmann, W. *Archs Int. Pharmacodyn.* 193, 340-356 (1971).

Reversal of the Na-K pump and apparent affinity for intracellular potassium

CENTRAL to all formulations of the transport mechanism of the membrane Na-K pump should be the description of cation binding and release. The acceptance sites for transport by the pump, external sites for K and internal sites for Na, have been extensively studied, and linked to the activating sites of the associated Na,K-ATPase¹. By contrast, little is known about the discharge sites of the pump, although proposed characteristics of these sites have vital roles in various transport and enzyme schemes, such as cyclical conversions of discharge sites for one cation into acceptance sites for the other. We have studied the apparent affinity for K at its intracellular discharge sites by measuring the rate of ATP synthesis as a function of the internal K concentration in resealed red blood cell ghosts, where the Na-K pump is driven in reverse by the downhill efflux of K and influx of Na: the observed $K_{0.5}$ for internal K was 0.3 M.

Resealed ghosts were prepared from fresh, human red blood cells, following the procedure of Bodemann and Passow² and Bodemann and Hoffman³. Cells, washed three times in 160 mM choline chloride-10 mM Tris-HCl (pH 7.4), were suspended at 50% haematocrit in isotonic Tris-HCl (166 mM, pH 7.4) at 0 °C, and then rapidly mixed with 10 volumes of ice-cold haemolysing solution⁴ containing 1 mM ATP, 2 mM ADP, 9 mM MgCl_2 , 10 mM iodoacetate, and 5 mM ^{32}P -phosphate. After 5 min the salt concentration was restored to 160 mM by adding 3.2 M KCl (or KCl plus choline chloride at a total concentration of 3.2 M), to give final KCl concentrations of 60-160 mM. After a further 5 min the ghosts were resealed by incubating for 60 min at 37 °C. The ghosts were then washed five times in 160 mM choline chloride-10 mM Tris-HCl. Most ghost cells prepared in this manner are relatively impermeable to cations⁵ and capable of active transport⁶ of Na and K.

To measure incorporation of ^{32}P -phosphate into ATP, the resealed ghosts, at a final haematocrit of 3%-5%, were incubated at 37 °C in 160 mM NaCl-10 mM Tris-HCl (pH 7.4) in the presence and absence of 0.2 mM ouabain. At timed intervals samples were removed and the ghosts washed twice at 4 °C in 160 mM choline chloride-10 mM Tris-HCl. The packed ghosts were haemolysed in a solution containing 1.4 mM ATP and 2 μM phosphate. (To estimate the initial level of internal K and ^{32}P -phosphate, ghosts that were resealed but not incubated were treated the same way.) Aliquots were then removed to determine the K and haemoglobin contents. To precipitate the protein in the haemolysate ice-cold trichloroacetic acid was added (final concentration 5%), and the mixture centrifuged.

Table 1 Incorporation of ^{32}P -orthophosphate into organophosphate by reversal of the Na-K pump in resealed red cell ghosts

Expt	% Of total initial radioactivity in organophosphate		
	Total	Plus ouabain	Ouabain-sensitive
1	2.19	1.73	0.46
2	3.41	2.97	0.44
3	2.05	1.91	0.14
4	2.73	2.17	0.56
5	3.51	3.15	0.36

Ghosts were resealed in a solution containing 160 mM KCl, plus other constituents as given in the text. The ghosts were then incubated for 15 min at 37 °C in 160 mM NaCl-10 mM Tris-HCl, in the presence and absence of 0.2 mM ouabain. The ouabain-sensitive incorporation of ^{32}P into organophosphate is assumed to represent synthesis of ATP by reversal of the Na-K pump.

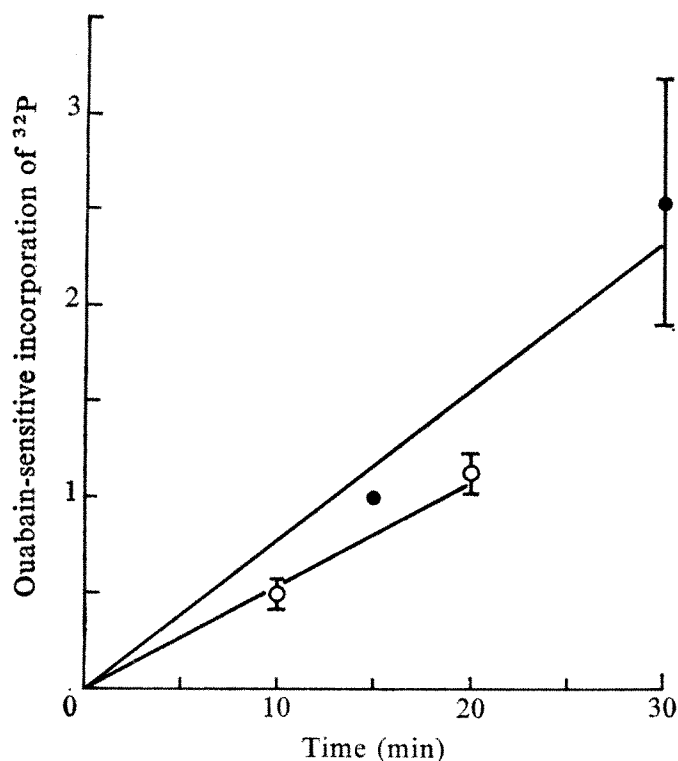


Fig. 1 Time course of incorporation of ^{32}P -orthophosphate into organophosphate by reversal of the Na-K pump. Red cell ghosts were resealed in solutions containing KCl at 160 mM (●) or 80 mM (○), plus other constituents as given in the text. At the times indicated during incubation, samples of the ghosts were taken for determination of ^{32}P -labelled organophosphate and of K concentration. In each experiment, the ouabain-sensitive incorporation of ^{32}P into organophosphate (as a fraction of the initial content of ^{32}P -orthophosphate) was normalised to the value at 15 min for ghosts resealed in 160 mM KCl. Results shown are from four experiments with ghosts resealed in 160 mM KCl (mean final [K] in the ghosts, $95 \text{ mM} \pm 8$, s.e.m.). In three of these experiments ghosts resealed in 80 mM KCl were also used (mean final [K], $54 \text{ mM} \pm 6$). Vertical bars indicate s.e.m. values (except for the value to which the others were normalised). The lines were fitted by eye.

The supernatant material was adjusted to pH 5–6 with Tris, unlabelled phosphate added as carrier, and inorganic phosphate extracted as the phosphomolybdate complex⁴. Radioactivity remaining after the extractions, ' ^{32}P -organophosphate', was then measured by liquid scintillation counting.

In these conditions ghosts resealed in 160 mM KCl and incubated for 15 min in 160 mM NaCl incorporated about 3% of the initial ^{32}P -phosphate into the organophosphate fraction (Table 1). Of this incorporation, about 14% was inhibited by ouabain (0.4% of the initial ^{32}P -phosphate), and this synthesis is assumed to reflect reversal of the Na-K pump^{1,5}. In similar experiments Garrahan and Glynn⁴ found 0.5% of the initial ^{32}P incorporated after 15 min, and 40% of this was ouabain-sensitive (0.2% of the initial ^{32}P).

In a preliminary experiment the extract was separated by ion-exchange chromatography⁴. ouabain-sensitive incorporation into material eluting with authentic ATP was demonstrated, together with a smaller fraction eluting with ADP. Garrahan and Glynn⁴ similarly found that the bulk of incorporated ^{32}P eluted with ATP; the labelling of ADP was attributed to the action of adenylate kinase. In the same experiment ouabain-sensitive influx of ^{22}Na was also demonstrated⁶. This flux could not result from Na-Na exchange since the ghosts, containing a high K content, were virtually free of internal Na.

The time course of ouabain-sensitive incorporation of ^{32}P into organophosphate was nearly linear for 20–30 min, with ghosts resealed in either high or low KCl media

(Fig. 1), although there was considerable error in the estimation of incorporation at 30 min. In these and subsequent experiments the rate of incorporation in each case is normalised to that of ghosts resealed in 160 mM KCl and incubated for 15 min, measured concurrently, defined as 1.0. Normalisation was necessary because of the variation in absolute incorporation between different preparations of ghosts (Table 1). The measured K concentrations were less than those of the resealing media (Fig. 1), but the loss did not occur during the experimental incubation: there was no detectable decrease in K content between 0 and 30 min.

Since it was possible to estimate in 15 min the initial rate of ouabain-sensitive incorporation, the effect of the internal K concentration on this rate was examined (Fig. 2). Although there were again large errors associated with the estimates of incorporation, there was a positive correlation between the measured K concentration within the ghosts and the rate of synthesis. Moreover, these data, replotted in double-reciprocal form (Fig. 2, inset) fit well a linear relationship, giving a $K_{0.5}$ of 0.3 M for K-activation of ATP synthesis through reversal of the Na-K pump.

This $K_{0.5}$ is two to three orders of magnitude greater than the apparent affinity for K at sites inhibiting the binding of ATP^{7,8}, sites that have been interpreted⁹ as representing the internal discharge sites for K. By contrast, this $K_{0.5}$ for pump reversal is only twofold greater than the K_i for K as

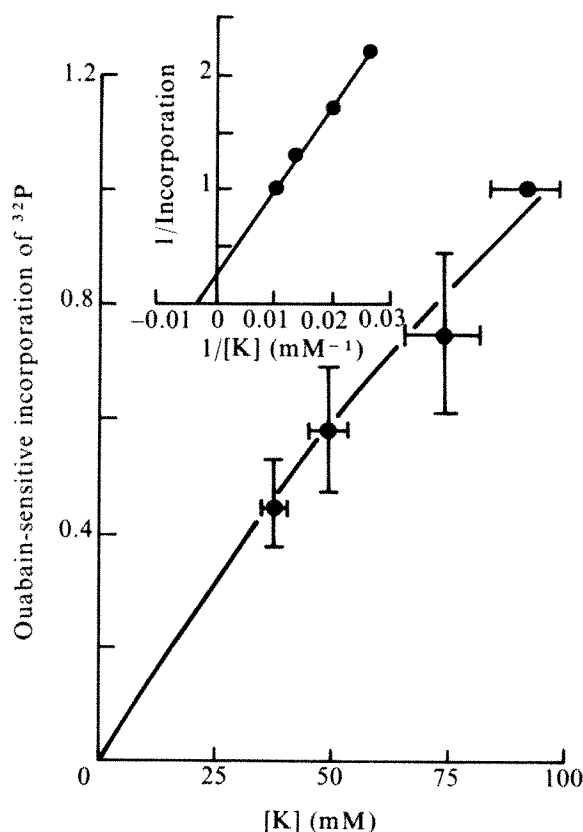


Fig. 2 Incorporation of ^{32}P -orthophosphate into organophosphate in resealed ghosts containing various K concentrations. Ghosts were incubated for 45 min at 37 °C. The horizontal and vertical bars indicate s.e.m. values for, respectively, the measured K concentrations in the ghosts and the ouabain-sensitive incorporation of ^{32}P into organophosphate (except for the values at the highest K concentration, to which the others were normalised). The results shown are from four experiments, each with ghosts containing four different K concentrations, plus a fifth experiment with ghosts at two K concentrations. In the inset, these data are replotted in double reciprocal form, and the straight line was fitted by the method of least squares; the calculated $K_{0.5}$ is 297 mM.

a non-competitive inhibitor of K-dependent phosphatase activity of the Na,K-ATPase¹⁰, an effect considered¹⁰ as product-inhibition through occupancy of discharge sites for K.

For K-K exchange through the pump the concentration of internal K for half-maximal activation is only 10 mM (ref. 11), well below the $K_{0.5}$ found here. This discrepancy may reflect mechanistic differences between the two processes; for example, K influx occurs with K-K exchange but not with pump reversal, so if influx becomes rate-limiting before internal K sites (involved in both exchange and reversal) are saturated, then different responses to internal K might be seen. Alternatively, K-K exchange may involve cyclical changes in K sites, rather than a simple shuttling through K channels. Since the K_i for K as a competitor to Na at the internal Na sites is 9 mM (ref. 12), close to the $K_{0.5}$ for K in K-K exchange, it is tempting to consider that efflux during K-K exchange involves those Na sites. Further quantitation of Na-K interactions¹¹ as well as elucidation of the role of inorganic phosphate¹³ will be needed before the nature of the exchange mechanism can be fully resolved.

For the Na-K pump the data can be accommodated in formulations featuring K discharge sites ($K_{0.5}$ approximately 300 mM) that may be cyclically converted to acceptance sites for Na (K_i for K about 9 mM), the latter possibly also serving in K-K exchange as acceptance sites for K ($K_{0.5}$ approximately 10 mM). In any case, our results clearly demonstrate the presence of internal low-affinity sites for K in conditions in which Na acceptance sites would be expected (that is, high nucleotide content and physiological K concentration). The observations thus support formulations¹⁴⁻¹⁶ in which distinct sites and channels for Na and K co-exist, perhaps on different subunits, perhaps with interconversion of these two classes of channels with each pump cycle.

This work was supported by grants from the US PHS and NIH.

J. D. ROBINSON
E. S. HALL

Department of Pharmacology,
State University of New York,
Upstate Medical Center,
766 Irving Avenue,
Syracuse, New York 13210

P. B. DUNHAM

Department of Biology,
Syracuse University,
Syracuse, New York 13210

Received 9 June; accepted 26 July 1977.

- 1 Glynn, I. M. & Karlsh, S. J. D. *A. Rev. Physiol.* **37**, 13-55 (1975).
- 2 Bodemann, H. & Passow, H. *J. Membr. Biol.* **8**, 1-26 (1972).
- 3 Bodemann, H. & Hoffman, J. F. *J. gen. Physiol.* **67**, 497-525 (1976).
- 4 Garrahan, P. J. & Glynn, I. M. *J. Physiol., Lond.* **192**, 237-256 (1967).
- 5 Glynn, I. M. & Lew, V. L. *J. Physiol., Lond.* **207**, 393-402 (1970).
- 6 Sachs, J. R., Ellory, J. C., Kropp, D. L., Dunham, P. B. & Hoffman, J. F. *J. gen. Physiol.* **63**, 389-414 (1974).
- 7 Hegyvary, C. & Post, R. L. *J. biol. Chem.* **246**, 5234-5240 (1971).
- 8 Robinson, J. D. *Biochim. biophys. Acta* **397**, 194-206 (1975).
- 9 Post, R. L., Kume, S. & Rogers, F. N. in *Mechanisms in Bioenergetics* (eds Azzone, G. F., Ernster, L., Papa, S., Quagliariello, E. & Siliprandi, N.) (Academic, New York, 1973).
- 10 Robinson, J. D. *Biochim. biophys. Acta* **384**, 250-264 (1975).
- 11 Simons, T. J. B. *J. Physiol., Lond.* **237**, 123-155 (1974).
- 12 Garay, R. P. & Garrahan, P. J. *J. Physiol., Lond.* **231**, 297-325 (1973).
- 13 Glynn, I. M., Lew, V. L. & Lüthi, U. *J. Physiol., Lond.* **207**, 371-391 (1970).
- 14 Robinson, J. D. *Archs Biochem. Biophys.* **156**, 232-243 (1973).
- 15 Garrahan, P. J. & Garay, R. P. *Curr. Top. Membr. Transp.* **8**, 29-97 (1976).
- 16 Robinson, J. D. *Biochim. biophys. Acta* **482**, 427-437 (1977).

Analgesic activity of lipotropin C fragment depends on carboxyl terminal tetrapeptide

A SERIES of peptides with morphine-like activity has been identified in extracts of pituitary¹⁻³ and brain⁴⁻⁷. With the exception of Leu-enkephalin, all those characterised represent some fraction of the 31-residue C fragment—a specific activation product of lipotropin^{1,8}. Several attempts have been made to define the receptor requirements of opiate peptides^{9,10} and to make potent and stable agonists^{5,11,12}, but essentially all the studies have been based on the easily synthesised NH₂-terminal pentapeptide of C fragment, Met-enkephalin. Unlike the enkephalins, C fragment has a high potency in its central actions¹³⁻¹⁶, *in vivo* and *in vitro*, and it is relatively stable to brain peptidases¹⁷⁻¹⁹. C fragment is the only naturally occurring peptide to possess strong analgesic properties and have a long lasting action²⁰. It is clear that specific regions of the molecular structure, not present in γ and α endorphin or Met-enkephalin, have a role in the expression of its central activity. We present here evidence that the COOH-terminal tetrapeptide of C fragment, Lys-Lys-Gly-Gln, is responsible for the unique affinity of C fragment for brain opiate receptors and that the presence of all four residues is essential for the high analgesic potency.

Lipotropin, C fragment (61-91) and C' fragment (61-87) were isolated from porcine pituitary¹. The 61-89 peptide and the ϵ -N-pentacitraconyl derivative of C fragment were prepared as described in Table 1. Opiate binding assays were carried out by measuring the ability of peptides to reduce the specific binding of ³H-naloxone and ³H-dihydromorphine to opiate receptors in washed synaptosomal membranes from rat brain. Analgesic potencies were determined by a tail flick assay using rats with indwelling cannulae²¹.

The affinity of C fragment (lipotropin 61-91) for brain opiate receptors, assessed by displacement of ³H-naloxone, was decreased by removal of the COOH-terminal glutamine and glycine residues (Table 1). C' fragment (61-87), which lacks two lysine residues in addition to glutamine and glycine, exhibited a much lower affinity; its ability to displace ³H-naloxone was 40 times less than that of C fragment.

Table 1 Binding of C fragment and derivatives to brain synaptosomal membranes

Peptide	Concentration required to displace 50% of bound opiate	
	³ H-naloxone (10 ⁹ M)	³ H-dihydromorphine (10 ⁹ M)
C fragment	2.6	2.2
C fragment (61-89)	7 ± 2 (4)	2.2 ± 0.5 (4)
C' fragment (61-87)	100	42
[CT ₅] C fragment	4,000	710
Acid-treated [CT ₅] C fragment	3.0	2.5

Desglutamine desglycine C fragment (61-89) was obtained by prolonged carboxypeptidase A digestion of citraconylated C fragment. The product was isolated by gel filtration on Sephadex G50 in 200 mM N-ethylmorpholine acetate at pH 8.5. ϵ -N-pentacitraconyl C fragment ([CT₅] C fragment) was prepared by trypsin digestion of citraconylated porcine lipotropin. The peptide was purified by gel filtration on Sephadex G50 in 20 mM Tris-HCl at pH 8.5 and by ion-exchange chromatography on DEAE-Sephadex A25 at pH 8.5. Citraconyl groups were removed from peptides by incubation with 100 mM pyridine acetate at pH 4.0 for 24 h at 37 °C. Volatile buffers were removed by evaporation in a vacuum and by desalting on Sephadex G25. Peptide purity was established by amino acid analysis and NH₂- and COOH-terminal determinations. Opiate binding assays were performed using displacement of radiolabelled alkaloids from brain synaptosomal membrane preparations as a measure of the relative affinity of a given peptide⁹. The percentage error of the mean of a determination was generally better than 3%. The standard deviations shown include results obtained from two preparations of the 61-89 peptide.

Similar results were obtained when displacement of ^3H -dihydromorphine was used as an index of receptor binding, but the decrease in affinity associated with removal of the COOH-terminal residues from C fragment was less marked. Thus, desglutamine desglycine C fragment (61–89) exhibited a receptor affinity comparable to C fragment and removal of the two lysine residues reduced affinity 19-fold.

The results indicate an important role for the paired lysine residues, and to a lesser extent glycine and glutamine at the COOH-terminus, for the binding of C fragment to brain opiate receptors. A requirement for positive charges on the lysine residues was suggested by study of the binding properties of a pentacitraconyl derivative of C fragment, in which the five lysine amino groups were masked by anionic substitution. The affinity of the citraconyl derivative, measured by displacement of naloxone, was three orders of magnitude less than the affinity of C fragment.

It was notable that the potency of ϵ -N-pentacitraconyl C fragment in displacing ^3H -dihydromorphine was greater than its potency in displacing ^3H -naloxone (Table 1) and in this respect the citraconylated derivative resembles Met-enkephalin. This is consistent with the finding that modifications in the COOH-terminal region of C fragment have more effect on the inhibition of naloxone binding than on the inhibition of morphine binding.

The analgesic activities exhibited by C fragment, the 61–89 peptide and C' fragment reflected their abilities to displace ^3H -naloxone in the binding assay. Removal of the glutamine and glycine residues from the COOH-terminus of C fragment reduced the analgesic potency (Fig. 1). The effect of removing the two lysine residues, shown in the properties of C' fragment, was to cause a severe loss of potency; the 61–87 peptide possessed approximately 1/500th the activity of C fragment.

Although the potency of C' fragment was low, its analgesic action persisted for several hours. In contrast, the weak analgesic action of Met-enkephalin is transient^{22,23}. It is clear that the duration of analgesia, and not the potency, is related to *in vivo* stability. Met-enkephalin, for example, undergoes rapid degradation in the presence of brain homogenates²⁴ whereas C fragment and C' fragment are comparatively stable¹⁷. The long peptides seem to possess preferred conformations which protect the NH₂-terminal region from enzymatic attack²⁵. In particular it is notable that C' fragment resembles C fragment in having a high stability to

attack by aminopeptidases, which would indicate that the two peptides possess similar conformations in solution. Our results suggest that the four residues at the COOH-terminus of C fragment contribute to receptor affinity by participating in a specific interaction within the peptide-receptor complex and not by stabilising an intrinsic conformation of the peptide molecule.

The COOH-terminal residues of C fragment, Lys-Lys-Gly-Gln, are highly resistant to the action of carboxypeptidases in brain¹⁸. Furthermore, loss of all four residues leads to a large reduction in analgesic potency and removal of additional residues has little further effect on binding affinity⁹ or analgesia²⁰. Thus, the COOH-terminal region of C fragment fulfils a dual function: it confers stability on the peptide against proteolysis and it provides a structure important for receptor affinity.

We thank D. E. Massey and Ann Smith for technical assistance.

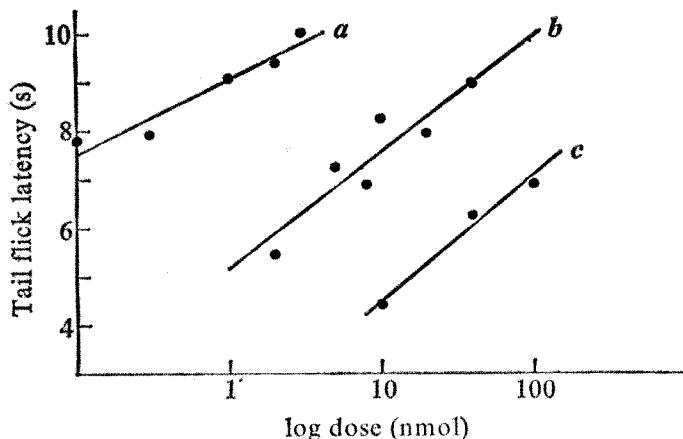
M. J. GEISOW
J. F. W. DEAKIN
J. O. DOSTROVSKY
D. G. SMYTH

National Institute for Medical Research,
Mill Hill,
London NW7, UK

Received 21 June; accepted 18 July 1977.

- Bradbury, A. F., Smyth, D. G. & Snell, C. R. in *Peptides: Chemistry, Structure and Biology* (eds Walter, R. & Meinhofer, J.) 609–615 (Science, Ann Arbor, 1975).
- Bradbury, A. F., Smyth, D. G. & Snell, C. R. in *Polypeptide Hormones: Molecular and Cellular Aspects*, CIBA Found. Symp. No. 41, 61–75, (Elsevier-North Holland, Amsterdam 1976).
- Graf, L., Barat, E. & Patty, A. *Acta Biochem. biophys. Acad. Sci. Hung.* **11**, 111 (1976).
- Hughes, J. *et al. Nature* **258**, 577–579 (1975).
- Bradbury, A. F., Feldberg, W. S., Smyth, D. G. & Snell, C. R. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 9–17 (Elsevier-North Holland, Amsterdam, 1976).
- Simantov, R. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2515–2519 (1976).
- Guillemin, R., Ling, N. & Burgus, R. C. *r. hebdom. Séanc. Acad. Sci., Paris* **282**, 783–785 (1976).
- Bradbury, A. F., Smyth, D. G. & Snell, C. R. *Biochem. biophys. Res. Commun.* **69**, 950–956 (1976).
- Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M. & Hulme, E. C. *Nature* **260**, 793–795 (1976).
- Lazarus, L. H., Ling, N. & Guillemin, R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2156–2159 (1976).
- Pert, C. B., Bowie, D. L., Fong, F. T. W. & Chang, J. K. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 79–86 (Elsevier-North Holland, Amsterdam, 1976).
- Coy, D. H. *et al. Biochem. biophys. Res. Commun.* **73**, 632–638 (1976).
- Feldberg, W. S. & Smyth, D. G. *J. Physiol., Lond.* **260**, 30–31P (1976); **265**, 25–27P (1977).
- Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2895–2898 (1976).
- Bradbury, A. F., Smyth, D. G., Snell, C. R., Deakin, J. F. W. & Wendlandt, S. *Biochem. biophys. Res. Commun.* **74**, 748–754 (1977).
- Gent, J. P., Smyth, D. G., Snell, C. R. & Wolstencroft, J. H. *J. Pharmac.* (in the press).
- Austen, B. M. & Smyth, D. G. *Biochem. biophys. Res. Commun.* **76**, 477–482 (1977).
- Geisow, M. J. & Smyth, D. G. *Biochem. biophys. Res. Commun.* **75**, 625–629 (1977).
- Austen, B. M., Smyth, D. G. & Snell, C. R. *Nature* (submitted for publication).
- Feldberg, W. S. & Smyth, D. G. *Br. J. Pharmac.* **60**, 445–454 (1977).
- d'Amour, F. E. & Smith, D. L. *J. Pharmac. exp. Ther.* **72**, 74–79 (1941).
- Belluzzi, J. D. *et al. Nature* **260**, 625–626 (1976).
- Büscher, H. H. *et al. Nature* **261**, 423–425 (1976).
- Hambrook, J. M., Morgan, B. A., Rance, M. J. & Smith, C. F. C. *Nature* **262**, 782–783 (1976).
- Austen, B. M. & Smyth, D. G. *Biochem. biophys. Res. Commun.* **77**, 86–94 (1977).

Fig. 1 Log dose-response curves for analgesia produced by intraventricular injection of peptides in the rat. a, C fragment 61–91; b, 61–89 peptide; c, C' fragment (61–87). Peptides were dissolved in 0.9% saline and injected in a volume of 5 μl through indwelling lateral ventricular cannulae. Tail-flick latencies were measured before and at intervals up to 3 h subsequent to administration of the peptides. The ordinate values give the tail-flick latency at 30 min after injection. There were no significant differences between groups in baseline latency (2–4 s).



Proinsulin conversion to desalanyl insulin by α_2 -macroglobulin-bound trypsin

HARPEL and Mosesson¹ observed that α_2 -macroglobulin-bound trypsin hydrolysed low molecular weight ester and amide substrates at rates comparable with those of free protease, but degradation of high molecular weight proteins was markedly inhibited. To account for this finding, Barrett and Starkey² introduced the novel concept of steric entrapment of endopeptidases by α_2 -macroglobulin. Neurath and Walsh³ have recently reviewed the role of proteolytic enzymes in the conversion of a variety of enzymes, hormones, and other physiologically active proteins from inactive precursors to active forms by limited

proteolysis. Many of these physiologically active proteins exist in the bloodstream in both precursor and active forms. We report here that proinsulin, a large polypeptide of 9,000 molecular weight, is rapidly hydrolysed by α_2 -macroglobulin-bound trypsin. This finding suggests that proteases bound to α_2 -macroglobulin may be involved in limited proteolytic cleavage associated with activation of precursor molecules as well as the degradation of biologically active polypeptides in the circulation.

Proinsulin was chosen as a model for investigation of the hydrolysis of polypeptide hormones by α_2 -macroglobulin-bound proteases because it is a polypeptide of known sequence¹ and representative size. Tryptic conversion of proinsulin to desalanyl insulin proceeds by way of two intermediate forms of proinsulin (insulin-Arg and insulin-Arg-Arg) with specific cleavages in the C-peptide region^{5,6}. Proinsulin, the intermediate forms, and desalanyl insulin can be resolved by polyacrylamide gel electrophoresis at pH 8.9 (ref. 4).

The digestion of porcine proinsulin (gift of Dr R. E. Chance, Lilly) was performed at 37 °C with an enzyme: substrate ratio of 1:200. A control incubation of proinsulin with α_2 -macroglobulin was included to assess the stability of proinsulin at 37 °C as well as the possible presence of endogenous protease-inhibitor complexes in the purified preparation of α_2 -macroglobulin. The gel electrophoresis pattern for digestion of proinsulin by α_2 -macroglobulin-bound trypsin or free enzyme is shown in Fig. 1. Chance *et al.*⁴ have shown that free trypsin rapidly digests proinsulin by way of two intermediate products to yield desalanyl insulin, which cannot be resolved from insulin in this gel electrophoresis system⁷. The intermediate forms seen in lanes 7–10 of Fig. 1 are presumed to be insulin-Arg and insulin-

Arg-Arg on the basis of previous investigations of the tryptic hydrolysis of proinsulin^{4–7}. The lighter appearance of the band for desalanyl insulin is due to some further digestion of trypsin, as well as to more rapid diffusion of smaller hydrolysis products from the gel, as can be seen by comparing the stain intensity of proinsulin (lane 1, 25 μ g) with that of insulin (lane 11, 50 μ g). By comparison of densitometer tracings of the proinsulin band at different times of hydrolysis, we estimate that proinsulin is digested by α_2 -macroglobulin-bound trypsin at approximately 10–15% of the rate of hydrolysis by the free enzyme.

Chance *et al.*⁴ have previously observed that trypsin hydrolyses proinsulin more rapidly than insulin or desalanyl insulin. The pattern of tryptic hydrolysis of proinsulin is also altered by binding of the enzyme to α_2 -macroglobulin (see Fig. 1). An intermediate product is apparent which is not present in detectable levels when proinsulin is digested with free trypsin. In addition, the major intermediate during digestion with trypsin does not accumulate to such a large extent during digestion with α_2 -macroglobulin-bound trypsin. This finding may reflect a more rapid diffusion of desalanyl insulin (molecular weight (MW) 5,600) and the intermediate products (MWs 6,050 and 5,900) compared with proinsulin (MW 9,000) into the enzyme-inhibitor complex.

Inactivation of the small peptide hormones angiotensin (MW 1,045) and lysine vasopressin (MW 1,116) by α_2 -macroglobulin-bound trypsin was reported previously⁸. Inactivation of these hormones involves proteolytic cleavage of two residues from the N terminal of the former peptide, while the latter is inactivated by cleavage of a C-terminal lysine amide, both of which might represent partial diffusion of a small peptide chain into the inhibitor-enzyme complex. In addition, plasmin bound to α_2 -macroglobulin has been

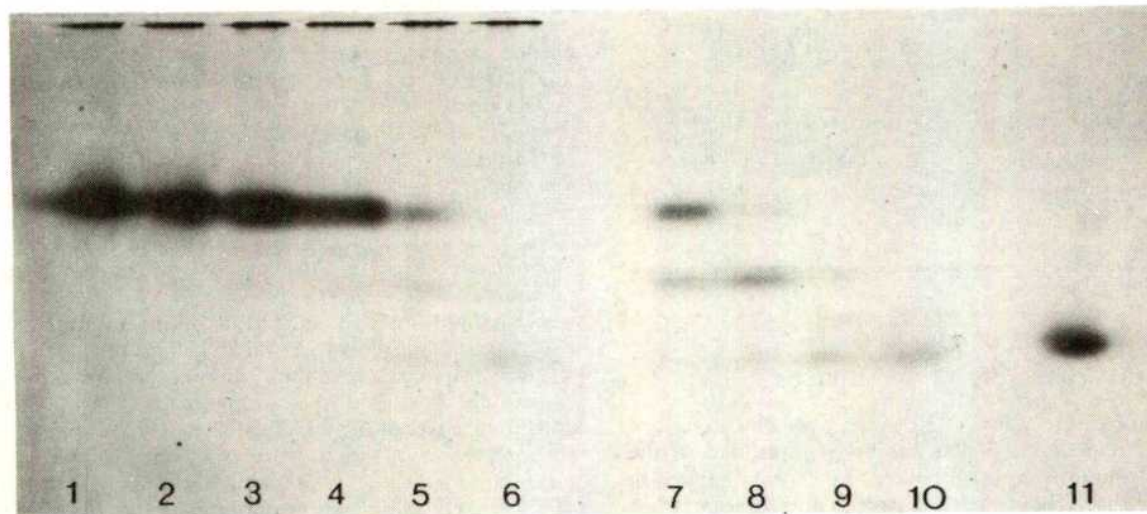


Fig. 1 Conversion of proinsulin to desalanyl insulin by α_2 -macroglobulin-bound trypsin. Lanes 1 and 2, proinsulin and α_2 -macroglobulin at 0 and 120 min; lanes 3–6, α_2 -macroglobulin-bound trypsin digestion of proinsulin at 15, 30, 60 and 120 min; lanes 7–10, trypsin digestion of proinsulin at 7, 15, 30 and 60 min; lane 11, insulin standard. Human α_2 -macroglobulin was purified from fresh plasma by DEAE-cellulose chromatography and gel filtration on Sephadex G-150 and Ultrogel ACA 22, a combination Agarose-polyacrylamide gel filtration resin (LKB). Bovine trypsin (Worthington) was treated with 1-tosylamido-2-phenylethylchloromethyl ketone to eliminate contaminating chymotryptic activity. Trypsin was pre-incubated with a 1.5-fold molar excess of α_2 -macroglobulin to produce an enzyme-inhibitor complex as follows: a 100- μ l aliquot of trypsin (0.1 mg ml⁻¹) was incubated with 300 μ l of α_2 -macroglobulin (1.2 mg ml⁻¹) in 40 mM Tris-HCl (pH 9.5) for 30 min at 37 °C. A control solution of enzyme and buffer was incubated in identical conditions. The extent of inhibition was measured using Remazol brilliant blue dyed hide powder (RBB-hide) as substrate as previously described¹⁰. Using this method, 0.1% of the original trypsin activity would have been detected. In the inhibition conditions used, however, no trypsin activity could be measured at 30 min, 2 h, or 24 h of incubation at 37 °C, while the control trypsin solution retained at least 90% activity after 24 h of incubation. Digestion mixtures consisted of a 40- μ l aliquot of the preincubation mixture of trypsin and α_2 -macroglobulin or trypsin alone with 100 μ l of proinsulin (2 mg ml⁻¹ in 40 mM Tris-HCl, 20 mM CaCl₂, pH 9.5). Sample aliquots (20 μ l) from the digestion mixtures were added to 16 mg of solid urea to form an 8 M urea solution and stored at -60 °C until electrophoresis. Polyacrylamide slab gel electrophoresis was performed at pH 8.9 using a modification⁷ of the original procedure described by Davis¹¹ in which 7 M urea was added to the resolving and stacking gels to improve resolution. The resolving gel contained 15% acrylamide with 0.4% *N,N'*-methylene-bis-acrylamide. Electrophoresis was performed for 16 h at 75 V in a BioRad Model 220 slab gel electrophoresis apparatus. Gel slabs were stained for 1 h in 0.1% amido black in 10% acetic acid, 20% ethyl alcohol and rapidly destained in 10% acetic acid to minimise diffusion of the peptide products from the gel. Using this procedure, the intensity of the bands remained constant for approximately 24 h.

shown to hydrolyse fibrinogen by degrading the A α -chain at a rate approximately 0.1% of that of the free enzyme¹. Our findings suggest that the inhibitor-enzyme matrix is substantially permeable to diffusion of peptides of moderate size. The tryptic cleavage position in proinsulin which produce desanyl insulin are at Arg-33 and Arg-63 in a polypeptide chain of 80 amino acid residues. Thus it seems that α_2 -macroglobulin-bound trypsin at approximately 10–15% variety of peptide hormones with molecular weights smaller than or comparable with that of proinsulin.

We have recently reported the presence of a pancreatic endopeptidase (elastase 2) in human blood and the elevation of this enzyme in sera of patients with acute pancreatic inflammation⁹. Pancreatic cationic trypsin has also been detected in human serum in this laboratory and will be the subject of a future communication. We suggest that some of the pathophysiological events associated with acute pancreatic inflammation may be due to hydrolysis of polypeptide hormones by circulating complexes of pancreatic proteases bound to α -macroglobulin.

This work was supported by the Medical Research Service of the Veterans Administration.

COREY LARGMAN
JANICE H. JOHNSON
JAMES W. BRODRICK
MICHAEL C. GEOKAS

Enzymology Research Laboratory,
Veterans Administration Hospital,
Martinez, California 94553, and
Department of Medicine,
University of California School of Medicine,
Davis, California 95616

Received 4 May; accepted 11 July 1977.

- ¹ Harpel, P. C. & Mosesson, M. W. *J. clin. Invest* **52**, 2175–2184 (1973).
- ² Barrett, A. J. & Starkey, P. M. *Biochem. J.* **133**, 709–724 (1973).
- ³ Neurath, H. & Walsh, K. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3825–3832 (1976).
- ⁴ Chance, R. E., Ellis, R. M. & Bromer, W. W. *Science* **161**, 165–167 (1968).
- ⁵ Chance, R. E. *Diabetes: Proc. Seventh Cong. Int. Diabetes Fed., Buenos Aires, 1970* (eds Rodriguez, R. R. & Vallance-Owen, J.) 292–305 (Excerpta Medica, Amsterdam, 1971).
- ⁶ Steiner, D. F., Hallund, O., Rubenstein, A., Cho, S. & Bayliss, C. *Diabetes* **17**, 725–736 (1968).
- ⁷ Yip, C. C. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1312–1315 (1971).
- ⁸ Rinderknecht, H. & Geokas, M. C. *Biochim. biophys. Acta* **295**, 233–244 (1973).
- ⁹ Geokas, M. C., Brodrick, J. W., Johnson, J. H. & Largman, C. *J. biol. Chem.* **252**, 61–67 (1977).
- ¹⁰ Rinderknecht, H., Silverman, O., Geokas, M. C. & Haverback, B. J. *Clin. chim. Acta* **28**, 239–246 (1970).
- ¹¹ Davis, B. *Ann. N.Y. Acad. Sci.* **121**, 404–427 (1964).

Structural analysis of purified platelet-activating factor by lipases

PLATELET-activating factor (PAF) is a newly described mediator of anaphylaxis which has been implicated in the deposition of immune complexes in acute serum sickness in rabbits^{1–4}. It is released by leukocytes and tissues, most probably by the basophils or mastocytes from various mammalian species, under the influence of various substances known to activate these cells^{5,6}. The study of the chemical nature of the compound responsible for the PAF activity led us to the preparation of large amounts of PAF from hog leukocytes⁷. Several fractionation steps of the crude material yielded a highly active chloroform-soluble fraction. We usually started these procedures with 100 l of hog blood which yielded 100 l of PAF, the biological activity of which could be detected at the 1- μ l level. Nevertheless, we have not yet obtained sufficient homogeneous material to apply the current methods for structural analysis (mass spectrometry, magnetic nuclear resonance, hydrolysis reactions and so on). We therefore used different lipases to get some insight into the structure of PAF. We present here the results of these experiments which suggest that PAF is a 1-lyso-glycerophospholipid.

PAF was obtained by procedures described previously^{7,8} modified for use on bulk quantities of hog blood. Throughout the purification steps, PAF activity was detected by aggregation of rabbit platelets⁹, in the presence of 10 μ M indomethacin⁷. Briefly, leukocytes from 100 l of blood were prepared by differential centrifugations followed by sedimentation in 2.5% gelatin in Tris-buffered saline (TBS), pH 7.4. (TBS contained Tris (hydroxymethyl)-amino-methane, 1×10^{-2} M; NaCl, 1.37×10^{-1} M; pH adjusted to 7.4 or 8 with HCl. Tyrode's contained TBS plus MgCl₂, 1×10^{-3} M; CaCl₂, 1×10^{-3} M; KCl, 2.6×10^{-3} M.) PAF affixed to bovine serum albumin (BSA) was obtained by overnight incubation of the leukocytes in Tris-buffered Tyrode's at pH 9.5, containing 0.25% BSA. PAF-BSA (100 l) was exchanged in batch on to QAE-Sephadex-A50 at pH 9.5; PAF was then eluted by washing the beads with 80% and 100% ethanol. After concentration under vacuum, the ethanol-water residue was extracted with chloroform. The lipid (3–4 g) thus obtained was chromatographed on a silicic acid column developed with chloroform, chloroform-methanol mixtures, and pure methanol. PAF-active, phospholipid-rich (major phospholipids were phosphatidylcholine, sphingomyelin and lysophosphatidylcholine, as shown by thin layer chromatography (TLC) and chemical ionisation mass spectrometry), fractions eluted with pure methanol, were further purified by high pressure liquid chromatography on a silicic acid column (Varian, Micropak Si-5) with chloroform-methanol-water as solvent. The active fraction (1–2 mg of lipids) was eluted between sphingomyelin and lysophosphatidylcholine; it migrated between these phospholipids on silica gel TLC, with a solvent containing by volume 100 : 5 : 1 of CHCl₃ : CH₃OH : H₂O. (Development was by use of iodine, Dittmer⁸ or Dragendorff reagents.) Final recovery was 25% of starting activity.

Three preparations of PAF were treated with the lipases: (1) crude PAF-BSA, dialysed overnight against TBS; (2) the most active fraction obtained after the first silicic acid chromatography, which exhibited three or four spots on TLC; and (3), the purest fraction described above.

The lipases used in this study were lipase from *Rhizopus arrhizus*, phospholipases A2 from either porcine pancreas or *Crotalus*⁸, phospholipases C from *Bacillus cereus*⁹, and D from cabbage¹⁰. Dr Zwaal (from Van Deenen's Laboratory, Utrecht) kindly provided purified phospholipase A2 from *Naja naja* and *Crotalus* and a phospholipase C from *Staphylococcus aureus* specific for sphingomyelin (sphingomyelinase)¹¹.

The conditions and the results of the treatment of PAF with lipases are shown in Table 1. The phospholipases A2 (from various origins) and the phospholipases C and D completely abolished the ability of PAF (preparations 2 and 3) to aggregate rabbit platelets. In contrast, the lipase from *R. arrhizus*, which hydrolyses exclusively the fatty acid ester bond at the 1-position of phosphoglycerides^{12,13}, and sphingomyelinase C, were totally ineffective in this respect. It should be noted that PAF-BSA (preparation 1) was insensitive to the action of phospholipases A2, but fully sensitive to phospholipases C and D. Controls for optimal conditions for the action of lipases were hydrolysis of phosphatidylcholine or, for sphingomyelinase, sphingomyelin, followed by TLC. We also verified that PAF treated with phospholipases A2, C and D, in the presence of EDTA, not only retained its full activity, but also migrated on TLC with the same *R_f* as untreated PAF. Finally, the purest PAF fraction (preparation 3) was first treated with sphingomyelinase C, then exposed to lipase from *R. arrhizus* as well as to phospholipases A2 and C. The results were the same as those reported above, indicating that treatment with sphingomyelinase, besides not affecting PAF activity, does not seem to modify the structure of the molecule.

The lack of effect of lipase from *R. arrhizus* and the sensitivity of PAF to phospholipases A2, C and D seems to

Table 1 Action of lipases on PAF activity

Lipases (concentrations per ml)	Medium	PAF activity (units ml ⁻¹)
Lipase (<i>R. arrhizus</i>)* 0.1 mg	Borate 0.1 M pH 6.5 CaCl ₂ 10 mM Deoxycholate 1 mg ml ⁻¹ BSA 0.4 mg ml ⁻¹	148
A2 (Porcine pancreas* or <i>Crotalus</i>) 0.1 mg	TBS pH 8 CaCl ₂ 10 mM	0†
A2 (<i>N. naja</i>), purified† 0.05 unit	TBS pH 8 CaCl ₂ 10 mM	0†
A2 (<i>Crotalus</i>), purified† 0.5 unit	TBS pH 8 CaCl ₂ 10 mM	0†
C (<i>B. cereus</i>)* 0.001 mg	TBS pH 8 CaCl ₂ 10 mM	0†
D (cabbage)* 0.01 mg	Acetate buffer 0.1 M pH 5.6 CaCl ₂ 50 mM	0†
C (<i>S. aureus</i>)† Sphingomyelinase, purified 4 units	TBS pH 8 CaCl ₂ 10 mM MgCl ₂ 0.25 mM	136

Experimental conditions were 15 min incubation at 37 °C except for lipase from *R. arrhizus* which was incubated for 18 h at 30 °C with constant agitation and phospholipase D, incubated 1 h at 20 °C. These experiments were repeated five times with identical results. PAF activity is expressed in units, as defined in refs 3 and 6. Starting solution of PAF contained 140 units ml⁻¹.

* From Boehringer-Mannheim.

† Gift from Dr Zwaal, Utrecht, The Netherlands.

‡ Same experiments done in the presence of 0.01 M EDTA: PAF activity was identical to that of starting solution.

favour PAF being a 1-lyso (or 2-acyl)-glycerophospholipid. Methylation by diazomethane for 10 min at room temperature¹⁴ did not affect the activity and the *R_f* of PAF, a result which suggests the presence of a choline polar head group. We therefore attempted to produce a PAF-like substance by hydrolysis of a variety of commercially available phospholipids with lipase from *R. arrhizus*, in order to obtain 1-lyso derivatives. We occasionally detected an aggregating activity shown to be due to production of arachidonic acid by its *R_f* on TLC and its inhibition by indomethacin. No PAF-like substance was obtained.

2-Acyl-glycerophospholipids are known to be unstable, and to isomerise to 1-acyl compounds¹⁵. This rearrangement, which occurs to some extent during chromatographic separation of the phospholipid, could account for the relatively low yield of our procedures, and could also represent an inactivation process of a potentially harmful mediator. The PAF-containing fraction, at the present state of purification, seems to be associated, in the chromatographic systems used, with sphingomyelin and lyso-phosphatidylcholine. The complete structure of PAF will be definitively known when more of the pure compound becomes available, making a more direct approach possible. But as the specificity for phospholipids of the enzymes used in this study is well established^{12,13} our present data leads us to postulate a phospholipidic structure for PAF, unique amongst the mediators of anaphylaxis.

This work was supported by contract 75-7-0942 from the Délégation Générale à la Recherche Scientifique et Technique and ATP 76-63 from INSERM.

J. BENVENISTE
J. P. LE COUEDIC

INSERM U25, Hôpital Necker,
161 rue de Sèvres, 75730 Paris Cedex 15

J. POLONSKY
M. TENCE

Institut de Chimie des Substances Naturelles,
CNRS, 91190 Gif-sur-Yvette, France

Received 9 March; accepted 14 July 1977.

*To whom correspondence should be addressed.

- Siraganian, R. P. & Osler, A. G. *J. Immun.* **106**, 1244-1251 (1971).
- Henson, P. M. & Cochrane, C. G. *J. exp. Med.* **133**, 554-571 (1971).
- Benveniste, J., Henson, P. M. & Cochrane, C. G. *J. exp. Med.* **136**, 1356-1377 (1972).
- Benveniste, J., Egido, J. & Gutierrez-Millet, V. *Clin exp. Immun.* **26**, 449-456 (1976).
- Benveniste, J. *Nature* **249**, 581-583 (1974).
- Camussi, J., Mencia-Huerta, J. M. & Benveniste, J. *Immunology* (in the press).
- Cazenave, J. P., Benveniste, J. & Fraser-Mustard, J. *Fedn Proc.* **36**, 454 (1977).
- Dittmer, J. C. & Lester, R. L. *J. Lipid Res.* **5**, 126-127 (1964).
- Meth. Enzym.* **32B**, 147-154 (1974).
- Meth. Enzym.* **35B**, 226 (1975).
- Zwaal, R. F. A., Roelofs, B., Comfurius, P. & Van Deenen, L. L. M. *Biochim. biophys. Acta* **406**, 83-96 (1975).
- Slotboom, A. J., De Haas, G. H., Bensen, P. P. M., Burbach-Westerhuis, G. J. & Van Deenen, L. L. M. *Chem. Phys. Lipids* **4**, 15-29 (1970).
- Semeriva, M., Benzonana, G. & Desnuelle, P. *Bull. Soc. Chim. Biol.* **49**, 71 (1967).
- Crone, H. D. *Biochim. biophys. Acta* **84**, 665-680 (1964).
- De Haas, A. J. & Van Deenen, L. L. M. *Biochim. biophys. Acta* **106**, 315-326 (1965).

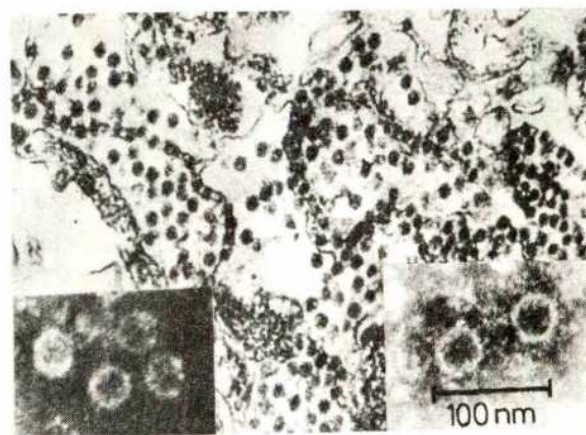
New oncogenic papova virus from primate cells

THE taxonomic group of papova viruses comprises a number of small icosahedral virus species, all isolated from mammals. The members of this group, such as papilloma, polyoma and the so-called vacuolating Simian virus 40 (SV40) contain double-stranded circular closed DNA. All are oncogenic, capable of transforming cells *in vitro*. Some viruses of this group which are called BK¹ and JC² have been isolated from human patients—BK-virus is apparently widespread, as more than 80% of the human population harbour antibodies against this virus³. We describe here a new member of the papova virus group, designated HD-virus, which we have isolated in our laboratory from primate cells (VERO-cells—a permanent line derived from *Cercopithecus aethiops* monkey kidney cells⁴—which have been routinely passaged for several years in our laboratory). HD-virus is capable of rapidly transforming cells *in vitro*.

Electron micrographs of ultra-thin sections of the cells (Fig. 1) reveal particles that correspond in size and morphology to SV40, although their diameter seems slightly smaller.

Incubation of growing VERO-cells (1 d after seeding) with either ³H-thymidine or ³²P-orthophosphate leads to the synthesis of radioactively labelled superhelical HD-viral DNA which can be selectively isolated and purified in a dye buoyant density gradient⁵. Stationary cells (6 d after seeding) reveal, in contrast, a smaller amount of HD-DNA (one-fifteenth of the amount obtained 1 d after seeding of the cells). The size of HD-DNA was estimated by comparison with the electrophoretic mobilities of SV40 and BK-DNAs in Agarose gels to be equivalent to 5,100 base pairs (molecular weight 3.1 × 10⁶ compared with 3.6 × 10⁶ for SV40 DNA). Thus, the HD-DNA is smaller than SV40

Fig. 1 Electron micrograph of an infected VERO-cell. Inserts: micrographs of negatively stained virions released from infected cells by freezing and thawing and concentrated by centrifugation.



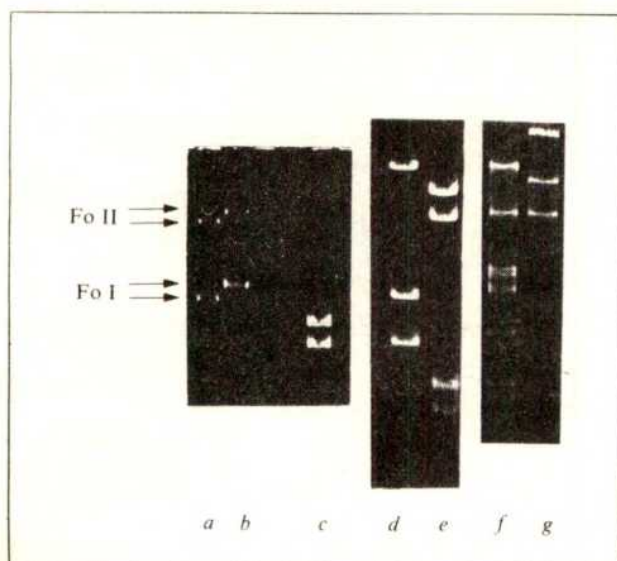


Fig. 2 Gel electrophoresis of DNA of HD, BK and SV40 virus. HD (a) and SV40 (b) form I and form II DNAs are shown in a and b. Fragments produced by digesting HD-DNA with *EcoRI* are depicted in c. A mixture of *HindII* + III was used to digest HD (d, g) BK- (e) and SV40-DNA (f). After electrophoresis the DNA was stained with ethidium bromide and photographs were taken under ultra violet light (a-e Agarose gel; f, g, polyacrylamide gel).

DNA by 14% (Fig. 2a, b). The susceptibility to various restriction endonucleases also distinguishes the HD genome from other papova virus genomes such as BK (Fig. 2e) and SV40 (Fig. 2f). One double-strand cut is produced by endonuclease *HindII* while two cuts are made by the enzymes *HindIII*, *EcoRI* (Fig. 2c) *HpaI* and *HpaII*. On the other hand, HD-DNA is not attacked by endonuclease *BamHI*. The molecular sizes of the three HD-DNA fragments shown in Figs 2d, g are 1.90 , 0.70 , and 0.50×10^6 respectively. Treatment with restriction enzymes and gel electrophoresis were performed as before⁶.

Unlike BK, JC, polyoma, and SV40 which all share some base-sequence homology with each other^{8,9,10} HD-virus proved in DNA-DNA hybridisation studies to be unrelated to the other viruses (Table 1). Using the stringent conditions of hybridisation in $2 \times \text{SSC}$ and 50% formamide at 37°C we found, in agreement with the data of others⁸, 10–16% homology between BK and SV40, while HD and SV40 displayed only 1.2% homology. BK and HD-DNA show as little as 0.2% homology. We were unable to demonstrate any homology between HD and polyoma virus in these conditions. Furthermore, anti-SV40 T antigen and

anti-SV40 capsid sera fail to react with HD-infected VERO-cells as judged by immunofluorescence.

VERO-cells which harbour HD-virus do not display any recognisable cytopathogenic effect (c.p.e.). They are routinely passaged at a weekly 1:4 split ratio in minimum essential medium (MEM) supplemented with 10% calf serum. Because of the lack of c.p.e. we have not been able yet to establish a plaque assay for titration of the HD-virus. Thus far we have been using the synthesis of HD-superhelical FO I DNA as a measure of infectivity. Using this assay it could be shown that lysates of our VERO-cells that were obtained after freezing and thawing followed by DNase and ether treatment retained the ability to induce the synthesis of HD-FO I DNA. For this purpose 10^7 VERO-cells were frozen and thawed twice in 50 ml of MEM, the cellular debris was removed by centrifugation (10 min, 4,000 r.p.m., Christ Heraeus Minifuge), and $100 \mu\text{g}$ of pancreatic DNase (Serva) were added for 60 min at 37°C after adjusting the medium to 10 mM MgCl_2 . Then an equal volume of ether was added, the mixture was shaken for 5 min, and the medium was separated from the ether. Residual ether was removed by bubbling N_2 through the solution. Ten ml, representing one-fifth of the virus-containing solution were used after passing it through a Millipore

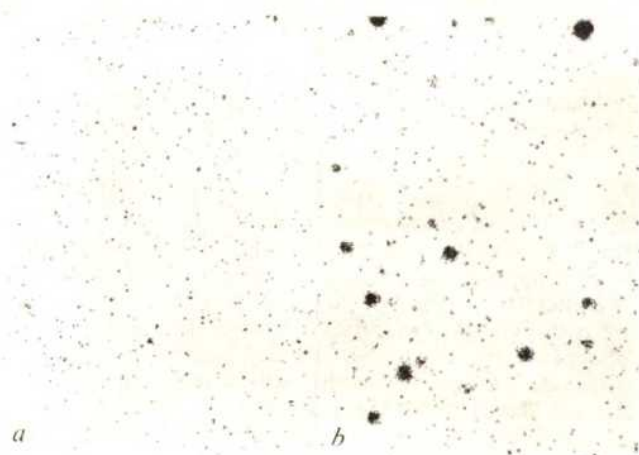


Fig. 3 Soft agar assays of a, uninfected VERO-cells (McMaster) and b, the same cells after infection with cell lysate containing HD-virus.

Millex filter (pore diameter $0.22 \mu\text{m}$) for infection of approximately 10^7 cells (1-h adsorption time). Four days later the cells were labelled with ^3H -thymidine ($500 \mu\text{C}$ per 50 ml) for 1 d and the superhelical DNA was purified as before. We found that HD-DNA replicates, although poorly, in RITA cells, another permanent line derived from *Cercopithecus aethiops* (Italdiagnostics). Other cell lines such as 3T3, WI38, CV-1 and human embryonic lung proved to be non-permissive for the replication of HD-DNA.

To test whether VERO-cells that have been maintained in other laboratories also contain HD-virus or HD-viral DNA we have attempted to label such cells using the procedure described above. We were unable to demonstrate the presence of HD-FO I DNA in VERO-cells from the Institute for Virology, Universität Erlangen, and from the Department of Pathology, McMaster University, Hamilton, Ontario. VERO-cells from McMaster University totally lack HD-DNA and fail to grow in soft agar¹¹. VERO-cells grown in our laboratory contain HD-DNA and 0.1–0.5% grow to form colonies in soft agar. When the Canadian VERO-cells were infected with lysate of our VERO-cells (prepared as described above) about 1% of the cells formed colonies in soft agar (Fig. 3). The number of colonies was not increased by doubling or tripling the virus-containing inoculum. This

Table 1 DNA-DNA hybridisation between HD and other papova DNAs

³ H-DNA in solution	DNA on filter (1 μg)	³ H-c.p.m. input	³ H-c.p.m. bound	% Bound to filter*
HD	SV40	9×10^4	0	0
	BK		200	0.2
	HD		56,300	62.4
SV40	SV40	1.29×10^5	75,500	58.5
	BK		21,040	16.3
	HD		1,602	1.2
BK	SV40	6.5×10^4	6,950	10.7
	BK		49,460	76.0
	HD		0	0
Polyoma	SV40	2.2×10^4	250	1.1
	BK		0	0
	HD		0	0

Viral DNAs were labelled⁶ and purified⁶ and filter hybridisation assays⁷ were performed essentially as before.

*Corrected for nonspecific binding to T4-DNA.

might be explained by the presence of a limited number of cells, which are capable of responding to infection with the formation of colonies in soft agar. Between 0.7 and 1% of the infected cells grew to form colonies in medium containing 2% calf serum. Only 0.04% of the uninfected cells gave rise to colonies in the same conditions. This indicates a reduced serum requirement for the infected cells^{12,13}. We believe, therefore, that HD-virus can transform VERO-cells.

We have thus isolated what seems to be an entirely novel papova virus and we intend to screen human and other tumours for evidence of its distribution.

We thank K. Bosslet, T. de Ledezma, M. Pernfuss and C. Waldeck for technical assistance and J. Tooze for help in preparation of the manuscript. This work was supported by a grant of the Bundesminister für Forschung und Technologie.

Note added in proof: While this paper was in press we have found that there exists an additional size class of HD-FO I DNA which is by 6% larger than the HD-DNA described here.

WALDEMAR WALDECK
GERHARD SAUER

Institut für Virusforschung,
Deutsches Krebsforschungszentrum,
6900 Heidelberg, Germany

Received 11 March; accepted 12 July 1977.

- 1 Gardner, S. D., Field, A. M., Coleman, D. V. & Hulme, B. *Lancet* i, 1253 (1971).
- 2 Padgett, P. L., Walter, D. L., Zu Rhein, G. M., Eckroade, R. J. & Dessel, B. H. *Lancet* i, 1257 (1971).
- 3 Gardner, S. D. *Br. med. J.* 1, 77 (1973).
- 4 Yasumura, T. & Kawakita, Y. *Nippon Rinsho* 21, 1201 (1963).
- 5 Radloff, R., Bauer, W. & Vinograd, J. *Proc. natn. Acad. Sci. U.S.A.* 57, 1514 (1967).
- 6 Waldeck, W., Chowdhury, K., Gruss, P. & Sauer, G. *Biochim. biophys. Acta* 425, 157 (1976).
- 7 Kuhn, C., Waldeck, W. & Sauer, G. *Z. Krebsforsch.* 83, 65 (1975).
- 8 Khoury, G., Howley, P. M., Baron, G. & Mullerkey, M. F., *Proc. natn. Acad. Sci. U.S.A.* 72, 2563 (1975).
- 9 Osborn, J. E., Robertson, S. M., Padgett, B. L., Walter, D. L. & Weisblum, B. *J. Virol.* 19, 675 (1976).
- 10 Ferguson, J. & Davis, R. W. *J. molec. Biol.* 94, 496 (1976).
- 11 Macpherson, I. & Montagnier, L. *Virology* 23, 291 (1964).
- 12 Clarke, G. D., Stoker, M. G. P., Ludlow, A. & Thornton, M. *Nature* 227, 798 (1970).
- 13 Dulbecco, R. *Nature* 227, 802 (1970).

Type C RNA virus production and cell competence for normal differentiation in myeloid leukaemic cells

STUDIES with various species have shown that type C viral genomes can be integrated and vertically transmitted as chromosomal elements within the cellular genomes (for review see refs 1-6) and that nucleic acid sequences derived from these viruses can be detected in the DNA⁷⁻¹⁰ of normal cells (for review see ref. 11). It has been suggested that in addition to their role in tumour formation, type C RNA viruses may also have a role in normal cell differentiation^{2,3}. We suggest here that one way in which these viruses may influence differentiation, is by modifying the competence of cells to be induced to differentiate by a normal regulator.

Normal differentiation-inducing regulators have been identified in cells of the haematopoietic system and these include the protein erythropoietin¹² which induces differentiation of erythrocytes and the protein that we now call MGI (macrophages and granulocyte inducer) (refs 13, 14) which induces differentiation of macrophages and granulocytes^{13,15-18}. The isolation of clones of haematopoietic cells with different degrees of competence for differentiation by one of these proteins, should be a useful experimental system to test the relationship between type C RNA viruses and cell competence for differentiation by a specific regulator. We have isolated such clones from

mouse myeloid leukaemia, which differ in their competence to be induced to differentiate by MGI (refs 13, 19-23). One type of clone, MGI⁺D⁺, can be induced by MGI to undergo normal cell differentiation, including the formation of rosettes for cell surface Fc and C3 receptors^{20,21}, the synthesis and secretion of lysozyme²² and differentiation to mature macrophages and granulocytes^{13,19}. A second type of clone, MGI⁺D⁻, can be induced for Fc and C3 rosettes and lysozyme but not to differentiate to mature cells and a third type, MGI⁻D⁻, was not inducible for any of these properties (Table 1). Some, but not all the properties associated with cell differentiation can also be induced in some of these myeloid leukaemic clones by the steroid hormones dexamethasone, prednisolone or oestradiol^{20,21,24} and compounds such as cytosine arabinoside, actinomycin D, 5-iododeoxyuridine²⁰ or dimethylsulphoxide²². The protein inducer¹³⁻¹⁸ that we call MGI has also been referred to by others as colony stimulating factor²³ or activity²⁰. We show here that the competence of myeloid leukaemic clones to be induced to differentiate to mature macrophages and granulocytes by the normal regulator MGI, is associated with a higher production of type C virus.

Table 1 Competence for the induction of differentiation in clones of myeloid leukaemic cells by the differentiation-inducing protein MGI

Cell type	Clone numbers	Inducibility by MGI			
		Rosettes Fc	Rosettes C3	Lysozyme	Mature macrophages and granulocytes
MGI ⁺ D ⁺	9,11,12	+	+	+	+
MGI ⁺ D ⁻	5,13,19	+	+	+	-
MGI ⁻ D ⁻	1,6,10	-	-	-	-

(+), Inducible, (-), non-inducible. Cells were incubated with conditioned medium containing MGI as described²³. MGI⁺D⁺ clones 9,11,12 and MGI⁺D⁻ clones 5,13,19 (refs 20, 21) were derived from a myeloid leukaemia originating in a SL mouse²⁶. MGI⁻D⁻ clones 1,6,10 (refs 21-23) were derived from three independently induced myeloid leukaemias after X-ray irradiation of SJL/J mice. Before treatment with MGI, cells from all the clones grew in suspension as myeloblasts to promyelocytes and produced myeloid leukaemia after inoculation into isologous adult mice. The cells were cultured in Eagle's medium with a fourfold concentration of amino acids and vitamins (H-21, GIBCO) and 10% inactivated (56 °C, 30 min) foetal calf serum.

All the nine clones tested of the three types shown in Table 1 were found to be producing type C virus. Typical type C particles were observed by electron microscopy in sections of the cultured cells and at a buoyant density of 1.15gcm⁻³ in a sucrose equilibrium density gradient of pellets from the culture medium. To quantitate the amount of virus released by the different clones, we measured the reverse transcriptase activity⁴ of partially purified and concentrated virus pellets obtained from the culture fluids²⁸. The virus production measured by this enzyme activity with the template oligo(dT):poly(rA), and cellular growth rate of three clones representative of the different types of myeloid leukaemic cells are shown in Fig. 1. The MGI⁺D⁺ and the MGI⁺D⁻ clones had similar growth rates, while the MGI⁻D⁻ clones grew somewhat faster. Virus production in all the clones was not significantly different for the first 2 d after seeding. When the cells entered the logarithmic phase of growth, however, virus production increased 16-fold in the MGI⁺D⁺ clone and only 2-fold in the MGI⁺D⁻ and MGI⁻D⁻ clones. Optimum virus production was obtained with all three types of clones at day 4 and then declined when the cells approached the stationary phase of growth. A similar relationship between type C RNA virus production and cellular growth has been observed in cultures of

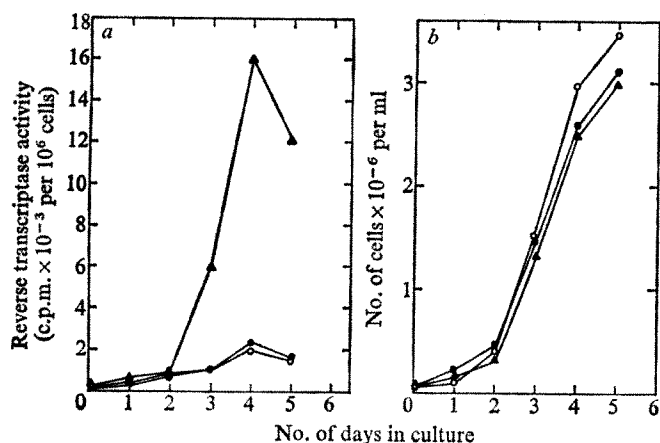


Fig. 1 Reverse transcriptase activity in the tissue culture medium (a) and number of cells (b) at different days after seeding of the three types of myeloid leukaemic clones. ▲, MGI⁺D⁺ clone 9 and ●, MGI⁺D⁻ clone 5 were seeded at 4×10^5 cells and ○, MGI⁻D⁻ clone 6 at 2×10^5 cells per 10-cm Nunc tissue culture Petri dish in 10 ml modified Eagle's medium (see Table 1) and 10% inactivated foetal calf serum. To assay for reverse transcriptase activity⁴, the cells were pelleted from 20 ml of medium and the medium clarified of cell debris²⁸ by centrifugation at 10,000 r.p.m. in a SS-34 rotor in a RC-5 Sorval centrifuge at 4 °C. The clarified medium was layered on a 5-ml cushion of 20% (v/v) glycerol in TNE buffer (0.1 M NaCl, 0.01 M Tris pH 7.6, and 0.001 M EDTA) and centrifuged at 40,000 r.p.m. for 60 min in a 60Ti type rotor at 4 °C in a Beckman ultracentrifuge. The resultant pellets were suspended in 0.1 ml TNE buffer. Enzyme activity was measured using [the synthetic template oligo(dT):poly(rA)] (Collaborative Research). Oligo(dT) and poly(rA) were pre-annealed at a ratio of 1:10 in 0.15 M NaCl, 10 mM Tris-hydrochloride (pH 7.8) at 37 °C for 5 min. Enzyme reaction mixtures (50 µl) consisted of 2 µM Tris (pH 7.8) 6 µM NaCl, 0.1 µM MnCl₂, 0.4 µM DTT, 0.015% NP/40, 5 µg oligo(dT):poly(rA), 0.001 µM unlabelled TTP, 2 µCi H³-dTTP, and 10 µl of the suspended virus pellet in TNE buffer. Reactions were carried out for 30 min at 37 °C and terminated by addition of 0.1 ml 0.2 M sodium pyrophosphate and 1 ml 10% trichloroacetic acid. Samples were collected on 0.45 µm Millipore filters rinsed with 5% cold trichloroacetic acid and counted in a toluene-based scintillation fluid in a Tri-carb Packard fluid scintillation counter. The values represent the means of three independent experiments.

other cell types²⁹⁻³². Since virus production in our experiments reached an optimum at day 4, all the following experiments were carried out on the fourth day after seeding.

Studies with the 9 clones have shown (Fig. 2), that only the three MGI⁺D⁺ clones, which can be induced to differentiate to mature macrophages and granulocytes, produced high levels of virus, while the partially inducible MGI⁺D⁻ and the non-inducible MGI⁻D⁻ clones produced lower levels of virus. The amount of virus produced by the MGI⁺D⁺ clones was about 4-5-fold higher than the highest amount produced by any of the MGI⁺D⁻ or MGI⁻D⁻ clones (Fig. 2). Three subclones isolated from MGI⁺D⁺ clone 9 and three subclones from MGI⁺D⁻ clone 5 were assayed for reverse transcriptase activity. All the MGI⁺D⁺ subclones showed the same high enzyme activity in the culture medium as the parental clone 9, while all the MGI⁺D⁻ subclones showed the lower enzyme activity of the parental clone 5.

The higher enzyme activity in the culture fluids from MGI⁺D⁺ clones was further identified as type C virus reverse transcriptase, by sucrose equilibrium density gradient centrifugation of the partially purified virus preparations from the three types of myeloid leukaemic clones. The peak of the reverse transcriptase activity at the buoyant density of 1.15 g cm^{-3} was 10-fold higher with MGI⁺D⁺ clone 9 than with MGI⁺D⁻ clone 5 and MGI⁻D⁻ clone 6. The virus specific nature of the enzyme was also confirmed by using, in addition to oligo(dT):poly-

(rA), two other synthetic templates, oligo(dG):poly(rC), a viral specific template, and oligo(dT):poly(dA), which is utilised poorly by the viral RNA directed DNA polymerase and is a good template for the cellular DNA-DNA polymerases³³⁻³⁶ (for review see ref. 37). The results have shown that with pellets obtained from culture fluids of all the 9 clones, the highest activity with oligo(dG):poly(rC) was again found with the MGI⁺D⁺ clones and there was almost no activity with oligo(dT):poly(dA). Incubation of cells for 1 d with 50 units of purified mouse interferon (from Dr I. Gresser, Institut de Recherches Scientific sur le Cancer, Villejuif), gave 70%-80% reduction in the enzymatic activity that could be recovered from the culture medium. This is in agreement with previous results showing that interferon can inhibit the secretion of type C virus³⁸⁻⁴⁰, and provides additional evidence for the viral nature of the activity measured.

Indirect immunofluorescence tests⁴¹ with an antiserum against murine leukaemia virus group-specific (MULV-gs) antigens (from Dr M. Haas⁴²) showed that all three types of clones were positive for these viral antigens and that all the cells were stained. After acetone fixation, MGI⁺D⁺ clones seemed to be the most brightly stained and titration of MULV-gs antigens by absorption of immunofluorescence with cell extracts⁴² has indicated, that the MGI⁺D⁺ clones had a higher amount of MULV-gs antigens. Unfixed cells tested for cap formation also showed a higher frequency of caps for MULV-gs antigens on MGI⁺D⁺ cells (Table 2).

Table 2 MULV-gs antigens. Titre of cell extracts tested by immunofluorescence absorption, and cap formation on unfixed cells

Cell type	Clone no.	Immunofluorescence absorption, antigen dilution of cell extracts						% Cells with cap
		0	1:2	1:4	1:8	1:16	1:32	
MGI ⁺ D ⁺	9	—	—	—	—	++	+++	90±5
MGI ⁺ D ⁻	5	—	—	+	++	+++	+++	10±3
MGI ⁻ D ⁻	6	—	+	++	+++	+++	+++	2±1

MULV-gs antigen titre of cell extracts was tested by absorption of immunofluorescence. Cells at 4 d after seeding were sonicated in Earl's balanced salt solution to give a 20% cell extract. Serial dilutions of this extract (40 µl) were then incubated overnight with an equal volume of Lewis rat anti-MULV-gs antiserum⁴² at 4 °C. Residual immunofluorescence after incubation with fluorescent rabbit anti-rat IgG (Miles Yeda) was scored on an acetone fixed NIH/3T3 Moloney virus producing mouse cell line on a scale from —(no immunofluorescence) to +++ (strong immunofluorescence obtained without absorption). Absorption tests were performed with a concentration of antiserum (1:32) two doubling dilutions below the end-point for the acetone fixed NIH/3T3 Moloney virus producing mouse cells. Cap formation on cells 4 d after seeding was tested after incubation of unfixed cells with rat anti-MULV-gs antiserum (1:8) for 15 min and then for 30 min with fluorescent rabbit anti-rat IgG. There was no immunofluorescence after incubation with normal rat serum instead of the anti-MULV-gs antiserum.

Our results show that mouse myeloid leukaemic cells that can be induced to differentiate to mature macrophages and granulocytes by the normal inducer protein MGI, produced a higher amount of type C RNA virus than clones of myeloid leukaemic cells that can be only partially or not at all induced to differentiate by this normal regulator. The amount of virus production was, therefore, associated with the competence of the leukaemic cells to undergo differentiation to normal mature cells. It will be of interest to identify the virus or viruses produced. Type C RNA viruses have been observed in myeloid leukaemic cells from human and rats⁴³⁻⁴⁵, and it will also be of interest to determine in these two other species, the relationship between the amount of virus production and ability of the cells to be induced to differentiate by the normal regulator MGI.

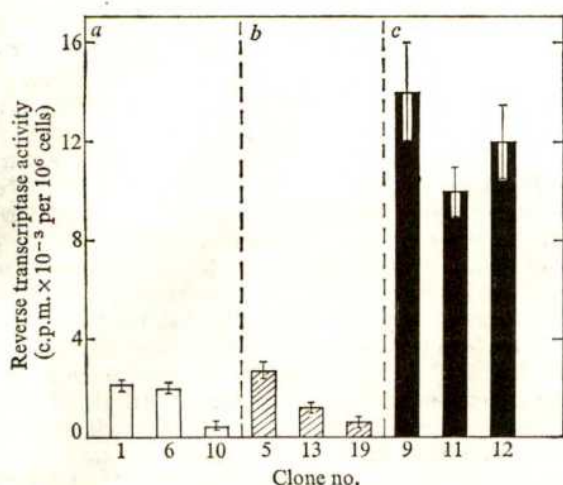


Fig. 2 Reverse transcriptase activity in the culture fluids of nine myeloid leukaemic clones. MGI⁺D⁺ clones 9,11,12 (c) and MGI⁺D⁻ clones 5,13,19 (b) were seeded at 4×10^5 cells and MGI⁻D⁻ clones 1,6,10 at 2×10^5 cells (a) per 10-cm tissue culture Petri dish. The culture medium was collected 4 d after seeding and reverse transcriptase activity measured as described in the legend to Fig. 1. The means and standard errors are based on six independent experiments.

Friend erythroleukaemic cells can be induced to differentiate partially by dimethylsulphoxide⁴⁶ and some other polar compounds⁴⁷ and the competence for this partial induction of erythroid differentiation was not associated with the amount of virus production³². However, the erythroleukaemic cells studied had lost the ability to respond to erythropoietin⁴⁸, the normal regulator of erythroid cell differentiation, whereas the MGI⁺ myeloid leukaemic cells, including those that can respond to dimethylsulphoxide²², have not lost the ability to respond to MGI, the normal regulator of differentiation to macrophages and granulocytes. It may also be that the viruses produced by the Friend erythroleukaemic cells differ from those produced by the myeloid leukaemic cells and that not all type C viruses are related to cell competence for differentiation.

One possible explanation for the relationship that we have found in our myeloid leukaemic cell system may be the association of viral sequences with regulatory sites for differentiation. The increased virus production could then indicate a different state of the regulatory sites in the competent compared with the non-competent cells. The higher virus production by the competent cells could either be a by-product or directly influence these regulatory sites. Another possibility is that the increased virus production by the competent cells is associated with an appropriate arrangement of surface receptors for MGI that is required for the induction of normal cell differentiation. The differences in cap formation by MULV-antigens (Table 2) and con A (refs 13, 49) indicate that it will be of interest to determine whether, as in cells transformed by sarcoma viruses⁵⁰, there are also differences in receptors for other polypeptide hormones. The myeloid leukaemic clones used in our studies seem to be a particularly favourable experimental system to investigate further these possibilities of the role of type C viruses in relation to cell competence for differentiation by a normal receptor.

We thank Dr M. Haas for help in experiments using anti-MULV-gs antiserum and Mrs Haya Dorf for technical assistance.

DAN LIEBERMANN
LEO SACHS

Department of Genetics,
Weizmann Institute of Science,
Rehovot, Israel

Received 18 May; accepted 11 July 1977.

- Gross, L. *Oncogenic Viruses* (Pergamon, New York, 1970).
- Huebner, R. J. & Tadaro, G. J. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1087-1094 (1969).
- Temin, H. M. *J. natn. Cancer Inst.* **46**, III-VII (1971).
- Temin, H. M. & Baltimore, D. *Adv. Virus Res.* **17**, 129-186 (1972).
- Toozé, J. *The Molecular Biology of Tumour Viruses* (Cold Spring Harbor Laboratory, New York, 1973).
- Gillespie, D., Saxinger, W. C. & Gallo, R. C. *Prog. Nucleic Acid Res. molec. Biol.* **15**, 1-108 (1975).
- Gelb, L. D., Aaronson, S. A. & Martin, M. A. *Science* **172**, 1353-1355 (1971).
- Baluda, M. A. *Proc. natn. Acad. Sci. U.S.A.* **69**, 576-580 (1972).
- Neiman, P. E. *Virology* **53**, 196-203 (1973).
- Gelb, L. D., Milstein, J. B., Martin, M. A. & Aaronson, S. A. *Nature new Biol.* **244**, 76-79 (1973).
- Aaronson, S. A. & Stephenson, J. R. *Biochim. biophys. Acta* **458**, 323-354 (1976).
- Krantz, S. B. & Jacobson, L. O. *Erythropoietin and the Regulation of Erythropoiesis* (University of Chicago Press, Chicago, 1970).
- Sachs, L. in *Harvey Lectures* **68**, 1-35 (Academic, New York, 1974).
- Landau, T. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2540-2544 (1971).
- Pluznik, D. H. & Sachs, L. *J. cell. comp. Physiol.* **66**, 319-324 (1965).
- Pluznik, D. H. & Sachs, L. *Expl Cell Res.* **43**, 553-563 (1966).
- Ichikawa, Y., Pluznik, D. H. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **56**, 488-495 (1966).
- Bradley, T. R. & Metcalf, D. *Aust. J. exp. Biol. med. Sci.* **44**, 287-299 (1966).
- Fibach, E., Hayashi, M. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 343-346 (1973).
- Lotem, J. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3507-3511 (1974).
- Lotem, J. & Sachs, L. *J. Immun.* **117**, 580-586 (1976).
- Krystosek, A. & Sachs, L. *Cell* **9**, 675-684 (1976).
- Azumi, J. & Sachs, L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 253-257 (1977).
- Lotem, J. & Sachs, L. *Int. J. Cancer* **15**, 731-740 (1975).
- Metcalf, D. *J. cell Physiol.* **74**, 323-332 (1969).
- Austin, P. E., McCulloch, E. A. & Till, J. E. *J. cell Physiol.* **77**, 121-134 (1971).
- Ichikawa, Y. *J. cell Physiol.* **74**, 223-234 (1969).
- Lieber, M. M., Livingstone, P. M. & Todaro, G. F. *Science* **181**, 443-444 (1974).
- Humphries, E. H. & Temin, H. M. *J. Virol.* **14**, 531-546 (1974).
- Greenberger, J. S. & Aaronson, S. A. *J. Virol.* **15**, 64-70 (1975).
- Fischinger, P. J., Tuttle-Fuller, N., Huper, G. & Bolognesi, D. P. *J. Virol.* **16**, 267-274 (1975).
- Sherton, C. C., Evans, L. H., Polonoff, E. & Kabat, D. *J. Virol.* **19**, 118-125 (1976).
- Weissbach, A., Bolden, A., Muller, R., Hanafusa, H. & Hanafusa, T. *J. Virol.* **10**, 321-327 (1972).
- Baltimore, D. & Smoler, D. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1507-1511 (1970).
- Spiegelman, S. et al. *Nature* **228**, 430-432 (1970).
- Goodman, N. C. & Spiegelman, S. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2203-2206 (1971).
- Sarngadharan, M. G., Alloudeh, H. S. & Gallo, R. C. *Meth. Cancer Res.* **12**, 3-47 (1976).
- Liebermann, D., Voloch, Z., Aviv, H., Nudel, U. & Revel, M. *Molec. Biol. Rep.* **1**, 447-451 (1974).
- Swetly, P. & Ostertag, W. *Nature* **251**, 642-644 (1974).
- Metz, D. H. *Cell* **6**, 429-439 (1975).
- Hilgers, J., Nowinski, R. C., Geering, G. & Hardy, W. *Cancer Res.* **32**, 98-106 (1972).
- Haas, M. *J. natn. Cancer Inst.* **58**, 251-257 (1977).
- Mak, T. W., Manaster, J., Howatson, A. F., McCulloch, E. A. & Till, J. E. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4336-4340 (1974).
- Gallagher, R. E., Salahuddin, S. Z., Hall, W. T., McCredie, K. B. & Gallo, R. C. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4137-4141 (1975).
- Greenberger, J. S., Aaronson, S. A., Rosenthal, D. S. & Moloney, W. C. *Nature* **257**, 143-144 (1975).
- Friend, C., Scher, W., Holland, J. G. & Sato, T. *Proc. natn. Acad. Sci. U.S.A.* **68**, 378-382 (1971).
- Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. & Marks, P. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 862-866 (1976).
- Kluge, N., Gaeddicke, G., Steinheider, G., Dube, S. & Ostertag, W. *Expl Cell Res.* **88**, 257-262 (1974).
- Lotem, J., Vlodavsky, I. & Sachs, L. *Expl Cell Res.* **101**, 323-330 (1976).
- Todaro, G. J., DeLarco, J. E. & Cohen, S. *Nature* **264**, 26-31 (1975).

Post-transcriptional control of avian oncornavirus transforming gene sequences in mammalian cells

TRANSFORMATION of cells by RNA tumour viruses generally results in a stable association of the viral genome with the morphologically altered cell. Studies with temperature-sensitive mutants of these viruses have clearly demonstrated that viral-specific gene functions are required for establishment and maintenance of the transformed phenotype¹⁻⁶. For avian oncornaviruses the transforming (sarcoma) gene sequences are required for both transformation of cells in tissue culture and the production of tumours in animals⁷⁻⁸. In all of the oncornavirus-infected mammalian cell systems studied to date regulation of expression of the avian RNA tumour virus transforming gene sequences seems to be under the influence of transcriptional control mechanism(s) because appreciable differences in the amount of sarcoma-specific RNA can be detected in cells exhibiting normal and transformed phenotypes. For instance, the amount of sarcoma-specific RNA was substantially reduced in most revertant subclones of Rous sarcoma virus (RSV)-infected hamster cells compared with the original transformed clones⁹⁻¹¹. Although several revertant RSV-hamster cells exhibited less dramatic differences in sarcoma-specific RNA, it nevertheless seemed that the transformed

phenotype was directly related to the extent of transcription of the virus transforming gene sequences in this transformed/revertant cell system. A similar correlation between the amount of sarcoma-specific viral RNA and the malignant phenotype was recently reported for murine sarcoma virus-infected cells¹².

Here we present data indicating that mammalian cells may also regulate the expression of viral transforming gene sequences by post-transcriptional mechanisms. This conclusion is based on the observation that revertant subclones of RSV-infected field vole cells contain as much, if not more, sarcoma-specific RNA as the transformed cells from which they were derived. Our preliminary data also indicate that the post-transcriptional restriction of expression of viral transforming genes in revertant vole cells does not result from either selective transport of sarcoma-specific RNA from the nucleus to the cytoplasm, or the lack of association of this RNA species with polyribosomes.

An established diploid cell line from the European field vole, *Microtus agrestis* was transformed by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) by Dr P. Vogt of the University of Southern California. The procedures for the isolation of clones of RSV-transformed mammalian cells and revertant subclones have been described elsewhere^{13,14}. The morphology of typical normal, transformed, and revertant vole cell types can be seen in Fig. 1. Not only did the revertant subclones resemble normal cells in their 'flat' fibroepithelial-like morphology but they also failed to grow in soft agar (R. Krzyzek, unpublished observations).

We have previously demonstrated that both transformed and revertant field vole cells contain similar genome-equivalents of virus-specific DNA¹⁵. These studies were performed by determining the influence, if any, that unlabelled DNA from RSV-transformed, and reverted vole cells has on the reassociation kinetics of the more complex double-stranded DNA component synthesised by the RSV RNA-directed DNA polymerase *in vitro*¹⁶. However, these methods cannot completely exclude the possibility that a small portion (<20%) of the viral genome is deleted from the DNA of the reverted cell because the virus-specific DNA probe used in these studies may not represent the entire viral genome¹⁶. Consequently it was conceivable that the reversion phenomenon could result from the complete or partial deletion of the RSV-transforming gene sequences. To determine whether the RSV-transforming gene sequences were retained in vole cells during reversion we have

performed further hybridisation analysis with a specific radiolabelled DNA probe complementary to the RSV-transforming gene sequences (cDNA_{src})¹⁷. As shown in Table 1 the cDNA_{src} probe hybridises equally well with DNA obtained from either transformed or revertant field vole cells. The extent of hybridisation is not complete in our conditions of hybridisation even with DNA from RSV-transformed avian cells. The reason for this is not clear; however this problem is not unique to our situation but has been observed by other investigators as well¹⁸. Even if complete hybridisation was achieved, this would not negate the possibility that the transforming gene in the revertant cell type was inactive due to a very small gene deletion or a point mutation which would not be detectable by the hybridisation techniques used. Therefore we performed studies to determine whether the transforming gene sequences present in the revertant vole cells were biologically active and capable of inducing transformation. As indicated in Table 2, the entire infectious RSV(D) genome could be rescued from transformed and revertant cells by either fusion with permissive cells or transfection of avian cells with DNA purified from both cell types. Although we have not computed precise frequencies of rescue, the efficiency of rescue of virus from transformed and revertant cells by both techniques was quite similar.

Table 1 Hybridisation of cDNA_{src} to transformed and revertant field vole cell DNA

Source of cellular DNA	Hybridisation (%)
RSV-infected duck	49.5
RSV-transformed field vole	
Clone 1	43.0
Clone 22	33.0
Revertant field vole	
Subclone 866	42.0
Subclone 4	44.6
Field vole liver	0.0

Cellular DNA was prepared as previously described¹⁹ and fragmented to a length of 300–400 nucleotides by treatment with 0.3 N NaOH for 30 min at 100 °C. Hybridisation analysis was performed in 30- μ l reaction volumes containing 0.6 M NaCl, 0.02 M Tris-HCl (pH 7.4) 0.002 M EDTA, 0.05% SDS, ³H-cDNA_{src} (1,500 c.p.m.) and a vast excess of fragmented, denatured cellular DNA (12 mg ml⁻¹) to C₀t values of approximately 5×10^4 mol s l⁻¹. All reactions were incubated for 70 h at 68 °C and the extent of hybridisation was determined by resistance to S₁ nuclease^{20,21}. % hybridisation was corrected for the intrinsic S₁ nuclease-resistance of cDNA_{src} (13.0%).

Since the revertant vole cells contain the entire biologically active sarcoma virus genome, it was of interest to determine whether an alteration in the transcriptional expression of the proviral DNA genome was responsible for phenotypic reversion. Our previous studies had indicated that revertant field vole cells contained similar amounts of virus-specific RNA as the transformed cell types (R. Krzyzek, A.F.L., P. Vogt & A.J.F., submitted for publication). The cDNA probe used in these studies was a relatively uniform representation of the viral RNA genome and indicated that the bulk (~70%) of the RSV-specific nucleotide sequences were expressed as virus-specific RNA in both transformed and revertant cells. However, this probe was not sufficiently sensitive to determine whether differences in transcription of the viral transforming gene occurred in these cell types. Therefore, to determine directly the expression of the RSV-transforming gene sequences in transformed and revertant vole cells we used the ³H-labelled cDNA_{src} probe to quantitate sarcoma-specific RNA in these cells. From Fig. 2 it can be seen that the rates of hybridisation of the cDNA_{src} probe to total cell RNA obtained from both transformed and revertant field vole cells was not appreciably different,

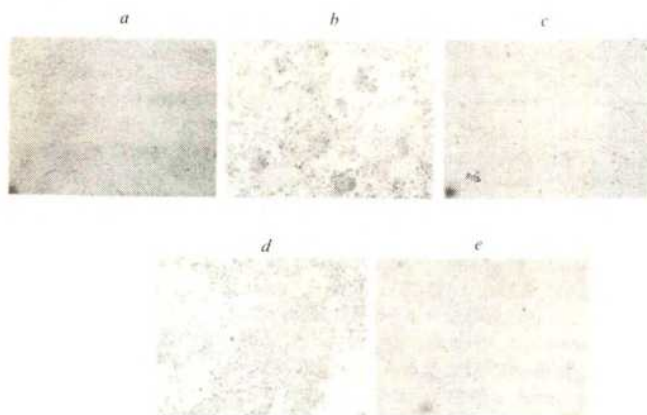


Fig. 1 Morphology of RSV-transformed and revertant field vole cells. *a*, Normal field vole cells; *b*, RSV-transformed field vole cells (clone 1); *c*, Revertant field vole cells (subclone 866); *d*, RSV-transformed field vole cells (clone 22); *e*, Revertant field vole cells (subclone 4). All photographs were taken at 80 \times magnification.

Table 2 Rescue of Rous sarcoma virus from transformed and revertant field vole cells by DNA transfection and Sendai virus-mediated fusion with susceptible chicken cells

Cell type	Transfection	Sendai virus-mediated fusion
RSV-transformed field cells		
Clone 1	+	+
Clone 22	ND	+
Revertant field vole cells		
Subclone 4	ND	+
Subclone 866	+	+
Uninfected field vole cells	-	-

Cellular DNA was prepared from field vole cells by the method of Haase *et al.*²². Transfections were performed by coprecipitation of DNA with calcium phosphate^{23,24}. Briefly, DNA dissolved in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate (HEPES)-saline-phosphate buffer at 20 $\mu\text{g ml}^{-1}$ was adjusted to 0.125 M CaCl_2 and allowed to precipitate at room temperature for 30 min. Aliquots (0.5 ml) of the DNA-calcium phosphate mixture containing approximately 10 μg of DNA were then added to chick secondary fibroblasts ($0.5\text{--}10^6$ cells per 60-mm tissue culture plate) previously washed twice with (HEPES)-saline-phosphate buffer. After 30 min incubation at room temperature, 2.5 ml of growth medium containing 10% calf serum but without tryptose phosphate broth was added to the cultures which were then incubated for 4 h at 37 °C. The growth medium containing the DNA-calcium phosphate mixture was then removed and replaced with growth medium containing tryptose-phosphate broth with 10% calf serum. Foci of transformed cells appeared within 10 to 14 d. When revertant or RSV-transformed field vole cell DNA was treated with pancreatic DNase (10 $\mu\text{g ml}^{-1}$, 30 min at room temperature) before the addition of CaCl_2 , no transformation of chick fibroblasts was observed.

Rescue of RSV from field vole cells by Sendai-mediated fusion with chick fibroblasts was performed as described by Boettiger²⁵. The procedure involved adding 0.1 ml of β propiolactone-inactivated Sendai virus (200–400 haemagglutination units) to cultures of chick secondary fibroblasts ($0.5 \times 10^6\text{--}1.0 \times 10^6$ cells per 35-mm tissue culture plate) previously washed twice with cold growth medium without serum. The cultures were then incubated for 15 min at 4 °C, with cold growth medium without serum and 0.1 ml containing 5×10^6 field vole cells previously treated with mitomycin C (10 $\mu\text{g ml}^{-1}$, 2 h at 37 °C) was added. After incubation for 40 min at 37 °C, growth medium was added containing 10% calf serum. Foci of transformed cells appeared within 4–7 d after fusion. No transformation was observed if treatment of chick fibroblasts with Sendai virus was omitted. Coincident with the appearance of foci was the production of virus as assayed by particle count and focus formation. Identification of the virus as RSV subgroup D was accomplished serologically by demonstrating that infectivity of the rescued virus was neutralised by RSV-D-specific antisera.

ND, Not done.

indicating the presence of similar amounts of sarcoma-specific RNA in both cell types. In fact revertant subclone 866R actually contained more sarcoma-specific RNA than both the transformed clones. Furthermore, the maximum hybridisation achieved from both cell types was identical, indicating that similar extents of transcription of the sarcoma-specific sequences had occurred in transformed and revertant cells. The extent of hybridisation with RNA from transformed and revertant field vole cells was identical to that observed with both RNA from transformed permissive cells and purified 70S RNA from the same strain of RSV used to synthesise the cDNA_{src} probe. No hybridisation to the cDNA_{src} probe was observed with RNA from normal field vole cells (Fig. 2).

In an effort to determine whether the sarcoma-specific RNA sequences were distributed similarly in transformed and revertant field vole cells, nuclear, cytoplasmic and polyribosomal RNA from these cells were hybridised to the cDNA_{src} probe (Figs 3 and 4). The rates of hybridisation obtained with the nuclear and cytoplasmic fractions indicated that the amounts of sarcoma-specific RNA present in the revertant cells were no less than that found in the corresponding fractions of the transformed cells. Thus it seems unlikely that the reverted state is related to an alteration in the intracellular localisation of sarcoma-specific RNA in these cells. Similar results were obtained for RNA isolated from polyribosomes

from both cell types indicating that the same amount of sarcoma-specific RNA is associated with polyribosomes in the revertant and transformed field vole cells (Fig. 4).

The hybridisation analyses presented above between RNA from transformed and revertant vole cells and a cDNA probe representing sarcoma-specific RNA sequences indicate that the phenomenon of reversion in these cells is not a function of transcriptional regulation of the

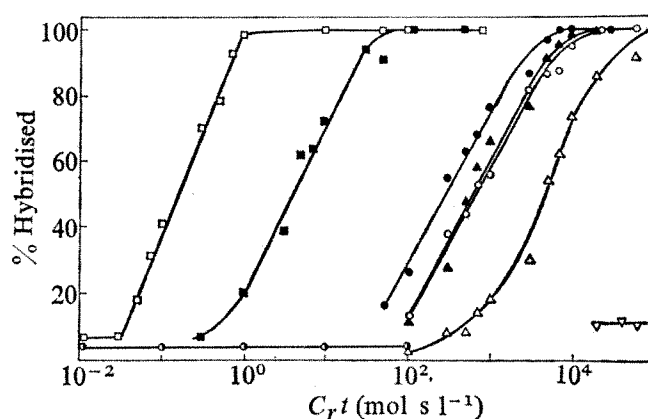


Fig. 2 Detection and quantitation of sarcoma-specific RNA¹ in transformed and revertant field vole cells. Total cellular RNA was extracted from vole cells with sodium dodecyl sulphate-phenol at 56 °C as previously described²⁶. Nucleic acids were precipitated with ethanol and collected by centrifugation at 13,000g for 30 min. The pellets were dissolved in DNase buffer (0.01 M Tris-HCl (pH 7.4), 0.01 M MgCl_2 at a concentration of 0.5 to 1.0 mg ml^{-1} . Residual DNA was removed by digestion with 20 μg of RNase-free pancreatic DNase ml^{-1} for 2 h at room temperature²⁷. The preparations were then adjusted to 0.01 M EDTA, 0.1 M Tris-HCl (pH 9.0), 0.5% SDS, extracted three times with two volumes of phenol-chloroform-isoamyl alcohol (50:50:1), precipitated twice with ethanol, and resuspended in TE buffer (0.02 M Tris-HCl, pH 7.4, 0.01 M EDTA). The conditions for the synthesis of virus-specific DNA *in vitro* with detergent-activated RSV are essentially those described by Stehelin *et al.*¹⁷. Reaction mixtures contained 200 $\mu\text{g ml}^{-1}$ of virus protein; 0.1 M Tris-HCl (pH 8.1); actinomycin D (100 $\mu\text{g ml}^{-1}$); 0.008 M MgCl_2 ; 2% (v/v) β -mercaptoethanol; unlabelled dATP, dCTP and dGTP (each 2×10^{-4} M); ^3H -dTTP (6.6×10^{-6} M, 0.33 mCi ml^{-1}); and Nonidet P-40 (0.08% v/v). After incubation for 4 h at 37 °C, the reaction mixture was supplemented with the addition of unlabelled dATP, dCTP, and dGTP to 4×10^{-4} M, further incubated for 12 h and then terminated by the addition of EDTA to 0.01 M. The enzymatic product was extracted with SDS-Pronase-phenol, treated with 100 $\mu\text{g ml}^{-1}$ pancreatic RNase for 1 h at 37 °C in 0.003 M EDTA to disrupt RNA:DNA hybrids, re-extracted with SDS-Pronase-phenol and concentrated by ethanol precipitation. cDNA complementary to the RSV transforming gene sequences (cDNA_{src}) was then purified from the total viral-specific DNA product by selective hybridisation to avian leukosis virus essentially as described by Stehelin *et al.*¹⁷. The transformation specific nature of this probe was confirmed by demonstrating that cDNA_{src} hybridises to transforming sarcoma virus RNA but not to non-transforming leukosis virus RNA. Hybridisation of ^3H -labelled cDNA_{src} to vole cell RNA was performed in a 10- μl reaction mixture containing 0.01 M Tris-HCl (pH 7.4), 0.6 M NaCl, 0.001 M EDTA, single-strand ^3H -cDNA (1,500 c.p.m.), 1.0 mg ml^{-1} yeast tRNA and the indicated amount of RNA. The reaction mixtures were sealed in 30- μl microcaps and incubated for either 24 or 70 h at 68 °C. Hybrid formation was detected by treatment of samples with single-strand specific S_1 nuclease^{20,21}. The results of hybridisation analysis were expressed as a function of $C_r t$ (concentration of RNA in $\text{mol l}^{-1} \times$ time of incubation in s). The percent hybridisation was corrected for the intrinsic S_1 nuclease resistance of cDNA_{src} (13%) and normalised to 100% values (actual hybridisation averaged 70–75%). Each point represents the average of three separate determinations. \square , RNA 70S RNA; \blacksquare , RSV-transformed duck cells; \circ , RSV-transformed field vole clone 1; Δ , RSV-transformed field vole clone 22; \blacktriangle , revertant field vole subclone 4; \bullet , revertant field vole subclone 866; \triangle , uninfected field vole; \odot , avian leukosis virus 70S RNA.

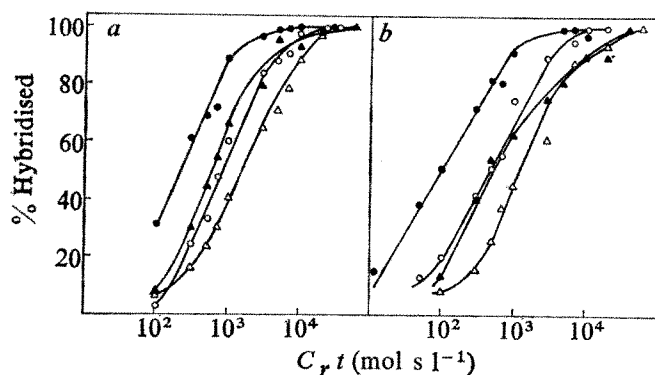


Fig. 3 Detection and measurement of sarcoma-specific RNA in the nucleus and cytoplasm of transformed and revertant field vole cells. Nuclear and cytoplasmic RNA from vole cells was prepared as follows. Frozen cell pellets were thawed rapidly at 37 °C and resuspended in RSB buffer (0.01 M Tris-HCl (pH 8.3), 0.01 M NaCl, 0.0015 M MgCl₂) at approximately 5×10^7 cells ml⁻¹. The cell suspension was adjusted to 0.5% Nonidet P-40, incubated at 4 °C for 5 min at which time cell disruption was completed by ten strokes of a Dounce homogeniser. The nuclei were pelleted by centrifugation at 700g for 10 min, washed once with RSB buffer and then several times with RSB buffer containing 1% Nonidet P-40 and 0.5% sodium deoxycholate. The nuclear washes were pooled with the cytoplasmic fraction which was further clarified by centrifugation at 13,000g for 5 min. The nuclei were lysed by resuspension in buffer containing 0.01 M Tris-HCl (pH 7.4), 0.5 M NaCl, 0.006 M MgCl₂, 0.6 mM CaCl₂ and adjusted to 50 µg of RNase-free pancreatic DNase ml⁻¹. After incubation at room temperature for 20 min, EDTA was added to 0.01 M. Nuclear and cytoplasmic fractions were then incubated for 30 min at 37 °C in the presence of 0.5% SDS and 500 µg of Pronase B (self-digested) ml⁻¹. The preparations were adjusted to 0.05 M sodium acetate (pH 5.1), 0.01 M EDTA and 1% SDS, extracted three times at 56 °C with an equal volume of phenol saturated with 0.05 M sodium acetate (pH 5.1), 0.01 M EDTA, and precipitated twice with ethanol. Nucleic acids were pelleted at 13,000g for 30 min, resuspended in DNase buffer and digested with RNase-free DNase as described above. The samples were re-extracted with phenol-chloroform-isoamyl alcohol and precipitated with ethanol as described for the preparation of total cellular RNA.

Contamination of cytoplasmic fractions by nuclear RNA was less than 1% as determined by previously published procedures²⁸. As far as cytoplasmic contamination of nuclear RNA was concerned, no obvious cytoplasmic material was present in the nuclei preparations as determined by contrast-phase microscopy. Furthermore, nuclear RNA preparations contained less than 5% 18S ribosomal RNA which is indicative of cytoplasmic contamination²⁹. Hybridisation of nuclear and cytoplasmic RNA to cDNA_{src} was performed at 68 °C for 70 h as described in Fig. 1. Each point represents an average of three separate determinations. *a*, Cytoplasmic RNA; *b*, nuclear RNA. ○, RSV-transformed field vole clone 1; △, RSV-transformed field vole clone 22; ▲, revertant field vole subclone 4; ●, revertant field vole subclone 866.

RSV-transforming gene sequences since little or no difference in either the amount or the extent of transcription can be detected in these two cell types. Since the viral transforming gene sequences are potentially active in the revertant cell these data suggest that the control of the transformed phenotype in this cell system is under the influence of some post-transcriptional control mechanism(s). The fact that no differences in the amount of sarcoma-specific RNA found associated with polyribosomes could be detected between the transformed and revertant cell type further indicates that this post-transcriptional regulation does not involve either restriction of transport of sarcoma-specific RNA from the nucleus to the cytoplasm, or the lack of formation of sarcoma-specific polyribosomes in the revertant cell type. Although previous studies on other transformed-revertant cell systems have indicated that regulation of expression of the sarcoma gene sequences can occur at the transcriptional level⁹⁻¹², the data presented here is the first indication that the expression of this particular gene sequence

can also be regulated post-transcriptionally. Thus the mere presence of sarcoma virus-specific RNA in eukaryotic cells may not be sufficient to initiate transformation following infection of cells by RNA tumour viruses since the host cell must play an important part in determining which particular gene sequences are ultimately expressed. This may explain the situation that exists in normal avian cells which contain low levels of sarcoma-specific RNA but are by no means transformed (D. Spector, H. Varmus & J. M. Bishop, personal communication) and may reflect the requirement of a host cell component that the sarcoma gene product must interact with in order to establish and/or maintain transformation. It is conceivable that this host cell component is absent in subclones of revertant vole cells. These transformed and revertant field vole cells should therefore provide us with a eukaryotic cell system with which to study the transformed phenotype and the epigenetic host factors that are operative and required either to regulate or maintain the expression of the viral transforming gene functions. Experiments are currently in progress to determine if viral-specific proteins, including the sarcoma gene product, can be detected in the revertant subclones. Retransformation of the revertant field vole cells by RSV is also being attempted. Collectively these studies should contribute to our understanding of the level of restriction of sarcoma

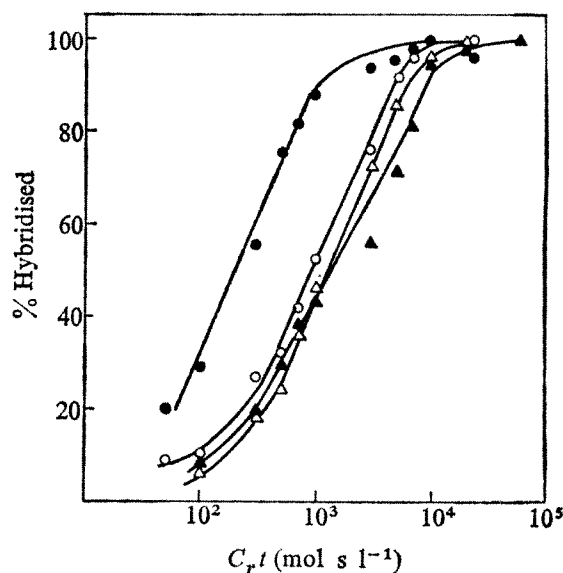


Fig. 4 Detection and measurement of polyribosome-associated sarcoma-specific RNA in transformed and revertant vole cells. Total polyribosomal RNA from transformed and revertant vole cells was prepared essentially according to the method of Gielkins *et al.*³⁰. Briefly, cells were washed with phosphate-buffered saline containing 200 µg of cycloheximide ml⁻¹, resuspended in buffer containing 0.05 M Tris-HCl (pH 8.5), 0.225 M KCl, 0.008 M MgCl₂, 0.002 M dithiothreitol, 500 µg of heparin, 200 µg of cycloheximide ml⁻¹ and 2% Nonidet P-40 and disrupted with a Dounce homogeniser. Nuclei and cell debris were removed by centrifugation and the 13,000g post-nuclear supernatant was layered on to a discontinuous sucrose gradient. Polyribosomes were pelleted by centrifugation, resuspended in 0.1 M Tris-HCl (pH 9.0) containing 0.5% SDS, extracted three times with phenol-chloroform-isoamyl alcohol (50:50:1) and precipitated twice with ethanol. Polyribosomal RNA was then digested with RNase-free DNase, re-extracted with phenol-chloroform-isoamyl alcohol and precipitated with ethanol as described in Fig. 1. In control experiments, treatment of the polyribosomal pellets with 30 mM EDTA for 15 min at room temperature released 90% of the sarcoma-specific RNA sequences consistent with its function as mRNA. Hybridisation of polyribosome-associated RNA to cDNA_{src} was performed for 70 h at 68 °C as described in Fig. 2. Each point represents an average of two to four separate determinations. ○, RSV-transformed field vole clone 1; △, RSV-transformed field vole clone 22; ▲, revertant field vole subclone 4; ●, revertant field vole subclone 866.

gene expression in this cell system and ultimately, elucidation of the precise nature of the cell function involved.

We thank S. Kanellos and D. Nelson for excellent technical assistance and L. Honza for typing this manuscript. We also thank Dr H. Varmus for invaluable discussions and communication of data before publication, and Drs J. Yunis and L. de la Mazafir for providing us with the normal vole cell line. We are indebted to Dr J. Gruber and the office of Program Resources and Logistics, Viral Cancer Program, Division of Cancer Cause and Prevention, National Cancer Institute for supplying us with valuable reagents. This investigation was conducted under Contract NO1-CP-61055 within the Virus Cancer Program of the National Cancer Institute. R. K. and A. L. are currently postdoctoral fellows supported by NIH postdoctoral fellowship CA 05231 and training grant CA 09 138 from the National Cancer Institute, respectively.

RICHARD A. KRZYZEK

ALAN F. LAU

ANTHONY J. FARAS

Department of Microbiology,
University of Minnesota Medical School,
Minneapolis, Minnesota 55455,

DEBORAH H. SPECTOR

Department of Microbiology,
University of California,
San Francisco, California 94122

Received 25 April; accepted 20 June 1977.

- 1 Martin, G. S. *Nature* **227**, 1021-1023 (1970).
- 2 Kawai, S. & Hanafusa, H. *Virology* **46**, 470-479 (1971).
- 3 Bader, J. P. *J. Virol.* **10**, 267-276 (1972).
- 4 Scolnick, E. M., Stephenson, J. R. & Aaronson, S. A. *J. Virol.* **10**, 653-657 (1972).
- 5 Graf, T. & Friis, R. R. *Virology* **56**, 369-374 (1973).
- 6 Wyke, J. A. & Linial, M. *Virology* **53**, 152-161 (1973).
- 7 Vogt, P. K. *Virology* **46**, 939-946 (1971).
- 8 Duesberg, P. H., Vogt, P. K., Maisel, J., Lai, M. & Canaani, E. *ICN-UCLA Symposium on Molecular Biology II*, 327-338 (Academic, New York, 1973).
- 9 Deng, C.-T., Boettiger, D., MacPherson, I. & Varmus, H. E. *Virology* **62**, 512-521 (1974).
- 10 Bishop, J. M., Deng, C.-T., Mahy, B. W. J., Quintrell, N., Stavnezer, E. & Varmus, H. E. *ICN-UCLA Symposium on Molecular and Cellular Biology, IV*, 1-20 (Academic, New York, 1976).
- 11 Deng, C.-T., Stehelin, D., Bishop, J. M. & Varmus, H. E. *Virology* **76**, 313-330 (1977).
- 12 Peebles, P. T., Scolnick, E. M. & Hawk, R. S. *Science* **192**, 1143-1145 (1976).
- 13 Toyoshima, K. & Vogt, P. K. *Virology* **39**, 930-931 (1969).
- 14 Friis, R. R., Toyoshima, K. & Vogt, P. K. *Virology* **43**, 375-389 (1971).
- 15 de la Maza, L. M., Faras, A. J., Varmus, H., Vogt, P. K. & Yunis, J. *J. expl Cell Res.* **93**, 484-487 (1975).
- 16 Varmus, H. E., Levinson, W. E. & Bishop, J. M. *Nature new Biol.* **233**, 19-21 (1971).
- 17 Stehelin, D., Guntaka, R. V., Varmus, H. E. & Bishop, J. M. *J. molec. Biol.* **101**, 349-365 (1976).
- 18 Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. *Nature* **260**, 170-173 (1976).
- 19 Collet, M. S., Kieras, R. M. & Faras, A. J. *Virology* **65**, 436-445 (1975).
- 20 Leong, J.-A., Garapin, A.-C., Jackson, N., Fanshier, L., Levinson, W. E. & Bishop, J. M. *J. Virol.* **9**, 891-902 (1972).
- 21 Sutton, W. D. *Biochim. biophys. Acta* **240**, 522-531 (1971).
- 22 Haase, A., Traynor, B., Ventura, P. & Alling, D. *Virology* **70**, 65-72 (1976).
- 23 Hillova, J., Mariage, R. & Hill, M. *Virology* **67**, 292-296 (1975).
- 24 Graham, F. L. & Van Der Eb, A. J. *Virology* **52**, 456-476 (1973).
- 25 Boettiger, D. *Virology* **62**, 522-529 (1974).
- 26 Girard, M. *Methods in Enzymology*, **XIIA**, 581-588 (Academic, New York, 1967).
- 27 Faras, A. J., Taylor, J. M., Levinson, W. E., Goodman, H. M. & Bishop, J. M. *J. molec. Biol.* **79**, 163-183 (1973).
- 28 Varmus, H. E., Guntaka, R. V., Deng, C. T. & Bishop, J. M. *Cold Spring Harbor Symp. quant. Biol.* **39**, 3874-3878 (1974).
- 29 Penman, S. *J. molec. Biol.* **17**, 117-130 (1966).
- 30 Gielkins, A. L. S., Salden, M. H. L. & Bloemendal, H. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1093-1097 (1974).

Cis-trans isomerisation in rhodopsin occurs in picoseconds

It has been believed for some time that the primary event in vision, the photochemical formation of bathorhodopsin, can be attributed to a *cis-trans* photoisomerisation¹. Recently this model has been questioned. Busch *et al.* proposed that the less-than-6-ps formation time of bathorhodopsin from rhodopsin does not allow significant isomerisation of the 11-*cis* chromophore to an all-*trans* isomer². This apparent difficulty with the *cis-trans* photoisomerisation model has prompted alternative models including (1) a mechanism involving deprotonation of the Schiff base nitrogen³, (2) proton transfer from the retinal methyl at position

five to opsin^{4,5} (involving the shifting of double bonds along the polyene chain to form a 'retro' type retinal), and (3) a photoinduced electron transfer to retinal from a protein donor group⁶. We have approached this question by performing picosecond absorption kinetic measurements on the formation time of bathorhodopsin from bovine rhodopsin and isorhodopsin. The essence of this experiment is that bathorhodopsin, being the common photo-product of rhodopsin (11-*cis* retinal) and isorhodopsin (9-*cis* retinal) must be an isomerised product of at least one of these pigments, but could be a product of both pigments (that is, basically all-*trans* retinal). Thus formation time measurements of bathorhodopsin from the two primary pigments can settle whether isomerisation can take place on the picosecond time scale.

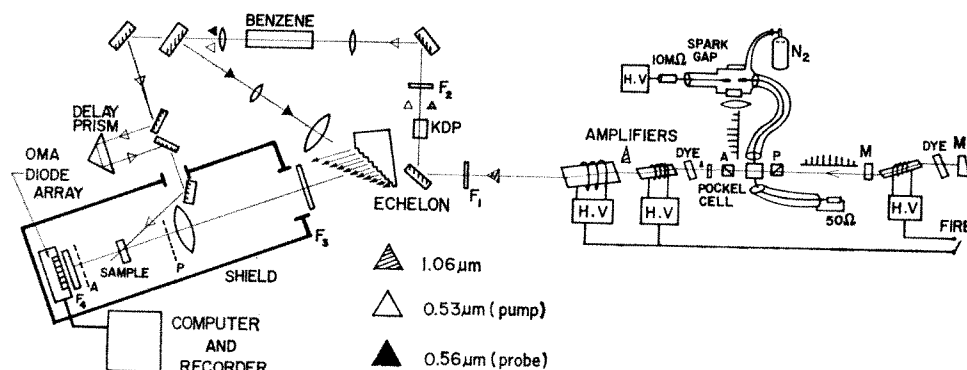
Rhodopsin is the visual pigment in disk membranes of vertebrate rod cells⁷. It is composed of the chromophore, 11-*cis* retinal, covalently linked through a protonated Schiff base to a small protein called opsin. On absorption of a photon by the chromophore, a consecutive series of thermal intermediates is formed ending in the release of all *trans* retinal and free opsin^{8,9}. The opsin binding site also accommodates a 9-*cis* retinal. This pigment, isorhodopsin, has the same thermal intermediates as rhodopsin. The first thermal intermediate, bathorhodopsin, has been shown to be generated from rhodopsin in less than 6 ps at room temperature and thermally decays in about 100 ns (ref. 2).

The picosecond apparatus is detailed in Fig. 1. Generated pump and probe pulses were at 530 and 561 nm respectively. Rod outer segment membrane fragments were isolated from dark-adapted bovine retinae essentially as described previously¹⁰. Solubilised rhodopsin was obtained by extracting the membrane fragments in 3% lauryldimethylamine oxide and 50 mM phosphate buffer (pH 6.6). Isorhodopsin was prepared photochemically¹¹ by taking some of the rhodopsin samples to liquid nitrogen temperature, irradiating using the 568.2-nm krypton laser light, and warming. All measurements were taken at room temperature, and the sample stirred between each laser pulse to avoid light induced sample changes in the laser light path. No average sample degradation was observed during the experiments as measured by standard ultraviolet-visible absorption spectroscopy.

In these measurements, care must be taken so as not to over drive the sample in the laser beam during the picosecond pulse. For example, significant amounts of isorhodopsin can be produced from a rhodopsin sample at high light levels. Calculations using an average intensity for the laser pulse, the quantum yields^{12,13} and absorption constants at the pump wavelength show that less than 10% of the rhodopsin sample in the laser path would be converted to isorhodopsin and similarly for isorhodopsin to rhodopsin. Also, for the pump intensities used, the yield of bathorhodopsin starting with isorhodopsin would be about half the yield of bathorhodopsin from rhodopsin, in agreement with the experimental results.

The time dependence of the laser-induced absorbance changes for the conversion of rhodopsin and isorhodopsin to bathorhodopsin are shown in Fig. 2. The salient feature of the curves is the rapid rise within 9 ps. Thus, the photochemical formation of bathorhodopsin from either rhodopsin or isorhodopsin occurs in less than 9 ps. The time to reach half the limiting absorbance change¹⁴ is about 3 ps. This is an indication of the formation time of bathorhodopsin. The formation time from rhodopsin is in agreement with the work of Busch *et al.*².

As briefly argued above, bathorhodopsin must be an isomerised product of at least one of the primary pigments. Proof for this is given by recent resonance Raman work (a technique sensitive to conformation¹⁵). Similarities between the resonance Raman spectra of the 11-*cis* protonated Schiff base in solution and rhodopsin, and between the 9-*cis* protonated Schiff base and isorhodopsin, show that the conformations of the retinals of rhodopsin and isorhodopsin are 11-*cis* and 9-*cis* respectively, and are not particularly distorted by the surrounding protein^{16,17}. In addition, the resonance Raman spectrum of photochemically produced isorhodopsin¹¹ is identical to that of regenerated 9-*cis* retinal¹⁸ and opsin, proving that complete photoisomerisation(s) about two double bonds takes place in the rhodopsin → bathorhodopsin → isorhodopsin phototransitions. Either at the



ing telescope lens system, a time delayed reflection echelon (14 steps at 4.5 ps per step), filters F_3 and F_4 to eliminate 530 nm, a focusing lens on sample 1.06 μm and 530 nm pulses are 9 ps and 7 ps respectively.

Fig. 1 Schematic representation of the experimental system. The components include: a Nd^{3+} glass oscillator with cavity mirrors M and saturable dye absorber; a single pulse selector consisting of two crossed polarisers, Pockels cell and spark gap; a half wave plate and 'clean-up' saturable dye absorber; two Nd^{3+} glass amplifiers (gain $\times 20$); filter F_1 to eliminate flash lamp; a second harmonic generator; filter F_2 to eliminate 1.06 μm ; a 15-cm benzene cell to produce stimulated Raman scattering at 561 nm; specially coated mirrors to separate the 530 nm and 561 nm beam paths; in the 561 nm path, an expand-

first or the second photoreaction or at both photoreactions a major isomerisation must take place. Thus, there is no doubt that bathorhodopsin, being a common photoproduct of both rhodopsin and isorhodopsin, is an isomerised product of at least one of the primary pigments.

Our picosecond results show that major photo-induced isomerisation(s) of retinal in bovine rhodopsin can take place on a picosecond time scale since both rhodopsin \rightarrow bathorhodopsin and isorhodopsin \rightarrow bathorhodopsin photoreactions take place in less than 9 ps. In agreement with our observations, Warshel¹⁹ has shown that picosecond isomerisation times are theoretically feasible in rhodopsin, although this theoretical approach relies heavily on the exact nature of the excited state potential surfaces which are difficult to obtain precisely.

Huppert *et al.*⁹ have performed kinetic measurements with protonated retinal Schiff bases in solution, and report nanosecond recovery kinetics which are ascribed to isomerisation times. From our results and theirs, it seems likely that in the visual pigments the photoisomerisation times are greatly accelerated by chromophore-protein interactions. That the protein significantly

modifies this retinal property is not surprising. In contrast to retinals in the visual pigments, protonated retinal Schiff bases in solution have blue-shifted absorption bands and much reduced, wavelength dependent quantum yields of photoisomerisation¹³.

Finally, our results do not describe the exact nature of bathorhodopsin. Rosenfeld *et al.*¹³, however, using the basic early arguments of Hubbard and Kropf¹ and Yoshizawa and Wald²⁰ concerning the properties of bathorhodopsin (or lumirhodopsin), recent resonance Raman data¹⁵, and recent artificial pigment work have recently argued that the chromophore of bathorhodopsin must have essentially a *trans* conformation but not necessarily a planar one. Our results remove any uncertainty from these arguments due to the picosecond isomerisation times.

We thank Professor Honig for useful discussions, and Princeton Applied Research for the use of an OMA. This work was supported in part by US NSF grants (P C M 75 13155A01 and BMS 75-03020) and grants from City University Research Award Programs and Philips Laboratories. B.H.G. is a Philips Fellow and R.R.A. an Alfred P. Sloan Fellow.

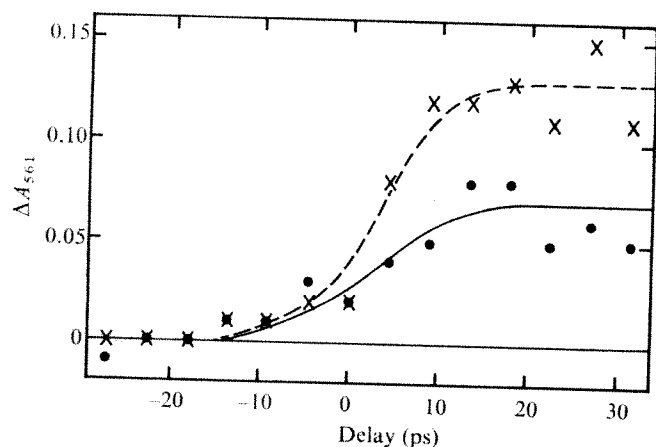


Fig. 2 Laser-induced absorbance changes at 561 nm as a function of time in detergent solubilised bovine rhodopsin (\times) and isorhodopsin (\bullet) at room temperature. Bathorhodopsin is the only intermediate during the bleaching of bovine rhodopsin known to absorb strongly at 561 nm. The energy of the 530-nm pump pulse was about 10^{-4} J; the energy of the 561-nm probe pulse was about 10^{-7} J. The beam sizes were about 1 mm² for the pump and 0.5 mm² for the probe. The samples (about 1.5 ml) were held in 0.5-cm cuvettes. The concentrations were about 4 A cm^{-1} at the absorption peaks near 500 nm; the ratios $A_{400}:A_{500}$ were about 0.3; and ratios $A_{530}:A_{500}$ were about 0.7 for rhodopsin and 0.5 for isorhodopsin. Each data point shown is the average of six (rhodopsin) and nine (isorhodopsin) laser shots. Typical mean standard deviations are ± 0.03 . The zero time is located using a 0.5 cm CS_2 Kerr optical shutter²¹ at the sample site. The half width at half maximum for the CS_2 shutter prompt response curve is about 6 ps.

B. H. GREEN

T. G. MONGER

R. R. ALFANO

B. ATON

R. H. CALLENDER

Department of Physics,
The City College of The City University of New York,
New York, New York 10031

Received 19 April; accepted 5 July 1977.

- Hubbard, R. & Kropf, A. *Proc. natn. Acad. Sci. U.S.A.* **44**, 130-139 (1958).
- Busch, G. E., Applebury, M. L., Lamola, A. A. & Rentzepis, P. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2802-2806 (1972).
- Thomson, A. J. *Nature* **254**, 178-179 (1975).
- Fransen, M. R. *et al. Nature* **260**, 726-727 (1976).
- van der Meer, K., Mulder, J. J. C. & Lugtenburg, J. *Photochem. Photobiol.* **24**, 363-367 (1976).
- Huppert, D., Rentzepis, P. M. & Klier, D. S. *Photochem. Photobiol.* **25**, 193-197 (1977).
- Wald, G. *Science* **162**, 230-239 (1968).
- Ebrey, T. & Honig, B. *Q. Rev. Biophys.* **8**, 124-184 (1975).
- Honig, B. & Ebrey, T. A. *Rev. Biophys. Bioeng.* **3**, 151-177 (1974).
- Hong, K. & Hubbell, W. L. *Biochemistry* **12**, 4517-4523 (1973).
- Oseroff, A. R. & Callender, R. H. *Biochemistry* **13**, 4243-4248 (1974).
- Dartnall, H. J. A. in *Handbook of Sensory Physiology*, V11, 1, 122-145 (1972).
- Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J. & Ebrey, T. G. *Pure Appl. Chem.* **49**, 341-351 (1977).
- Ricard, D., Lowdermilk, W. H. & Ducuing, J. *Chem. Phys. Lett.* **16**, 617-621 (1972).
- Callender, R. H. & Honig, B. A. *Rev. Biophys. Bioeng.* **6**, 33-55 (1977).
- Mathies, R., Oseroff, A. R., Freedman, T. B. & Stryer, L. in *Tunable Laser Applications* (eds Jaeger, T., Stokseth, P. & Mooradian, A.) (Springer, New York, in the press).
- Mathies, R., Freedman, T. B. & Stryer, L. *molec. Biol.* **109**, 367-372 (1977).
- Mathies, R., Oseroff, A. R. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1-5 (1976).
- Warshel, A. *Nature* **260**, 679-683 (1976).
- Yoshizawa, T. & Wald, G. *Nature* **197**, 1279-1286 (1963).
- Duguay, M. A. & Hansen, J. W. *Appl. Phys. Lett.* **15**, 192-194 (1969).

reviews

Darwin's papers

Frederick Burkhardt

The Collected Papers of Charles Darwin. (2 Volumes.) Edited by Paul H. Barrett, with a Foreword by Theodosius Dobzhansky. Pp. viii+277+326. (University of Chicago: Chicago and London, 1977.) £27.30; \$40 the set.

THESE volumes bring together 152 papers which Darwin published over a period of forty-seven years. They range in length from a paragraph to forty-eight pages. Thirty-three of them first appeared in *Nature*, between 1869 and 1882. Others were contributions to learned societies of which Darwin was a member: the Geological (12), Linnean (9), Zoological (4), Geographical (1), and the Royal (1). (His sole contribution to the Royal Society was his solution of the problem of the 'Parallel Roads of Glen Roy', a paper he later called a 'gigantic blunder'.)

The largest number of items (50) are from *The Gardeners' Chronicle and Agricultural Gazette*, the medium Darwin used for communication with plant and animal breeders. The rest of the papers are mainly from periodicals concerned with popular but serious discussion of scientific matters, like the *Natural History Review*.

An appendix lists fifty-two articles dealing with the species collected by Darwin on the *Beagle* voyage, in itself a considerable bibliographical contribution. All of Darwin's notes are preserved. Professor Barrett's notes are relatively few in number and are concerned with identifying persons and places and explaining technical terms.

The effect produced by the papers in their totality is dramatic in the way they make clear the range of Darwin's interests, his powers of observation, his imaginative questioning, his probing for facts that he hoped would corroborate his hypotheses, and his astonishing talent for experimentation.

The papers naturally vary considerably in scientific and historical significance. Some are famous, like the joint paper with Wallace in which Natural Selection was first announced, and his remarkable papers on plant physiology.

Some of the brief and apparently ephemeral items are now seen to be important in Darwin's work on his species theory. As Theodosius Dobzhansky points out in his appreciative and graceful Foreword, Darwin's short query about 'A Mouse-Coloured Breed

of Ponies' was really asking 'Have the species horse and ass descended from a common ancestor?'. Similarly, Darwin's experiments on the ability of seeds to survive immersion in salt water were designed to provide him with an explanation of the geographical distribution of species that could dispense with creationism and Forbes's vast continental extensions.

Although this is primarily a reference work, there is plenty of interesting and readable material in it. Darwin wrote many of the pieces in clear non-technical prose for those interested in such subjects as erratic boulders, the fine dust that falls on ships in the Atlantic, mimetic butterflies, the fertilisation of British orchids, the origin of certain instincts, or the biographical sketch of an infant.

Scholars will want to know how complete the collection is. The answer depends on what criteria are used in selection. Professor Barrett includes only papers published by Darwin in his lifetime. Richard Freeman, in his new edition of the bibliographical handlist of Darwin's works includes posthumous articles, memorials, and items privately printed and circulated by Darwin. On

Professor Barrett's criteria, each work has four items overlooked by the other. Using Mr Freeman's standards, but omitting repetitive printings, nine more might have been included. For example, Darwin's important 'Queries about the Breeding of Animals' is omitted by Barrett because it was printed and distributed privately, but the 'Queries about Expression' is included because it found its way into an Annual Report of the Smithsonian Institution. Again, Professor Barrett omits three items because the books to which they were contributed were published after Darwin's death. Since these circumstances in no way affect the authenticity of the writings or their importance, it seems arbitrary to deny them a place in a *Collected Papers*.

This editorial question aside, Professor Barrett deserves great credit for making these writings available in a convenient and reliable collection in which scholars and non-specialists alike will find both profit and pleasure.

Frederick Burkhardt, President Emeritus of the American Council of Learned Societies, is co-editor, with Sydney Smith, of the prospective Collected Letters of Charles Darwin.

Complex systems

The Foundations of Cybernetics. By F. H. George. Pp. xiv+286. (Gordon and Breach: London, Paris and New York, 1977.) £13.50.

THE last page of this book contains the following comment "... about the word Cybernetics. In America it is now slightly frowned upon, and although the word is currently more acceptable in the United Kingdom it is still suspect in some quarters. . . . The problem stems from the arbitrary divisions we have in our sciences, and the very slow conservative attitude of scientists towards the development of scientific ideas and classifications. . . ."

Up to a point the complaint deserves sympathy. Much can be gained from interdisciplinary work. There is, of course, little to stop the individual from drawing his own "arbitrary divisions". Whether his new subject becomes acceptable to the larger community depends on what it has to offer and the following it can attract.

Broadly, Cybernetics encompasses the study of complex systems. On the one hand there are the man-made systems of automation and computer technology and, on the other, those provided by nature, such as the brain of man and other physiological organs. There is, one hopes, a conceptual framework common to both. The man-made systems can serve as a source of analogies for the development of theory in psychology and physiology while, in the reverse direction, the living world can act as a source of inspiration to inventors.

The book is concerned with the, as yet undeveloped and unconnected, conceptual framework that may serve the two sides. There are chapters on feedback, finite automata, neural nets, logic, computers, information theory, and aspects of psychology and physiology. With a net cast so wide, it is not surprising to find that the treatment of some topics is superficial. This means that for information on any one

of the topics one could do better by turning to more specialised sources. As a book of its kind, however, it is one of the best.

It is clear from the evidence presented that Cybernetics still awaits its breakthroughs. As a body of theory it has little to offer and seems to have taken almost everything it has from other disciplines. The developments envisaged by Wiener, Ashby, and others, that can result from the interaction of ideas from biology and technology do, of course, continue to take

place; but, perhaps not, as they had hoped, under the heading of Cybernetics. Ironically, there is the consolation that the "arbitrary divisions" which, some thirty years ago, marked out this new field of science are not likely to become hard-set, and thereby to obstruct future changes in the internal boundaries of science.

V. Zakian

V. Zakian is Lecturer at the University of Manchester Institute of Science and Technology, Manchester, UK.

Heterocyclic chemistry

Stereochemistry of Heterocyclic Compounds. Part 2: Oxygen, Sulfur, Mixed N,O, and S, and Phosphorus Heterocycles. By W. L. F. Armarego. Pp. xviii + 494. (Wiley-Interscience: London and New York, 1977.) £32.65; \$54.

ALTHOUGH the literature of organic chemistry includes many comprehensive surveys of the stereochemistry and conformational analysis of carbocyclic compounds, scant attention has hitherto been given to the corresponding aspects of heterocyclic chemistry. Numerous discussions of isolated topics in this vast, comparatively neglected area are scattered throughout the review literature, but the present two-volume set appears to be the first monograph to attempt a review of this field as a whole. This second volume is devoted to the stereochemistry of oxygen, sulphur, mixed nitrogen, oxygen and sulphur heterocycles, and a final chapter deals with phosphorus heterocycles.

The organisation of the volume is the same as that of the first, even to the extent that the first brief, introductory chapter, which explains the order of discussion of the data, is identical in the two volumes. Each section begins with a discussion of the stereochemical aspects of the synthesis of compounds in the group, then follows a discussion of the configuration, and then the conformation, of the compounds, and finally stereochemical aspects of their reactions are discussed. The whole project is one of daunting proportions; this is amply illustrated by the fact that this second volume alone contains a bibliography of over 2,400 references, which cover the subject completely up to the end of 1974, and incompletely for 1975. Presumably because of the enormous volume of material to be reviewed, the final chapter has been contributed by M. J. Gallagher.

The coverage of this material in under 450 pages of text has resulted in inevitable restrictions; the style is of

necessity compressed, but it is clear, critical, and very readable. Where earlier authoritative reviews exist, the author has been concerned primarily with updating them. Important stereochemical principles and illustrations of syntheses and reactions are taken mainly from the behaviour of model compounds, and a discussion of the vast number of natural products is not attempted. In the present context this includes, for example, furanose and pyranose carbohydrates, penicillins and cephalosporins. Steroidal epoxides are also among the groups omitted; however, in all these areas, the excellent bibliography provides a ready entry into these aspects of the subject, as well as providing a comprehensive bibliography for readers who wish to pursue the subject matter of the text in greater depth.

The book is beautifully produced, and typographical errors are rare. The formulae have been very skilfully drawn by the author's talented amanuensis, the heteroatoms and substituent groups being inserted manually rather than in the usual type-face. This has presumably resulted in some economy in production, and it has also obviated one possible source of errors, without any sacrifice of production standards. There is a moderately detailed subject index, which also includes the names of authors mentioned in the text; there is no separate, complete, author index.

This volume certainly fills a very important gap in the review literature of heterocyclic chemistry, and since, with its companion volume on nitrogen heterocycles, it heralds the introduction of a new series of monographs on general heterocyclic chemistry, each volume in which will survey one aspect of the whole field of heterocyclic chemistry, it is to be particularly welcomed. It will be immensely useful to advanced undergraduates and to research workers in heterocyclic chemistry, and it surely belongs in every chemistry library and heterocyclic research laboratory.

J. E. Saxton

J. E. Saxton is Senior Lecturer in Organic Chemistry at the University of Leeds, UK.

Biological tools

Introduction to the Spectroscopy of Biological Polymers. Edited by D. W. Jones. Pp. xii + 328. (Academic: London and New York, 1977.) £11.60; \$25.50.

SPECTROSCOPIC techniques have played no less an important part in the structural studies of biological macromolecules than they have in more classical studies of simpler molecules, and the range of spectroscopies, if differing in emphasis, has been equally as wide. Consequently, an author or editor aiming to produce a book that covers all or most of the spectroscopic techniques used in studying biological polymers sets himself an unenviable task. He not only attempts a synthesis of several highly specialised subjects but does so, unlike his many predecessors who have undertaken similar enterprises in a purely chemical context, knowing he can make very few assumptions about his readers' knowledge of mathematics and physics.

The editor of this work has overcome the problem of the multidisciplinary nature of his subject by choosing an author eminent in each field to contribute a chapter on that speciality. He has sandwiched these chapters between a general introduction to molecular spectroscopy and a concluding chapter briefly describing some examples that illustrate the power of combining several spectroscopic techniques to tackle a specific biological problem. The approach throughout is descriptive and not mathematical, and several difficult physical concepts are explained clearly.

The most original aspect of the book is the strong emphasis placed on the use of vibrational spectroscopy. The chapter on Raman spectroscopy is particularly welcome as advances in laser technology over the past decade have made this technique a powerful biological tool. No less welcome and more surprising are the two chapters on infrared spectroscopy. They illustrate the potential of this technique especially when combined with Fourier-transform analysis. They also link the work done on biological polymers with that done on the industrially important synthetic polymers. The remaining chapters cover absorption, emission, optical rotation, circular dichroism, nuclear magnetic resonance, electron spin resonance and Mössbauer spectroscopy.

Michael T. Flanagan

M. T. Flanagan, until recently a member of the Biophysics Division of the National Institute of Medical Research, London, is now with Standard Telecommunication Laboratories, Harlow, UK.

Space-time proceedings

Asymptotic Structure of Space-Time. Edited by F. Paul Esposito and Louis Witten. Pp. vii+442. (Plenum: London and New York, 1977.) \$51.

In recent years it has increasingly become the practice for the proceedings of international scientific conferences to be published a year or so later in a single volume at an exorbitant price. Occasionally these collected works are very useful as a comprehensive review of a new or rapidly developing subject, and if well written and edited can provide students with a convenient access to up-to-date work. All too often, however, the proceedings are a hotchpotch of incomplete articles, bearing only a superficial connection to each other, and coexisting under the same cover merely to avoid offending some of the conference contributors.

The usefulness of conference proceedings is still further reduced when preprints of the papers are thoroughly circulated beforehand and all the publisher does is to bind together reproductions of these manuscripts (often containing barely legible hand-written mathematical symbols and a multiplicity of type faces) and offer the end-product for anything up to a few dozen dollars.

Many of these volumes are now being offered to university libraries labouring under stringent budget controls, so that would-be purchasers are becoming much more discerning in their requirements. They will be interested to know that *Asymptotic Structure of Space-Time* is, by these recent standards, very well put together. True, the price, at \$51, is regrettably high, but most of the manuscripts have been well prepared and typed, and the papers are of a fairly uniform style and standard. The conference took place in Cincinnati in June 1976, and covers a selection of important and fast-moving new developments in mathematical relativity and space-time structure. After a useful and well written introduction by R. Geroch the reader is treated to a discourse on cone space, (Brian Bramson), conformal bundle boundaries (B. G. Schmidt) and complex vacuum metrics (J. F. Plebanski and I. Robinson). I was disappointed that the theory of twistors did not receive some prominence, but there are two good papers on the related topic of H-space (E. T. Newman, K. P. Tod and M. Ko), although these are far too condensed.

The recent resurgence of interest in quantum aspects of gravitation is reflected in a (once again, too short)

paper on supersymmetry by P. van Nieuwenhuizen, together with an excellent and very readable review on quantum field theory in curved space and particle production by gravitational fields, by Leonard Parker. I found this one of the best presented of all the papers, and its list of 168 references is a masterpiece of literature research.

My overall impression of this book is that it is well edited and presented, and is nice to have available.

Paul Davies

Paul Davies is Lecturer in Mathematics at King's College, University of London, UK.

Methylmercury intoxication

Minamata Disease: Methylmercury Poisoning in Minamata and Niigata, Japan. Edited by T. Tsubaki and K. Irukayama. Pp. 310. (Kodansha: Tokyo; Elsevier: Amsterdam, New York and Oxford, 1977.) \$48.25; Dfl. 118.

METHYLMERCURY intoxication is now frequently called Minamata disease as it was first around the Minamata Bay that methylmercury caused a mass epidemic. One of the best parts of the book deals with the background and epidemiology of this and a similar epidemic in Niigata. The Minamata epidemic started in 1941 with individual cases and its epidemic proportion was noticed in 1956 when it was first believed to be of infectious origin. In 1958 Dr McAlpine, who saw some of the patients, called attention to the similarity between Minamata Disease and methylmercury intoxication. His paper written with Dr Araki and published in the *Lancet* in 1958, but not mentioned in the otherwise detailed historical and clinical account, turned the attention to mercury; and subsequently methylmercury was identified in sediments, seafood and in the drainage system of the Minamata factory.

Although the number of yearly cases declined, new cases were reported as late as 1972-73, which is not surprising as the mercury concentration in shellfish remained up to that period of about 5 p.p.m., but sometimes as high as 16 p.p.m. The situation was not very different in Niigata, where the ban on fishing in the Agano river did not prevent new outbreaks, which are discussed in this book as intoxications with delayed onset. Whether methylmercury is able to precipitate disease long after the cessation of exposure is an important problem, but inadequately handled in the whole book. There is also a certain reluctance to re-value past views: data based on the dithizone

method is interpreted without reservation although according to a table this method, compared with atomic absorption, generally underestimated tissue mercury levels by a factor of 3.

Translation is very uneven and often puzzling. A sentence like "After an interval of 14 years, one of the 4 cases came to necropsy, and was found to be grossly ataxic; blindness of the left eye and severe constriction of the visual field in the right eye persisted" is more than a puzzle, but it might illustrate the difficulty of interpreting some paragraphs with less blatant error. It might be that the translation is responsible for the surprising statement that mercury half-time in the brain was calculated from autopsy material.

Despite shortcomings, *Minamata Disease* is an important publication on the origin, epidemiology, pathology and clinical aspects of the two outbreaks in Japan. It would be a very much better book, however, if publications of this type were submitted to the same rules as any publication in a learned journal: revision based on the suggestions and criticisms of two referees.

Laszlo Magos

Laszlo Magos is Senior Scientific Officer at the MRC Toxicology Unit, Carshalton, UK.

BOOKS

ON PURE
AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £7.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Circle No. 08 on Reader Enquiry Card.

Functional groups

Chemistry of Functional Groups. Supplement A: Chemistry of Double-Bonded Functional Groups. Parts 1 and 2. Edited by S. Patai. Pp. 651 each part. (Wiley: London, New York, Sydney and Toronto, 1977.) Part 1 £29; \$52.50; Part 2 £29.50; \$55. Suppl. as a set £58.50; \$107.50.

SUPPLEMENT A to *Chemistry of Functional Groups* is the first in a set of planned supplementary volumes to this well established series. The aim of the Editor is to include in them the "missing chapters" from earlier volumes in addition to providing other chapters to extend and bring up to date the material in the earlier volumes. The two parts of this supplement A relate chiefly to earlier volumes in the series on alkenes (1964 and 1970), the carbonyl group (1966), the carbon-nitrogen double bond (1970), and the azo group (1975).

The first part contains chapters in which all of the above groups are considered, usually separately within each chapter. Reviews on dipole moments, configurations and conformations of molecules, liquid crystals, and thermochemistry provide a timely reminder of the value of an understanding of the physical properties and structures of organic molecules as a basis for a study of their reactions and reactivity. The chapters which follow on the mechanisms of elimination and addition reactions, electrochemistry, 1,3-dipolar cycloadditions, and the reactions of carbenes, demonstrate the rapid developments in these areas in recent years, and provide the reader with a clear picture of the advances that have taken place.

Part two commences with a review on the formation of unsaturated groups by heterolytic fragmentation. It is particularly useful in showing that numerous apparently different reactions conform to a common pattern of reactivity and lead to the formation of compounds with double-bonded functional groups. The remaining chapters are on more specific subjects, and are concerned with reviews of electrophilic additions to carbon-carbon double bonds, the olefin metathesis reaction, oxidation of $-C=C-$ and $-C=N$ groups, and transition metal-catalysed carbonylation of olefins, imidines and diamides (1,3,5-triazapentadienes).

All chapters in the supplement are of the nature of essay reviews not claiming to give encyclopaedic coverage. They are well written with clear diagrams and reaction schemes. Although

coverage is selective, the authors have been careful to choose illustrative examples that demonstrate the essential principles they wish to convey. Cross references are given to earlier volumes in the series as well as to other reviews and to many individual papers. In some chapters as many as 500 references are provided.

The main value of this series is that a reader on studying a particular chapter will get a clear overall picture of the aspect of chemistry that it covers, and will be able to explore more deeply by consulting the references provided. In this supplement, the chapters vary in the ground they attempt to cover; some survey enormous fields, others bring together reactions of a number of different functional groups within a coherent framework, and yet others cover very specific topics. Within the framework of the

series these two volumes represent valuable additions to their parent volumes published earlier. They will be useful to undergraduate and postgraduate students, and also to research workers in industry. The scope of the series makes its price beyond the pockets of individuals but these volumes, like the others in the series, will be most important additions to the libraries of teaching and research establishments specialising in organic chemistry. The series is likely to increase in value particularly when, in these inflationary days, the range of review journals and periodical reviews subscribed to by libraries have to be curtailed on financial grounds.

D. I. Davies

D. I. Davies is Reader in Organic Chemistry at King's College, University of London, UK.

Water and tropical agriculture

Climate, Water and Agriculture in the Tropics. By I. J. Jackson. Pp. xii+248. (Longman: London and New York; 1977.) £3.75.

SINCE temperature is always adequate for plant growth in the tropics, the main climatological limitation to agriculture is often inadequate rainfall. *Climate, Water and Agriculture in the Tropics* by Jackson therefore covers what is potentially an important field. The author has spent six years lecturing in Tanzania, so he has first-hand experience of the problems and is well qualified to write this particular book. He examines the characteristics of tropical rainfall and evaporation, together with their implications, especially as related to agriculture, land use and other aspects such as soil erosion and irrigation. Early chapters consider the origins of tropical precipitation, rainfall seasonality and variability, and rainfall intensity. The general atmospheric circulation of the tropics is discussed briefly. Only one chapter is devoted to evaporation, with a short discussion of methods of estimation. The final chapters are concerned with water-plant relationships and the book ends with a discussion of man's impact on the hydrological cycle.

The tropics are vast in area, and cover a great variety of climatic types. It is therefore impossible in a book of limited length to cover all aspects of tropical plant-water relationships. Although the coverage of the book is

wide, it does tend to concentrate on the humid tropics of Africa and Asia, with particular emphasis on the statistical aspects of rainfall. Considering its importance to the energy balance of tropical plants, evaporation is discussed somewhat briefly. Evaporation depends largely on the available radiation, but there is no discussion of the distribution of solar radiation in the tropical world. Given an adequate water supply, plant yields depend to some degree on the available solar radiation, which in the summer hemisphere can increase away from the equator, leading to the highest crop yields in the subtropics. This is another reason for introducing some discussion on tropical solar radiation patterns. Considering that the book is essentially about water and tropical agriculture, it is a pity that there is not a really substantial section on the problems of drought and desertification in the tropics. Both problems have assumed some importance in recent years, especially as there are suggestions that there is a feedback mechanism between desert formation by overgrazing, and decreasing rainfall.

The main strength of this book lies in its discussion of tropical precipitation; here it fills a gap in the available literature on this subject. The book is well produced and well illustrated, and includes an extensive list of references. It should prove invaluable to specialists concerned with tropical water resources, and also provide useful reading for students of the agriculture, climatology or geography of the tropics.

J. G. Lockwood

J. G. Lockwood is Senior Lecturer in the School of Geography at the University of Leeds, UK.

nature

15 September 1977

Destination without route-map

NOSTALGIA, as more than one wag has said, is not what it used to be. Nor, according to Denis Hayes, is the future. The entire world, he argues in his Worldwatch Institute book *Rays of Hope* (published this week in the United States*), stands at the edge of an "awesome discontinuity" in its production and use of energy—hence the book's subtitle, 'The transition to a post-petroleum world'. In trying to anticipate what that world could look like, it is undoubtedly an important sketch of a growingly complex picture; its flaw, if that is what it is, is that it ignores how the disputed views of the final canvas are to be reconciled and implemented.

Firmly in the now well-established tradition of Worldwatch publications, the lucidly written and remarkably broad-ranging exposition is a valuable synthesis of existing work, here in the burgeoning field of energy—indeed, it is a synthesis of a synthesis, incorporating Hayes' already-published arguments on conservation, nuclear power and solar energy. Designed also to influence as well as inform, it has a clear message to convey: the three energy sources on which to build a self-sustaining post-petroleum world, namely coal, nuclear and solar, have narrowed to just one—the sun; the technology is already available, now is the time to move.

Part of the argument is familiar enough. The fossil fuels outlook is discouraging: without a change, the current generation will consume most of the oil and gas. Resource, environmental and climatic constraints make coal just a transitional fuel. The nuclear outlook is even less encouraging: the threats of proliferation and terrorism come on top of problems of radiation, waste, uranium resources, breeders and safety. With fusion's promise weak, that leaves the safe sustainable sources—the sun, wind, water and 'plant power'—and a crying need for conservation.

To see it simply as a 'pro-solar' or as an 'anti-nuclear' book would be a mistake, however, though it is this almost to a fault and as convincing as anything that has taken the opposite point of view. The book's relentless onslaught on the wasteful use of energy, specifically in food production, transport and construction, together with its detailed technical picture of a more energy-efficient world is especially welcome. So too is its historical and global perspective, although parts

are plainly pitched to a US audience.

Where the argument is different, but also troubled, is in the character of its advocacy. Worldwatch's president, Lester Brown, says in a foreword that the question is not whether or not we make the transition, but whether it will be smooth or chaotic. Unfortunately Hayes seems to believe it is better to arrive than to travel, for he is as short and unenlightening on how politically to secure his alternative as he is long and rewarding on what, in energy terms, the world ought to look like under it.

On a subject this controversial, this is a rather glaring omission. To consider global energy problems without considering the global political economy is like scaling a mountain without allowing for the weather. But few books go even as far as Hayes does. It also remains unfortunately true that people let the unpredictability of the weather mask their appreciation of mountains, and he has undoubtedly taken account of this. Yet in a book whose underlying theme is world-wide change, questions are bound to go begging when the technical changes are teased out while the concomitant social changes are not. And that is exactly what happens.

We are told—but only told—of the 'weighty' depletion issue, which focuses on the discount rates that the markets and the politicians apply to, say, a future barrel of oil. We are told of the highly centralised technocratic and authoritarian societies that nuclear power fosters. We are also told that there is neither a 'quick fix' nor a *deus ex machina*, that the coming transition is unlikely to be smooth and painless, that there will be opposition. And we are told that the issues are not scientific and technical, but social, political and philosophical—that redistributions of land and of wealth must be enormous, both within and between countries.

This too will demand some authoritarian action, for, historically speaking, a more explosive political issue is difficult to find. But for Hayes it must be the subject of another book. So we are left with rays of hope instead of straws, and an argument for a least bad option. It is a pity. Perhaps, as he intimates, civilised societies must discover their own solutions and carve out their own paths. But in an interdependent and competitive world, events look more and more like intervening to do their own damage. This book was needed five years ago. Even with it, people will regret not being institutionally prepared as well as technically competent to survive the impending threat of holocaust.

*Denis Hayes, *Rays of Hope: The Transition to a Post-Petroleum World* (W. W. Norton, September 1977) \$3.95

A good time to raise standards

EVERY year we scan the statistics of The Universities Central Council on Admission (UCCA) for trends, and occasionally we get rapped over the knuckles for misinterpretation. This year's figures, just out, offer the usual happy hunting ground.

The statistics, as always a year late, refer to those who applied during the year 1975-76, generally for entry in October 1976. They show applications up again to around 142,000. Overseas candidates, particularly from the Commonwealth, continue to grow in number more rapidly than home candidates—in 1976 they accounted for one in seven of all applicants. Places were found for 74,000 applicants; the figure for total applicants, which is always about twice the number accepted, includes a large number who subsequently fail to gain adequate school qualifications, who withdraw, who are applying for the second time and so on. So it must not be inferred that there is a genuine shortage of places.

Engineering continues its run of popularity (which started well before the recent governmental campaign). Projections to 1977 suggest there are now at least 40% more applying for engineering than in 1972. And even mathematics, physics and chemistry—which lost popularity until 1975—may now be sharing again in the general rise in applications.

Simple head-counting, however, conceals some striking variations in quality. The relative paucity of candidates in mathematics, physics and chemistry does not seem to have led to a diminished standard in those accepted—at least as far as paper qualifications go. Indeed the school performances of accepted physicists rival those of accepted medical students—the latter a category in which universities can be very selective. Good scientists still seem to be coming forward. The position is somewhat different in engineering. Rising quality has not gone along with rising numbers, which may mean that schools have been urging more of their modestly accomplished pupils to consider engineering.

It is against this background that one must consider the suggestion made in the report that universities may soon have to allocate further resources to alleviate the 'critical situation' which seems to be developing in engineering places. Only two or three years ago, of course, we were being told by the engineers of half-empty departments: it is hard to believe the turn-around has been that dramatic. But maybe the universities, rather than create more spaces, should become more selective as time goes on, in the hope of creating a genuine impression amongst students not just that the country welcomes engineers, but that standards in engineering are high. □

Research funding: the new trichotomy

William S. Hillman comments on the relevance of the dichotomy of 'pure' and 'applied' science

THE dichotomy contrasting 'basic' and 'applied' research is still commonplace in discussions on funding. Such discussions often conclude that one mode is over-supported at the expense of the other. Yet on most reasonable definitions of the two, history suggests that neither prospers alone. The old dichotomy is at best misleading, and distracts attention from a more immediately dangerous aspect of current policies. That aspect invites analysis by trichotomy, if you will excuse such language.

Strictly speaking, nothing in the current situation is entirely new. Reasonably enough, the patrons of science have always wanted useful results, quickly if possible. But the main dispenser of patronage now is government, with its self-justifying administrative hierarchies, and it has finally become clear that the taxpayers' resources are finite. In addition, occasional spectacular successes, such as moon landings, have been inappropriately but invidiously contrasted with (for example) the 'failure' of NIH research to abolish cancer. Recent versions of the king's desire for the alchemists' gold have thus split research into at least three recognisable types: real, imitation (or administrative) and defensive. The terms are not quite self-explanatory, but they correspond roughly to real gold, fool's gold and the elusive quicksilver.

Real research is originated, organised and carried out by investigators who understand the field in question. It is aimed at problems which in the view of those investigators are both important and reasonably likely to reward the effort with some return in understanding, control or both. The 'applied/basic' dichotomy is meaningless here. A salient characteristic of real research is that it engages the best judgment of capable scientists. The subjective elements in this description (who is 'capable'?) do not obscure the distinction between it and the second category.

Imitation research, in contrast, starts with the belief that, because science has proved useful in the past, scientists should be able to do anything, right now, given the funds, proper organisation and (most important) what sports writers call desire. This is all regardless of whether the necessary concepts, techniques and people actually exist. The primary motive for organising imitation research is neither controlling nor understanding nature, but pleasing one's superiors. That those superiors should know little about the opportunities and limitations in specialised fields is inevitable, but many organisers of imitation research either share that ignorance or are simply cynical. Hence manifold reports, detailed timetables and complex flow charts are prominent features of the game, leading naturally to the synonym 'administrative research'. The overriding rule is that the players must avoid being corrupted by luxuries such as intellectual rigour, imagination and patience; to that end, frequent exercises in relevance, interdisciplinary communications modalities and urgency are scheduled.

What, finally, is defensive research? The notion is analogous to that of defensive driving in that both recognise the hazards of assuming people will behave sensibly; or to the developing style of defensive medicine, in which procedures that are legal safeguards for the physician may be wasteful for both the patient and society. Defensive research, in short, is what so many scientists must do who would prefer to be doing real research but lack private incomes. The result is that a core of real research becomes surrounded with a set of possibly quite worthless elements designed to impress those who want the gaudy imitation rather than await the real thing.

There is no space here for examples, but few readers will need them. Very likely some policy-makers, here and there, in one agency or another, will indignantly deny any knowledge of imitation or defensive research. But they are easily answered with a paraphrase of the ancient epitaph: if you need examples, look around you. □

From détente to development

Eric Burhop reports on the discussion between scientists at the 27th Pugwash Conference

ATTEENDANCE at the 27th Pugwash Conference, held in Munich on 24–29 August, was as always on an individual basis, facilitating the frank and uninhibited exchanges between scientists of very different backgrounds that has always been a feature of Pugwash. Two hundred and sixty participants from 54 countries met to discuss problems of universal concern: détente, human rights, energy and development. About 50 of them came from countries of the Third World. Those not attending on an individual basis included 18 observers representing governmental international organizations.

In his address to the opening session, Herr Matthöfer, Minister of Research and Technology of the Federal German Republic, remarked "Today Pugwash is a very familiar name, indeed a symbol of the responsible active efforts of scientists from all over the world to help solve the major world-wide tasks of humanity across political and ideological barriers".

For those who may not be so familiar with the history, Pugwash is the name of a small Nova Scotian village, birthplace of the Cleveland, Ohio, industrial magnate, Cyrus Eaton, who financed the first of these conferences held there in July 1957. The call for the holding of such a conference came in a remarkable statement drafted by Bertrand Russell in his incomparably clear and compelling language and beginning: "In the tragic situation which confronts humanity, we feel that scientists should assemble in conference to discuss the perils that have arisen as

a result of the development of weapons of mass destruction . . .". *The statement goes on to spell out to the world the grave and immediate dangers to the whole of humanity implicit in the development of the hydrogen bomb. It was signed by Einstein only two days before his death in May 1955 and by nine other eminent scientists from six countries. The Russell–Einstein Manifesto, as it has come to be known, was presented to the world at a press conference presided over by Joseph Rotblat and held in Caxton Hall, London, in July 1955.

Two roles

There have always been two conceptions of the aims of the Pugwash Conferences. The Russell–Einstein Manifesto looked to them to rouse public opinion to the dangers of the development of nuclear weapons by issuing statements which, coming from scientists of such eminence and integrity, could be ignored neither by peoples nor by governments. The other concept of the role of Pugwash as providing facilities for private discussions between the very experts from NATO and Warsaw Pact countries who were advising their governments on problems of weapons development and disarmament, was especially associated with the thinking of Leo Szilard.

Both Pugwash roles have had an important effect on relations between the two power blocs in the past. The mutual understanding of each other's thinking has made remote the possibility of stumbling into nuclear war by accident, through, for example, wrong interpretation of observations on a radar screen. Pugwash Conferences have been credited with playing a significant role in achieving the Partial Test Ban and Non-Proliferation Treaties and in the development of the theory of deterrence with its strange array of concepts such as first and second strike capability, mutually assured destruction and all the rest.

The general conferences are held

every year, the participants from each country or region being selected by local Pugwash groups. Every fifth year the whole Pugwash community, made up of every participant in previous Pugwash meetings, is eligible to attend. This quinquennial conference elects the principal officers and council of approximately 25 persons that will direct the work for the following five years, assesses the work of the previous quinquennium and plans the future programme. The Munich Conference was a quinquennial one, indeed the largest yet held.

Customarily the conferences work mainly through working groups which deal with specific topics, preparing reports for plenary sessions in the concluding stages of the conference. At the Munich Conference there were eight such groups dealing with nuclear arms control and disarmament; arms control and disarmament in the non-nuclear realm; coexistence, détente and cooperation between nations and systems; security of developing nations; development problems of the economically poor nations; energy, world resources and population trends; environmental hazards of global concern; science, scientists and society.

The prevailing judgment of the first working group was that the SALT talks are not progressing fast enough to prevent advances in military technology which negate the effect of agreements, based on SALT, already reached. The time for stopping the arms race is running out. There were powerful pleas to examine again basic concepts such as that of deterrence which can at best provide a breathing space. Pugwash should take the lead in pressing for a start on real disarmament and not restrict its approach to arms control measures.

Almost unanimous concern was expressed by several groups about President Carter's decision to go ahead with the development of the neutron bomb. The idea of a weapon specifically designed to kill people while preserving property largely intact has caused a wave of revulsion and it was suggested that Pugwash could recapture some of its old sense of urgency in an appeal to public opinion on issues such as this and on the reported development of a nuclear capability by South Africa.

Public statement

It is the practice of Pugwash that its conferences, symposia, workshops, etc, issue reports of discussions and recommendations for the benefit of Pugwash members but public statements formulating specific policies on such matters can come only from Council. (See box for the statement issued by Council on these questions,

Eric Burhop is Professor of Physics at University College, London, and President of the World Federation of Scientific Workers.



Russell at the 1955 press conference

**It is not widely known that originally Russell was himself sceptical about calling a conference. He envisaged simply the issuing of a statement. The proposal to add the call to scientists to meet in conference was made by Frédéric Joliot-Curie on behalf of the World Federation of Scientific Workers, of which he was President, in a letter to Russell in January 1955. The writer acted as go-between in the negotiations between Russell and Joliot-Curie.*

reflecting the discussions at the Munich Pugwash Conference.)

The discussions on détente predictably led on to problems of human rights in an atmosphere largely free of acrimonious interchange of charge and countercharge. Appalling departures from acceptable standards are reported from many countries while no country seems faultless. However, the approach to human rights problems should be guided by how to help the victims. They have been used all too often for propaganda purposes or even to attempt to destabilise governments. Discussions on human rights issues tend to be simplistic, the fact that human rights cannot be separated from human responsibilities being forgotten. Nor can the question be adequately discussed in isolation from its social and political framework.

The question of energy supplies practically dominated the discussions on world resources. There was general agreement that ultimately energy needs must be met from solar, wind, geothermal and safe nuclear fusion power but no consensus about how we are to get through the next 40 or 50 years. There was some but not much enthusiasm for the fast breeder solution and a feeling that there was still some time available before committing us to a full programme of fast breeder reactor development. If a determined programme of energy saving were coupled with control of population growth, one could realistically aim for

a situation in which, by the year 2020, the world population would not rise above 6.7×10^9 with an average energy use of 5 kWt (kilowatts-thermal)—8.0 kWt (rich nations), 3.2 kWt (poor nations)—within a global rate of energy use of only four times that of today. It is believed such a level of demand could be met without introducing breeder technology.

North-South divisions

The problems of development loomed large in the conference discussions. Indeed, any differences in the conference were far more between North and South than between socialist and non-socialist countries. Many delegates from the Third World do indeed feel that their problems are inadequately dealt with by Pugwash and stress that problems arising from the policies of the industrialised countries relative to the Third World, the role of multinational companies, and also to a lesser extent contradictions between Third World countries themselves may represent the most serious threat to peace. On the other hand, some participants looked back somewhat nostalgically to the early Pugwash Conferences when participants were all more or less expert in some aspect of the field of nuclear weapons and it was possible to issue sharp, incisive statements in this field, carrying great weight. A few felt that there would be a case for two separate types of Pugwash Conferences, one specifically de-

voted to disarmament and the other to Third World problems. This would surely be a retrograde step and could only sharpen North-South divisions and the overwhelming view was in favour of continuing as at present.

Pugwash has developed a certain mystique and influence of its own. Some may think it elitist and self-perpetuating; some may chafe at the confidentiality of the discussions, which is an essential feature of the whole Pugwash concept, but it seems that Pugwash is a powerful force for peace and understanding between nations. Thanks to a band of devoted scientists, too numerous to mention but from whom at the present time could be singled out Joseph Rotblat, Bernard Feld, Martin Kaplan, Moishe Markov and Dorothy Hodgkin, the ideas of Russell and Einstein have remained alive and the Pugwash movement has become a living movement with its members showing a genuine sense of loyalty to these ideas and of belonging to the movement.

Of the tasks ahead special emphasis should be placed on the participation of Pugwash in two vital UN meetings; the Special Session of the UN General Assembly on Disarmament in the spring of 1978, and the UN Conference on Science and Technology for Development in the summer of 1979. In both these areas the world community has grievously failed in the past. Here is perhaps an opportunity for a new beginning. □

Declaration by the Pugwash Council

In the light of discussions at the 27th Pugwash Conference on Science and World Affairs, the Council of the Pugwash Movement feels impelled to issue the following declaration:

The world is poised for a new, more intensive and more dangerous round of the arms race. Three features of the world scene account for our sense of heightened urgency and danger:

New weapons of mass destruction. The neutron bomb is proposed for development in the heart of Europe. It is sometimes called a 'clean' or damage-free bomb. On the contrary, both its lethal radiation effects and the short and long-term biological damage it would cause are substantially greater than for existing nuclear weapons of comparable size. There would be strong induced radioactivity from neutron capture in the soil. Because of its relatively small size, it would narrow the distinction between conventional and nuclear weapons and thereby make the use of nuclear weapons more likely.

But the neutron bomb is only one example of new types of weapons now coming into military arsenals: cruise missiles, mobile ballistic missiles, and

others. These are often provocative and thus destabilising. Their deployment is not verifiable with national means, and thus frustrate efforts at control. And the number of nuclear warheads continues to mount. *The deployment of all these new weapons must be stopped.*

Proliferation. There was serious concern at the conference over reports of an imminent nuclear-weapons test in South Africa. This concern was not allayed by the assurances by the South African Government that it has no intention to do so. The acquisition of nuclear weapons by South Africa would be a grave peril to the peoples of Southern Africa and the rest of the world. Developments there must be kept under intense continuing surveillance. Any collaboration with South Africa in the nuclear weapon field—whether on the governmental, commercial or scientific level—must stop. The United Nations should be urged to apply effective sanctions should South Africa be proven to be developing nuclear weapons.

But South Africa represents only the most urgent and immediate occasion for concern. At various points around the globe there have been, from time to time, disturbing reports of states seeking

nuclear weapons. We unequivocally condemn moves towards further nuclear weapon proliferation.

The failure of arms control. In spite of endless efforts, progress towards limiting armaments and stopping the arms race has been all but invisible. Everywhere there is a new sense of impasse. And this is particularly ominous in the SALT and MBFR negotiations. There is no military reason for their failure to make progress. No nation's security would be endangered, indeed it would be enhanced, by much lower levels of armaments. The obstacles are political and it requires only political will and political decisions to overcome them.

● We call on the heads of concerned governments and states all over the world, particularly of the USA and USSR, to halt new weapons deployment and reverse the arms race.

● We call on men and women everywhere to redouble their efforts to make their governments understand and act in the face of our common peril.

And we in Pugwash rededicate ourselves to the achievement of a world at peace.

GEOS

Salvaged results

Stuart Sharrock reports on some of the preliminary results from experiments aboard Geos

At a champagne breakfast on 20 April at Cape Canaveral, European Space Agency officials were gloomy. Instead of celebrating the successful launch of the Geos satellite they were contemplating the total failure of the whole mission. But within a few days the Geos ground control at Darmstadt had partially rescued the situation—although not in the planned geostationary orbit, the satellite would at least be spending some time in the right place.

This would have been a hollow victory, however, if it had meant no scientific results, so the experimenters had to retrieve what they could. In Munich last week they met to discuss their preliminary findings. Although there are some problems, remarkably, there are already some interesting results. After only four months it is clear that much of the scientific programme is going to be a success. But the role of Geos as the reference spacecraft of the International Magnetospheric Study (IMS) cannot be restored and the success of the experiments makes it imperative that the second spacecraft should be launched into geostationary orbit in the near future.

Geos was planned as the first geostationary satellite devoted solely to magnetospheric research. It followed

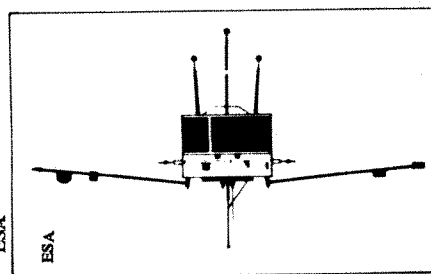
the ATS series of satellites which confirmed that a geostationary orbit was ideal for studying the magnetosphere. The intention was to launch Geos 1 into geostationary orbit early in 1977; the second identical satellite, Geos 2, was to be launched into an elliptical geosynchronous orbit in December 1979 by Ariane, ESA's own launch vehicle now under development. The second mission, codenamed Geosari, was to be complementary to the first, providing a wider spatial coverage of the magnetosphere. The only changes to the second payload would be improvements to the experiments found necessary as a result of experience gained during the first Geos mission.

The attractiveness of Geos as a tool for investigating the magnetosphere is based on the uniqueness of its planned orbit and the sophistication of its payload. Its position was planned to lie on magnetic field lines connecting northern and southern auroral zones. In addition, its geostationary orbit would have allowed average conditions to be established, deviations from the average indicating temporal rather than spatial variations. As the near-earth environment is not static this discrimination is important, but it can only be fully achieved by simultaneous use of more than one satellite such as the pair of satellites of the ISEE mission due for launch in October.

Although Geos has lost these advantages because it is not in geostationary orbit the possibilities opened up by the comprehensive nature of the experiment package still largely apply. It is still possible during part of Geos' new orbit to make simultaneous measurements on waves and particles whilst monitoring the background electric and magnetic fields and plasma density.

When the malfunctioning Delta launch vehicle failed to place Geos into the correct transfer orbit it was clear that injection into a geostationary orbit was not possible. ESA's Space Operations Centre at Darmstadt however managed to place it into a 12 h elliptic orbit with an apogee of around 7 earth radii using the apogee boost motor (see diagram). The orbit was chosen because the satellite is visible around apogee for about 10 hours per day from the ground receiving station near Darmstadt.

Initially the local time of the apogee of the orbit was in the evening sector, but it gets progressively earlier, drifting at a rate of 1 hour every 17.5 days. It has just passed the earth-sun line and



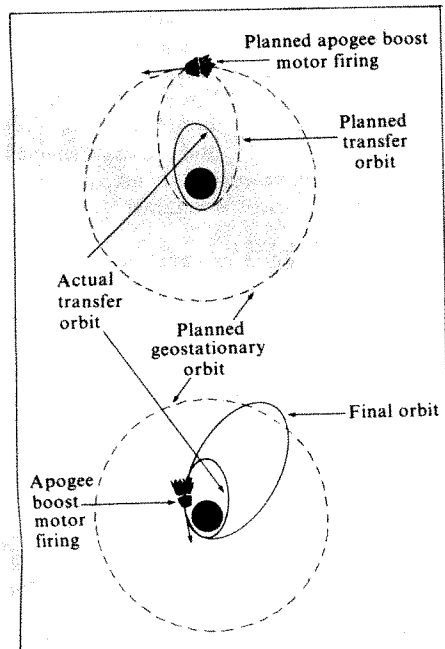
will start to move into the night sector around Christmas. The aurora are much more active in the night sector and so any correlated measurements with rocket campaigns will have to wait until 1978.

So far each experiment has been operated independently and there are still a few interference problems to be sorted out before the optimum mode of automatic operation of the experimental package will be known. But the initial data indicate that the Geos payload is sophisticated enough to provide reliable measurements of electric fields and plasma densities outside the plasmasphere. All data obtained so far are from the afternoon sector of the magnetosphere.

In perhaps the most ambitious experiment on board, an electron beam is fired from the satellite perpendicular to the local magnetic field (determined by another Geos experiment). It is deflected by the geomagnetic field in a circular path of several kilometres radius to return to the satellite close to its source. The distance between the source and the point of return depends on the electric field and the gradient of the magnetic field; the two contributions can be sorted out by varying the energy of the emitted electrons and the plasma drift velocity and direction can be determined. This is difficult to verify on earth as a vacuum chamber a few kilometres long would be required in a magnetic field of less than 100 γ . Unfortunately after about one week of operation the oxide cathode of the electron gun became poisoned but, even with only a few days data, the viability of the method was proved.

Another experiment measuring electric fields—in line with the Geos philosophy of providing redundant measurements of important parameters—has also been successful and interesting slow fluctuations have been seen which have yet to be correlated with data from the ULF wave experiment. The electrostatic wave experiment has been able to detect electrostatic waves with the help of information on the plasma frequency provided by an exploratory experiment using the first relaxation sounder to be launched above the ionosphere.

A variety of electromagnetic wave



Geos orbits: planned and achieved

phenomena have been observed, and some have been related with measurements at ground stations. It will be interesting to correlate these results with those from the various particle experiments but there are still some problems. Confusion over the number density and temperature of the plasma, however, may well be resolved by a determination of the energy spectrum of the various particles, but it needs further investigation of the contribution of the observed photoelectron cloud from the satellite itself and of the effects of spacecraft changing. These problems may be solved later in the year when Geos goes into eclipse.

Some experimenters have been unable so far to completely understand their results through the lack of reliable attitude data necessary to sort out spin modulation effects. But it should be only a matter of time before the relevant analysis programs are fully working.

The experiments measuring high energy particles are least affected by these problems. Preliminary results from a sophisticated mass spectrometer for measuring ion composition, indicate the presence of the doubly charged ions $^4\text{He}^{++}$ and $^{16}\text{O}^{++}$ deep in

the magnetosphere. The new orbit of Geos is almost a bonus in this case because ion compositions can be measured as a function of distance from the earth. Another unexpected bonus of the new orbit is the fact that Geos now spends considerable time near the geostationary satellite ATS-6. Earlier attempts to persuade NASA to drift ATS-6 past the planned geostationary position of Geos were unsuccessful but now the possibility of correlated measurements with similar instruments on the two satellites has become a reality.

The progress that has been made in such a short time after the disappointment of the failed launch is impressive and prospects for the future look bright. But most of the Geos programme in which coordinated measurements were planned with ground-based campaigns has had to be abandoned. Indeed the rocket campaigns from northern Sweden have been persistently plagued with bad luck. The rockets are supposed to pass through the transient auroral arcs and correlate their measurements with satellites poised at magnetically conjugate points above the equator. On the first launch ATS-6 was in position but the rocket exploded.

Geos should have been in position for the second rocket but delays had pushed back the Geos launch date and it was still on the ground. ATS-6 had meanwhile moved on. These difficulties are increased by the refusal of Norway to allow the rockets to overfly their territory and the consequently restricted rocket range makes it very difficult to hit the aurora. There are two rockets planned for 1978 and the rocket team hope their luck will change and that the second Geos spacecraft will have been launched into geostationary orbit.

Geos 2 has already been refurbished and will be of flight standard by the end of the year. It can either be launched into geostationary orbit by NASA early in 1978 using another Delta launcher, or it could wait until late 1979 to be sent up on ESA's own launcher Ariane. An early reflight on a Delta is what the experimenters want—from the way they have evaluated their data in such a short time they have in a sense earned it—and from some view points this appears the most likely option, but the extra cost to ESA will be about \$14 million and it is unclear where this will come from. ESA is expected to decide early in October. □

USA

Opening a biological pathway

The signing of the Panama Canal Treaty last week has implications for the ecology of the Atlantic and Pacific Oceans. Colin Norman reports

AMID the diplomatic fanfare and loud political protest which greeted the signing last week of the Panama Canal Treaty, muted rumblings of a scientific controversy were also faintly audible. The controversy centres on a little-noticed article in the Treaty committing the United States and Panama to conduct a joint study of the feasibility of building a sea-level canal within a few miles of the existing waterway.

The idea of carving out a direct, sea-level link between the Pacific and Atlantic Oceans has been investigated many times in the past, but political, economic and technical problems have so far kept it from becoming a reality. In fact, in spite of the renewed official interest in the idea, it is still considered unlikely that the channel will ever be dug. Nevertheless, the possibility is worrying some marine scientists, who fear that a sea-level canal would damage the ecology of the surrounding oceans.

The worry stems from the fact that

the tropical Pacific and Atlantic Oceans have been separated for perhaps five million years, since North and South America were joined by the isthmus or Panama. Consequently, marine life has evolved independently in the two oceans for long enough to ensure that few species are common to both. Blasting a sea-level link across Panama would open a biological pathway which may result in the migration of plants and animals into a new environment, a process which some marine scientists believe could have considerable impact on the oceans' ecology.

Very little migration takes place through the present canal because a freshwater lake in the middle of the waterway provides a barrier to most marine life, and the large number of locks along the canal means that there is little flow of water through the system. A sea-level canal would, however, be an entirely different prospect. Tidal ebb and flow through the canal, coupled with the fact that the average sea level on the Pacific side is slightly higher than that on the Atlantic side, could result in the transport of plants, animals, larvae and other organisms through the channel.

Among the more dramatic consequences that have been predicted as

a result of breaching the land barrier are the possibility that the yellow-bellied sea snake, a poisonous reptile which now lives only in the Pacific water, could become established in the Caribbean, and that the crown-of-thorns starfish might similarly invade the tropical Atlantic coral reefs. Other potential worries are that fish travelling through the canal might carry pathogens or viruses which are not known to native species. The central problem in such interchange is that the introduction of flora or fauna into a new environment might upset the ecological balance because there may be no natural predators and native species may not have evolved an effective defence mechanism against the newcomers.

Such possibilities are, of course, highly speculative, and some marine biologists contend that it is very unlikely that major environmental damage would result from opening a sea level channel. Nevertheless, there are several sobering examples of previous experience with the introduction of animals and plants into a new environment. For example, the building of a canal to bypass the Niagara Falls, providing a channel joining the Great Lakes to the Atlantic, has resulted in invasion of the lakes by a predatory fish-like creature called the sea lamprey. The lamprey has spread throughout the Great Lakes,

and decimated native species. Similarly, the introduction of the European rabbit into Australia and New Zealand was followed by a population explosion which caused serious damage to vegetation and topsoil.

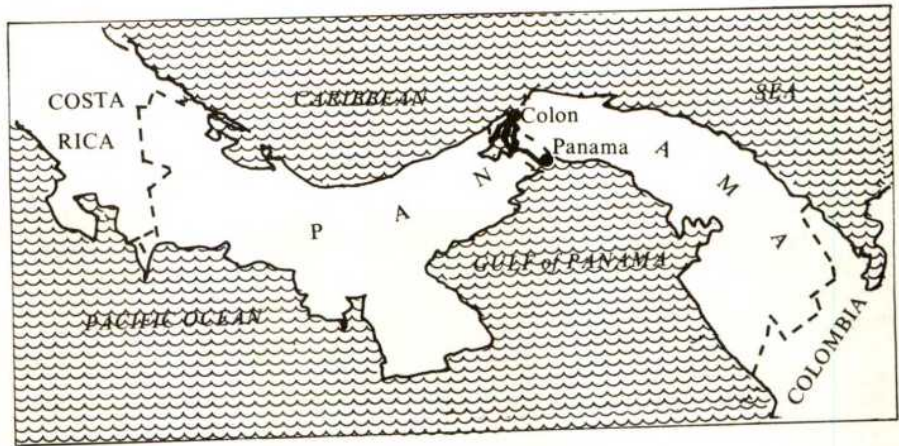
Such concerns were given wide publicity in the late 1960s, when the sea-level canal last came under serious study. A Presidential Commission, established in 1964, conducted a major feasibility study of such a canal, examining potential routes, engineering problems and the possibility of using nuclear explosives to blast out a channel. In 1970, the Commission recommended that the project should go ahead, along a route about 10 miles west of the present canal. Excavation should be by conventional means, the Commission said, and it calculated that the effort would cost about \$2,900 million in 1970 dollars.

Because of the high price tag, and in view of political problems in re-negotiating the existing Panama Canal Treaty, the Commission's recommendations were never implemented, and the whole idea swiftly dropped out of the limelight. It is, in fact, something of a surprise that the idea has been resurrected in the new canal treaty.

As part of the Commission's study, the National Academy of Sciences and the Battelle Memorial Institute were asked to conduct independent analyses of the likely ecological impact of opening a sea-level link between the Pacific and the Atlantic. The Academy's report, which was published as an appendix to the Official Commission Report, must rank as one of the most forthright statements of concern ever to emerge from that august institution. Calling the sea level canal "a gigantic natural experiment" which will have "unforseeable" consequences, the Academy called for a massive study of the tropical Pacific and Atlantic ecosystems before the project is allowed to go ahead. "Available information is altogether insufficient to allow reliable predictions of particular events resulting from the excavation of a sea-level canal in Panama", the Academy said.

The Battelle report was, however, more sanguine about the impact, noting that although there is scant knowledge of the ecology of the region, there is "no firm evidence to support the prediction of massive migrations from one ocean to another, followed by the extinction of thousands of species". The Commission itself was even less impressed with the warnings of disaster. It devoted a mere four pages of its report to a discussion of environmental concerns and concluded that the hazards, whatever they may be, would be "acceptable".

The argument for building a sea-level



Miraflores locks, preventing flow between Atlantic and Pacific

waterway is simply that the present canal is already heavily travelled and it is likely to reach its capacity in the 1990s. Moreover, its locks are too small to take the large freighters and supertankers which are now appearing on the oceans. Such limits were, in fact, realised, back in the 1930s, when work was begun on the construction of a series of larger locks alongside the present canal, but construction was stopped during the war and it was never resumed. (The new Panama Canal Treaty in fact calls for a study of the feasibility of completing the construction of the old locks as an alternative to building a new sea-level canal.)

More recently, a new argument has appeared: a sea-level canal will be needed to transport Alaskan oil and gas to the east coast of the United States. According to this argument, which has been advanced most prominently by Senator Mike Gravel of Alaska, the cost of building a new canal (about \$5,900 million at today's prices, Gravel reckons), should be measured against the cost of building a new pipeline system to transport Alaskan oil and gas from the oil-rich west coast to markets in the east. Once all the advantages are counted up, Gravel reckons that the canal will seem like a bargain. He has stated that he will seek congressional approval for the 1970 report to be up-

dated.

In anticipation of such renewed interest, Dr Frank Press, President Carter's Science Adviser last month asked the National Academy of Sciences to take a fresh look at its earlier report to the Commission to see whether recent scientific data are available to help predict more precisely the likely environmental consequences. Last week, the Academy Committee held two days of hearings and it is hoping to produce a report for Press by early October. Judging from last week's meeting, the Committee is likely to conclude that although a good deal of work has been done, knowledge of the Panamanian ecology is still too sparse to predict with certainty the impact of breaching the land barrier between the tropical Pacific and Atlantic oceans.

Asked last week when the joint US-Panama study envisaged in the treaty is expected to get under way, a State Department spokesman said that no details have yet been worked out. The first priority will be to steer the treaty itself through conservative shoals in the Senate. In view of the immense political problems involved in re-negotiating the treaty, the prospects for negotiating a new treaty to build a sea-level canal in Panama must, at this point, be considered remote. □

USSR

● Training enough highly qualified scientific manpower is a major problem of the Soviet economy. According to the current five-year plan, some 9.6 million new engineers, technologists, agronomists, doctors and other specialists will enter the labour pool by 1980, with increasing emphasis on graduates in molecular biology, environmental protection, atomic and electronic technology, electrical instrumentation, automation, agriculture and forestry. To train them the Soviet Union now has 859 universities and other higher educational institutions, and 4,303 technical colleges. The problem is not simply one of training, however; the proper deployment of scientific personnel is a constant topic of discussion.

Current planning policy calls for closer integration of research in industry. One way to bring this about is to involve university students in projects with an immediate industrial application. Speaking at the opening of the new Grodno State University last month, S. M. Carnau, Minister of Education of the Byelorussian SSR, noted with approval the "widespread attraction of students of senior courses" towards choosing as their term or diploma project a specific contract for research from an industrial enterprise or organisation. This, he said, has considerably extended the "geography" of application of young scientists' talents.

One potential source of close association between academic life and industrial needs would seem to be through the considerable numbers of external and evening students. In the present academic year, the 'full-time' university and technical college intake is estimated by *Pravda* as "more than a million". If the demand for new scientific personnel during 1981-85 equals or exceeds the 9.6 million of the current five-year plan (as all available figures indicate), and assuming a current annual university intake of 1.2 million, by the early 1980s almost one-third of the new graduates will come from external and evening courses.

Unfortunately, these 'part-time' graduates do not necessarily return to industry. The current 'press discussion' of the new Draft Constitution recently included a long letter from a certain Senior Engineer A. Akimova from Khabarovsk, suggesting that 'part-time' graduates should be compelled, by law, to return to their former 'collective'. Ms Akimova complains that in spite of the self-sacrifice of their fellow-workers, who

have to cover their duties when they are absent on paid leave taking examinations, and who "try to provide the conditions of study" during work breaks, many young graduates "on receiving the diploma of a specialist, straight away try to find a



more advantageous job". Ms Akimova's letter, like the majority of contributions to the 'debate', takes the form of a shop-floor proposal, but also like the majority of such letters, has wider implications than the complaint of a few disgruntled workers. It would clearly be to the advantage of the managers of industrial enterprises if they could be sure that the promising young workers involved in part-time education would remain at the disposal of their own production team after graduation, instead of disappearing into the general scientific labour pool.

● This year marks the fortieth anniversary of the first 'North Pole' drifting ice-floe station, in which a five-man team drifted for 274 days in the vicinity of the Pole. That expedition established a valuable research procedure which still continues; this summer 'North Pole' 22 and 23 continued the tradition.

In comparison with these long-term voyages, the recent dash to the Pole by the 'Arktika' icebreaker appears to have had only a limited role in gathering meteorological, geophysical and oceanological data. The expedition was clearly considerably prestigious, and its heroes will doubtless figure prominently in the forthcoming 60th Anniversary Celebrations of the October Revolution. But all in all, only 15 h were spent at the Pole, which included time spent in ceremonial flag-hoisting, rocket-firing and holding hands round the pole.

The main scientific value of the voyage would appear to relate to the design and performance of the

'Arktika' herself. According to A. I. Fokin, Deputy Minister of the Shipbuilding Industry, 100 scientific research institutions were involved in the design of the ship and equipment. A new navigational procedure was tested—the use of helicopters instead of the traditional crows'-nest lookout, to locate the best route through the ice. It is claimed that as a result, speed increased 50-100% and the 'Arktika' reached the Pole seven days ahead of schedule, even though the floes were at times 4 m thick. A new and even more modern icebreaker, the 'Sibir', whose cabins will have independent suspension, obviating the effect of hull motion, will be completed shortly.

The northern sea route, between European Russia and the Soviet Far East is of considerable importance to Soviet economic planning. Commenting on the success of the 'Arktika', Timofei Guzenko, the leader of the expedition and Minister of Merchant Marine remarked that the commercial use of the route is now a "real possibility". The immediate problem, he said, is to build ships capable of following the icebreakers through the paths they have cleared.

Meanwhile, the world's first research icebreaker, the 'Academician Otto Schmidt' is being built in Leningrad for the Institute of Arctic and Antarctic Research. The vessel will carry fourteen laboratories for oceanological, meteorological, hydrological, and ice research, a special unit for aqualung divers and a computer for the on-board processing of research results.

● Using 'Pisces-7' and 'Pisces-11' self-propelled diving craft, a limnological expedition has succeeded in reaching the bottom of Lake Baikal, the biggest and deepest rift-trench in Asia. According to Evgenii Mirlin, leader of the expedition, the main purpose was to apply the techniques developed for ocean-bed research to a study of the lake bed. The programme includes underwater photography of geological formations, and a study of the unique flora and fauna of the lake. Even at the greatest depths, said Mirlin, shrimps, Baikal bullheads, and the small semitransparent *Comphorus baicalensis* can be found.

The team spoke highly of the 'Pisces' craft, which, in addition to providing facilities for visual observations and still and TV photography, have special mechanical arms for collecting geological samples and specimens of water.

Vera Rich

IN BRIEF

A voice for geologists

Geologists in Britain who have felt for some time that they should have a collective voice for the profession have just founded an Institution of Geologists. The institution will aim to advance the profession and practice of geology by 'shaping opinion' in various centres of power and taking an interest in matters of professional standards, education, remuneration and so on. There are about 4,000 geologists in Britain, and a quarter of them have taken an interest in the founding of the institution.

The Geological Society of London, which will provide premises for the institution, was unable to perform these functions itself by virtue of being a learned society with charitable status. But in the long run many hope that the two bodies will operate jointly.

New line of questioning

The case of the dissident Moscow mathematician, Anatolii Shcharanskii, who has been held incommunicado since his arrest last March has now taken an ominous turn; last week, his fellow refusenik Vladimir Lazaris was called to the Lefortovo prison to answer questions about Shcharanskii's mental stability. Although Moscow activists feel it is too early to assess the significance of this new line of questioning, they feel that its proximity to the International Psychiatric Congress in Honolulu, at which the Soviet Union was censured for its use of psychiatric means of political repression, should not be ignored.

CFC policy

The UK government's policy not to ban the use of chlorofluorocarbons

(CFCs) in aerosols until much more research has been undertaken, is supported in a statement issued last week by the Clean Air Council, a statutory body set up to advise the Secretary of State for the Environment on air pollution. The Clean Air Council regards as tenuous the argument that CFCs lead to depletion of the ozone layer in the atmosphere allowing increased ultraviolet radiation to reach earth and possibly cause damage to health (for example, skin cancer).

Since measurements began in the 1920s, the amount of ozone has in fact increased by 5-10%. The statement points out that 'natural' variations are enormously greater than those so far calculated to be a result of the use of CFCs. While supporting further research on CFCs, the Council regards the moves in the USA to ban their use as "precipitate".

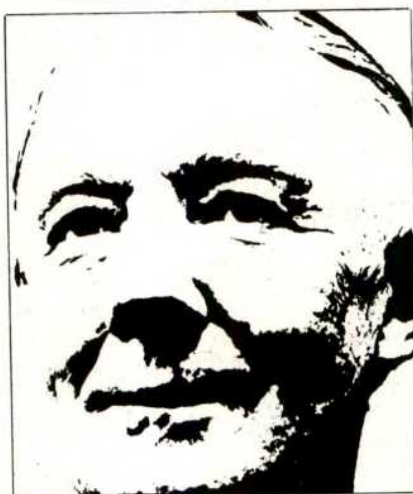
THE World Health Organisation (WHO) is rightly concerned with nutrition, and with the more efficient production and distribution of the earth's resources. WHO has fortunately stopped putting forward absurd statements, such as the one that more than half the world's population is starving, which damaged its earlier reputation. The situation in many countries is serious enough without dramatic exaggerations. Some millions of people die annually of starvation, and several hundreds of millions are underfed for at least a part of the year.

Yet there is no need for this suffering. The world produces more than enough for every member of its population to receive an adequate and healthy diet. If waste and greed could be abolished, there would already be enough to feed the extra three or four thousand million mouths expected by the year 2,000. People starve, or are underfed, because they are poor, and cannot compete in the world market with the consumers in rich countries. The most voracious and wasteful of these consumers are livestock, the battery hens, pigs in sweat houses and barley beef, which waste both calories and protein to add to the pleasure of western palates.

The richer countries also have nutritional problems, but these arise from over and not under eating. When we hear that one of our friends is 'on a diet' we immediately understand that he is trying to lose weight gained by previous over-indulgence. Slimming is becoming big business. A substantial part of the food processing industry is making

allegedly non-fattening products. Newspapers fill their columns with bizarre diets which are often so revolting that their victims will lose weight. Health farms charge over-fed

Cuisine minceur



KENNETH MELLANBY

pop stars more than five star hotels for organised starvation. Even reputable scientists explain how we may try to mislead our stomachs into believing that they have been generously indulged on a minimum number of calories.

A sophisticated attempt at making the best of both worlds—of gluttony and abstinence—is made by Michel Guérard in his new *Cuisine minceur* roughly translated as 'Slimming cookery'. The author runs one of the best, and most expensive, restaurants

in France. His book claims to be "an authoritative and authentic guide to a revolutionary new method which is sweeping the world of haute cuisine". It contains some good recipes; I was glad to learn how to produce a delicious *purée-mousse d'artichauts* and use up the glut of globe artichokes, one of the few vegetables spared by the plague of rabbits in my Huntingdonshire garden. But the new method seems mainly to eschew the more calorific dishes, and to use sugar substitute and *crème fraîche*, a tiresome preparation containing buttermilk. In fact in her introduction, Shirley Conran gives the game away, pointing out that M. Guérard's portions are small, for "if you eat large quantities of food, no matter what it is, you are not going to lose weight". Surely it would be sensible to avoid all this bother, and eat even smaller amounts of normal recipes. M. Guérard's dishes taste even better when made with real cream.

Clearly we should make the best of both worlds. If we in the West ate less we should be healthier, and there would be enough for the underfed nations; surely mankind is not too stupid to be unable to solve the economic difficulties. But we must learn that the only cure for over-eating is abstinence, not a fancy diet. Few are as naïve as the plump girl I heard lamenting her inability to eat enough starch-reduced bread, but the view that some foods encourage thinness is still widely held. High-protein diets are only slimming because they are so expensive. We should remember that the only sweetmeat with a truly non-fattening centre is the polo mint.

correspondence

Roots of 'enzyme'

SIR,—There can be no doubt that Kühne introduced the word *Enzym*, from which, of course, was formed its English equivalent. The reasons for his choice are discussed in at least two printed sources^{1,2}. But the actual construction of the word from Greek originals is less certain, in spite of some very definite statements in the literature.

Bayliss gave a translation of the relevant passage of reference 2, in two slightly different versions in *The Nature of Enzyme Action*, page 11–12 (Longmans, London 1925) and *Principles of General Physiology* (Longmans, London). The words concerned in the former are as follows " . . . but it merely states that *εν ζυμη* [in yeast] something occurs that exerts this or that activity, which is considered to belong to the class called fermentative". Nothing could be clearer: rarely do we have as precise knowledge as this of the thought processes of an author. However, Greek words do not occur in the original at all. They are an interpolation by the translator, whose immense authority and influence has led to almost universal acceptance of this version. The original runs at this point, " . . . sondern nur gesagt, dass in der Zyme etwas vorkomme, das diese oder jene zu den fermentativen gerechnete Wirkung habe . . .".

Bayliss's translation of this passage is, in other respects also, remarkably inaccurate. But unless there is concrete evidence of Kühne's intentions we are reduced to considering probabilities, and this direct construction from *εν ζυμη* still seems rather more likely than the alternative etymology put forward in *The Oxford English Dictionary*. According to this authority, the word was formed on modern Greek *ενζυμος*, meaning 'leavened', or more specifically the leavened bread used in the Eucharist of the Greek Orthodox Church. The very word 'enzyme' was used in English in 1850 in this sense. It seems unlikely that Kühne would have had any knowledge of modern Greek and, if not, this derivation appears to be impossible, even though it preserves a connection with the primitive meaning of the word 'ferment' which Kühne was seeking to replace.

As pointed out by Plantefol, in

Comptes Rendus de l'Académie des Sciences **226C**, 1976, a compound had already been formed in Greek meaning 'yeast in' rather than 'in yeast', so that Kühne's probable sequence of thought was somewhat unfortunate. But even Plantefol seems unaware of Bayliss's blunder. Plantefol also quite justly comments that 'enzyme' has merely taken over the functions of the pre-existing word 'diastase'. (In neither case is the etymology of any relevance to modern ideas of the nature and function of the substances concerned). It seems regrettable, if irretrievable, that this usurpation should have occurred.

Yours faithfully,

T. R. C. BOYDE

Department of Biochemistry,
University of Hong Kong,
Hong Kong

1a *Verhandlungen des Naturhistorisch-Medizinisch Vereins zu Heidelberg* **1** Pp 190–3 (1877). Formally anonymous report of a conference speech by Kühne. Recently reprinted with reference 1b in facsimile in *FEBS Letters*, supplement to **62** (1976).

1b Kühne, W. *Verhandlungen des Naturhistorisch-Medizinisch Vereins zu Heidelberg* **1** Pp 194–8 (1877).

2 Kühne, W. *Untersuchungen der physiologischen Institut der Universität Heidelberg* **1** Pp 291–324 (1878).

Proton-electron mass ratio

SIR,—Sirag (28 July, page 294) states that the proton-electron mass ratio is 1836.109, and derives a combinatorial prediction of 1836 for this quantity. The most recent editions of the *Review of Particle Properties* give 1836.1514 ± 0.0073 , some 21 standard deviations away. While the accepted value for the proton mass has changed significantly since about 1973, the value 1836 for the ratio m_p/m_e has been getting less and less plausible for at least the past 15 years.

The value $6\pi^3 = 1836.1181$ has been calculated, on very different grounds, to be this mass ratio (see *Physics Today*, page 17–19, August, 1971, for discussion). It is substantially closer to the most recent experimental value, but is still in disagreement by 4.6 standard deviations. It is also perhaps worth drawing attention to the apparent tendency of stable hadrons (and the muon) to have masses which are integral multiples of $3m_e$, the threefold electron mass. This has been discussed by R. Frosch in *Lett. Nuovo Cim.* **8**, 633 (1973), and at a meeting of the

Swiss Physical Society, Geneva, 8–9 October, 1976.

Yours faithfully,

JOHN F. CRAWFORD

SIN, Switzerland

Was Darwin the Bellman

SIR,—The uncanny resemblance of F. Waddy's engraving of Darwin on the cover of 21 July to Henry Holliday's picture of the Bellman in the 1876 edition of *The Hunting of the Snark* by Lewis Carroll (Macmillan) (see below) and the 1962 edition of *The Annotated Snark* by Martin Gardner (Penguin Books), must have caused many to suspect that Darwin was the Bellman. This suspicion is confirmed by the two tufts (of hair?) at the foot of Waddy's picture, strongly suggesting that Darwin, in

"Supporting each man on the top of the tide

By a finger entwined in his hair"
had inadvertently scalped two of the crew.

Was it really the Snark that the Bellman was after in his voyages in H.M.S. Beagle (see pp28–31 and p52 footnote 17 in *The Annotated Snark*)?

Yours faithfully,

H. J. A. DARTNALL

MRC Vision Unit, University of
Sussex,
Brighton, UK



news and views

Ultraviolet observation of a quasar spectrum

from B. E. J. Pagel

QUASARS of high redshift permit emission and absorption lines of the far ultraviolet region to be observed from the ground. But what would the spectrum of a quasar look like if it could be observed over a really wide range of (rest) wavelength, say from 0.09 to $1\ \mu\text{m}$? At first sight one might think that this question can be answered by putting together the spectra of several quasars spanning the full range of observed redshifts, and indeed a good deal of useful work has been done along these lines leading in particular to the discovery that the Lyman- α emission line of hydrogen is not much stronger than the red H α line although straightforward theories predict that it should be about an order of magnitude stronger. However, there are severe difficulties in piecing together the spectra of quasars with different redshifts because of the wide range in their intrinsic properties: thus (if one makes the most plausible assumption, that the redshifts are 'cosmological', that is, symptomatic of great distances) the quasars that are actually observed generally become intrinsically more luminous at the higher redshifts, their brightnesses are subject to uncertain cosmological corrections and they are seen through increasing amounts of intervening material in intergalactic space and possibly in clusters of galaxies and even in galaxies themselves.

Consequently it has been clear for some time that observations of the ultraviolet spectra of quasars would help to answer crucial questions concerning both the nature of quasars and the distribution of diffuse matter in the Universe. Furthermore, if 'standard candles' can somehow be identified among quasars, then there is the possibility of using the enormous distances to which they can be seen to obtain really decisive results in

cosmology. But quasars are faint (the brightest one by far being 3C 273 with apparent magnitude 13 and redshift 0.16) and have been beyond the range of past experiments with rockets and ultraviolet satellites, so that the first results were expected to come from the next generation of space observatories using large reflectors, particularly the IUE (to be launched at the end of this year) and the NASA Space Telescope (planned for 1983).

Some of the exciting results awaited from the next generation of space telescopes have now been anticipated, however, in a remarkable rocket experiment carried out by members of the Physics Department of Johns Hopkins University (Davidsen, Hartig and Fastie, this issue of *Nature*, page 203), in which a Faint Object Telescope (FOT) of 40 cm diameter (the largest ever to be flown in a sounding rocket) with a concave grating giving a resolving power of about 150 was used to observe the spectrum of 3C 273 between 1,080 and 1,465 Å in its rest frame.

Despite the limited resolution, Davidsen *et al.* have been able to draw a number of interesting conclusions from their spectrum of 3C 273. It shows Lyman- α and other strong emission lines seen in high redshift quasars and confirms the low ratio of Lyman- α to H α which may be due to dust in the line-emitting region or some other causes. The flux at the Lyman continuum limit (important as an input datum for photoionisation models of the line-emitting region) can be estimated from a small extrapolation of the observed continuum, and the absence of an absorption trough on the short side of Lyman- α enables the previous very stringent limit on the abundance of neutral hydrogen in intergalactic space to be extended to recent cosmological epochs.

A traditional preoccupation in extragalactic astronomy is the determination of the deceleration parameter q_0 , the basis of the method being to plot the redshifts of 'standard candles' against

their apparent brightness so that deceleration manifests itself as an upward curvature in the relation at the faint end. The favoured choice for standard candles is to use first-ranked elliptical galaxies in clusters, but these are restricted to redshifts less than about 0.5 and raise the problem of the effect on their luminosities of stellar evolution during the look-back time; so all that one can say with confidence from present data is that q_0 is fairly small, between -1 and $+1$, say. Indirect arguments, based on the large cosmic abundance of deuterium, suggest that q_0 is positive and under 0.1, well below 0.5 which is the boundary between a 'closed' and 'open' Universe.

Recent work on high redshift quasars has suggested that there may, in fact, be a method of finding standard candles among quasars, based on the equivalent width of Lyman- α , and perhaps one or two other emission lines, in those objects which are flat-spectrum radio sources. Since 3C 273 is a member of this class, Davidsen *et al.* have been able to use their measurement of Lyman- α to tie it in to the system of standard candles based on objects with redshifts up to 3.39 and thus to deduce that $q_0 = 1$ with an impressive internal precision, but with a possibility of systematic error which the authors are careful to point out. A further point that might be made is that the fact of their getting a reasonable value of q_0 at all by this method is evidence in favour of the cosmological interpretation of the redshift.

Probably the most significant result of all in the Johns Hopkins experiment relates to the nature of the absorption lines seen in quasars of high redshift. These fall into two classes: lines seen close to (and usually on the blueward side of) the emission lines, which can be very broad and are almost certainly a 'P Cygni' effect of absorbing gas coming out from the quasar itself (Class I); and Class II absorption lines (including occasionally the 21-cm line of neutral hydrogen), seen at much

B. E. J. Pagel is Professor of Astronomy at the University of Sussex, and is at the Royal Greenwich Observatory, Hailsham, Sussex.

lower redshifts than the emission lines, which are narrow, are formed under low-excitation conditions and can exist in many different redshift systems in the spectrum of one object. The nature of the Class II absorption lines is highly controversial. Riva hypotheses state on the one hand that they are due to material ejected from the quasar at speeds up to 0.6 c ; and on the other that they are due to diffuse interstellar gas in unseen intervening galaxies at the normal cosmological redshift. The second hypothesis is favoured by the distribution statistics of number of systems in one object and by the resemblance between the absorption-line spectra and that of the interstellar medium in our own neighbourhood, but requires the extent of interstellar gas in at least some galaxies to be larger than expected. The presence or absence of Class II absorption lines (which are chiefly

seen shortward of Lyman- α) in 3C 273 provides a critical test between these two hypotheses, because 3C 273 is as luminous as at least one of the high redshift quasars in which several absorption-line systems are seen and is presumably very similar to them physically so that it is equally capable of expelling matter; but it is so near to us that the probability of an intervening galaxy being present is negligible.

Although the spectral resolution of the experiment of Davidsen *et al.* is too low to detect individual absorption lines, the number of lines in high redshift objects is so great as to produce a depression of about 30% in the continuum below Lyman- α , and this can still be easily detected at low resolution. The observed depression in 3C 273 is only $2 \pm 6\%$, which provides an impressive argument in favour of the intervening-galaxy hypothesis. \square

Cell transformation with herpes simplex viruses

from Franklin H. Portugal

HERPES simplex virus, one type of which causes cold sores and another genital tract infections, is receiving increasing attention as a model for understanding how virus expression leads to host cell transformation. In certain conditions infection of mammalian cells with HSV leads to a non-productive infection in which only part of the viral genome is expressed. When mouse cells lacking the enzyme thymidine kinase are transformed by ultraviolet-irradiated HSV, the cells develop thymidine kinase activity which is coded by the virus and which can be distinguished from the host cell enzyme by various biochemical criteria. So far cells transformed in this way cannot be induced to release infectious virus, suggesting that a fragment of the viral genome, although unable to produce infectious virus, is able to maintain the virus-transformed phenotype. Nucleic acid hybridisation indicates that in mouse cells infected with irradiated HSV-1, either five copies of about 23% of the viral genome (Kraiselburd, Gage & Weissbach *J. molec. Biol.* **97**, 533; 1975) or four to six copies of a 10% fragment of the viral genome (Davis & Kingsbury *J. Virol.* **17**, 788; 1976) are present. Hamster cells transformed by irradiated HSV-2 seem to

contain one to three copies of between 8% and 32% of the viral genome (Frenkel *et al. J. Virol.* **18**, 885; 1976). It is not yet known whether the viral genes become integrated into the host chromosome or remain in the cytoplasm.

Maitland and McDougall (*Cell* **11**, 233; 1977) now report that they have been able to transform mouse cells lacking thymidine kinase activity with DNA fragments produced from HSV-2 DNA either by specific cleavage with restriction endonucleases or by mechanical shearing. The transformed cells were selected by their ability to grow in medium containing hypoxanthine, aminopterin, and thymidine, which indicates the presence of a thymidine kinase-positive cell. The cells contained the virus-induced enzyme as well as HSV-specific immunofluorescence. The DNA fragments produced by endonuclease cleavage can be located on a map of the HSV-2 genome and from this information, the thymidine kinase gene seems to be located in the long segment (L region) of the viral genome. Wigber *et al.* (*Cell* **11**, 223; 1977) report the transformation of thymidine kinase-negative mouse L cells with a *Bam*I endonuclease restriction fragment of HSV-1. Cells treated with *Eco*I restriction fragments were not transformed. Bacchetti and Graham (*Proc. natn. Acad. Sci.*

U.S.A. **74**, 1590; 1977) also report that thymidine kinase-negative human cells can be transformed by mechanically sheared fragments of HSV-2 DNA. Several lines of transformed cells have been established that grow continually in the selection medium, express thymidine kinase activity of viral origin, and differ in the stability of thymidine kinase expression.

As well as thymidine kinase, a recent study suggests that a new species of another enzyme, deoxycytidine deaminase, is induced when mammalian cells are lytically infected with HSV-1 (Chan *Proc. natn. Acad. Sci. U.S.A.* **74**, 1734; 1977). The results strongly suggest that this enzyme is also coded by the virus. This study is of particular importance because the induced enzyme may affect cytosine arabinoside, a drug commonly used to treat HSV infections, thus making it less effective.

The fact that specific fragments of the HSV genome, coding for clearly defined biochemical functions, can transform mammalian cells suggests that the HSV system will become an increasingly important model for defining those genes required for both biochemical and morphological transformation. In addition, this system has already begun to provide evidence for the mechanisms that control the viral genome in mammalian cells. This information will presumably be of use not only for specific chemotherapy of human HSV infections but also for understanding virus transformation in general, including oncogenic transformation. Furthermore, it may eventually become feasible to use the HSV model for DNA-mediated gene transfer between mammalian cells. \square

Electron storage rings as lasers

from John Pendry

A NEW mechanism for laser action has been demonstrated by a research group working at the Stanford linear accelerator (Deacon *et al. Phys. Rev. Lett.* **36**, 892; 1977). The device is built on the theory that an electron deflected by a magnetic field can emit stimulated as well as incoherent radiation. So far the device has worked in a pulsed mode extracting 7 kW peak power of laser radiation from the kinetic energy of the electron beam. It appears that only

John Pendry is Head of the Theoretical Physics Group at the Daresbury Laboratory.

Franklin H. Portugal is Associate Managing Editor of the Proceedings of the National Academy of Sciences.

about 0.2% of the electron's kinetic energy can be extracted in each pass through the magnets but it is planned to increase the efficiency by circulating the electrons in a storage ring, which should also raise the mean power available.

Conventional electron storage rings have a large amount of power pumped into them which is dissipated in the incoherent synchrotron radiation. Ultimately this new laser, fixed to a storage ring, could conceivably channel a substantial fraction of the power into stimulated radiation making it available as a well collimated beam of fixed wavelength.

In a conventional laser transitions are stimulated between electronic states of atoms. The power is limited by the pumping process for energising the atoms, and the frequency by availability of suitable electronic transitions. Only recently have continuously tunable lasers become available and the range of tuning is limited to typically less than 1% of the operating wavelength. In the new process massive amounts of energy are available in the kinetic energy of the electron beam and the wavelength of radiation can be simply varied by a large factor. It is expected that lasing action can be observed at wavelengths varying from a few 100 μm down to 0.1 μm .

The laser operates by passing the electron beam through a helical magnetic field. If the spontaneous radiation is confined in a cavity, it can stimulate further radiation, the gain peaking at a wavelength

$$\lambda_s = \frac{\lambda_q}{2\gamma^2} \left[1 + \frac{1}{(2\pi)^2} \frac{\lambda_q^2 r_0 B^2}{mc^2} \right]$$

where λ_q is the period of the helix, B the magnetic field strength, c the velocity of light, r_0 and m the classical electron radius and mass of the electron respectively; the electron energy is γmc^2 . The electron chases closely after the emitted radiation with almost the velocity of light so that pulses from successive periods of the helix fall very close together and the wavelength of radiation is much shorter than the helix period. A 3.2-cm period helix and 43.5 MeV electron beam generate 3.4 μm radiation. (The helix used in the experiment was 5.2 m long.) It is this relativistic shift of the wavelength which gives the laser its great tunability. The energy of the electron beam being proportional to γ , the operating wavelength, λ_s , scales inversely as the square of the energy.

The immediate difficulty is that the laser requires high currents to produce usable gain, posing problems of stability in storage rings operating at what are

rather low electron energies. The theory of storage rings is well understood and these difficulties can probably be overcome to a satisfactory extent. Less well understood is the perturbation that the huge radiation fields building up in the cavity will have on the electron orbits. Even in these preliminary experiments $\frac{1}{2}$ MW of peak power was present in the cavity and this amount of radiation could possibly upset the operation of a storage ring.

Devices producing large amounts of power over a wide spectral range are bound to find wide application. It is conceivable that one of the first uses might be as a tunable source of infrared radiation. Current sources tunable in the 20–200 μm range are of very low power, 10 mW or less, whereas the free electron laser has already functioned at an average power of 0.36 W. Spectroscopy in the far infrared would be vastly improved by the availability of even 1 W of power. Experiments on semiconductors would have the sensitivity to measure excitonic and impurity spectra. Many transient species occurring in fast reactions or in the stratosphere would become accessible to laboratory infrared spectroscopy, and infrared studies of surfaces would be much extended in their capabilities if the weaker absorbers of radiation could be detected.

If the free electron laser can be further developed to give very high powers approaching the megawatt c.w. range sufficient power would be available to consider photochemical reactions and isotopic separation on an industrial scale. In the laboratory large amounts of radiation could be used to shift populations of states on a macroscopic scale giving rise to non-linear phenomena. But it is probably true that the most valuable uses of such a source are yet to be discovered. \square

Planetary crater retention ages

from David W. Hughes

ABSOLUTE ages of planetary features can be determined in principle by dividing the observed crater number density N by the crater production rate, F . So age = N/F . William K. Hartmann (Planetary Science Institute, Tucson, Arizona) is of the opinion that 'the emphasis in funding, research and publication is all on the numerator and none on the denominator' in the above

David W. Hughes is a Lecturer in the Department of Physics at the University of Sheffield.

Correction

In the article 'Multispecific antibodies' (*News and Views* 268, 689; 1977) column 2, page 690, paragraph 2, line 4 should read: "The 'best' antibodies bind their antigens with avidities of 10^7 – 10^9 1 mol^{-1} but so far multispecificity has only been observed in the $\leq 10^6$ 1 mol^{-1} range."

equation. He aims to remedy this state of affairs by his recent paper in *Icarus* (31, 260; 1977).

First let us deal briefly with the numerator. The literature abounds with papers giving details of how N varies with diameter D . Researchers have obtained photographs of lunar and planetary features from Orbiter, Surveyor, Apollo and Mariner spacecraft and have simply counted N , the number of craters per square kilometer with diameters in the range D to \sqrt{D} km. N is proportional to D^n where $n = -2.0$ for lunar maria and the Tharsis plane of Mars, regions where there has been little disturbance. Erosion, which removes smaller craters, gives n values between -1.3 and -1.8 . On the slopes of Olympus Mons, the Martian volcano, $n = -2.4$, possibly due to small volcanic pits adding to the impact craters. To give some impressions of the numbers involved, a typical lunar maria which has been exposed to impact bombardment for about 3.4 aeons (an aeon is 10^9 yr) would have about 10,000 craters larger than 1 km in diameter and 10^{13} larger than 10 cm, in an area of 10^6 km^2 .

Hartmann deals with F , the crater production rate in considerable detail. F is a function of crater diameter, time from the origin of the Solar System, heliocentric distance and planetary size. The diameter dependence is fairly well understood and a similar power law dependence has been found for crater populations on young undisturbed plains on Mercury, Mars and the Moon. The dependence is consistent with the power law size distribution of asteroids, comets and meteorites, these being the impacting bodies responsible for the craters.

The time dependence is based on Apollo chronologies. F was at least hundreds of times higher than its present value before 4 aeons ago but has been within a factor of 10 of present day values during the past 3 aeons. Most people regard the time dependence as representing a smooth transition from the chaotic state of affairs during planetary accretion to the present day near steady state depletion of the asteroid and comet reservoirs. F would be expected to have roughly the same time dependence for all planets.

The change in F as one goes from one planet to another is virtually unknown and is the primary subject of Hartmann's paper. He bases his calculations on a study made by G. Wetherill (*Proc. Lunar Sci. Conf.* 6, 1539; 1975) who considered families of planetisimals in specific orbits and then proceeded to compute the relative number of impacts from each family onto the surfaces of the different planets. The first step is to consider a meteorite of a specific mass and to calculate the diameter of the impact crater it would produce on Mercury, Venus, Earth, Moon, Mars and so on. Obviously the meteorite's orbital velocity (and thus energy) will increase as it moves in towards its perihelion. The effect that the planetary gravitational attraction has on this velocity also varies (this is proportional to $\sqrt{M/R}$ where the planet has a mass M and a radius R). The more massive planets also have a larger effective collision cross section and can sweep up more impacting bodies. Four orbital families are considered, ancient planetisimals circling planets (these only hit planetary satellites), ancient planetisimals circling the Sun (these two families having low relative velocities), asteroidal and short period cometary bodies making up a medium velocity family and long period comets, a high velocity family. In the case of the Moon the impact velocity of a typical member of each of these families would be about 3, 6, 14 and 38 km s^{-1} respectively. The calculated F values from each source onto each planetary and satellite body turn out to be remarkably similar and Hartmann concludes that the terrestrial planets have crater production rates within a factor of 10 of each other. No planet seems to have a preferential source of impacting bodies. The Mercurian crater production rate is about 0.8 to 5 times the lunar rate indicating that Mercury's lava plains, which have N values equal to their lunar counterparts, are in fact younger. Venusian and terrestrial cratering rates are about 1 to 2 times the lunar rate, the surface of Venus having a retention age for 100 km scale craters of around an aeon. Martian production rates are between 1 and 7 times higher than lunar rates; more of this later. The satellites of the outer planets have crater production rates less than a tenth the rate of lunar cratering and should be considerably smoother. There seems to have been no major fluctuations in these rates over the past 3 aeons, and Hartmann concludes that the planetary crater retention ages of surface features formed during this time can be determined within an uncertainty factor of 3 in either direction.

The calculations discussed above have been applied specifically to

Martian crater counts. By scaling to a typical lunar maria the age of a Martian feature, T , is given by

$$T = 3.4 (N_{\delta} / N_{\text{L}}) (F_{\text{L}} / F_{\delta}) \text{ aeons}$$

The channels on Mars are found to have ages of 1 aeon (the uncertainty factor making this range between 0.3 and 3 aeons). The Tharsis plain, a broad equatorial region which surrounds four volcanoes, is slightly younger, about 0.8 aeons old. Olympus Mons is about 0.2 aeons old. These latter findings are contrary to recent assertions by G. Neukum and D. U. Wise (*Science* 194, 1381; 1976) who find that Olympus Mons is 2.5 aeons old and most Martian volcanic provinces older than 3 aeons. Hartmann puts forward a hypothetical Martian scenario in which liquid water was much more abundant on Mars before the Tharsis volcanism started. This volcanism caused a variation in the obliquity of Mars which switched greenhouse effects on and off forming and remelting permafrost layers. This continued until the current Tharsis surface was formed which fixed the obliquity at its present value and resulted in the present state of nearly permanent cold dryness.

Two things are needed to refine the study of crater production rates. First the number of velocity and orbital families considered must be increased and statistical calculations must be made to give a more accurate estimate of the final impact site of bodies in these families. Second, more asteroidal and cometary surveys must be undertaken so that more accurate estimates can be made of the numbers of bodies in each orbital family. With this knowledge the uncertainty factor of 3 in the crater retention ages can one hopes be much reduced. \square

Irrepressible superconductor

from Robert W. Cahn

THOSE who study superconductors with practical ends in mind have been much concerned to raise the transition temperature and the critical magnetic field: indeed, one sometimes has the impression that a transition temperature at or above ambient represents the philosopher's stone for some of today's physicists. There is however another, less regarded material property which limits potential applications, and that is the critical current density, itself a function of field and a structure-

sensitive property. Nb_3Al is one of the most promising A-15 superconductors because of its extremely high critical field ($H_{c2} = 295 \text{ kG}$ at 4.2 K), but the critical current density is modest, and various metallurgical tricks used by a number of investigators to modify the microstructure have in the past not been very effective in improving the situation. About 10^5 A cm^{-2} is the best that has been achieved. A completely new approach now promises the engineer an excellent new superconductor.

K. Lo, J. Bevk and D. Turnbull (*J. appl. Phys.* 48, 2957; 1977) of Harvard University decided to melt-spin the alloy. 'Melt-spinning' is the name given to a variant of splat-quenching which produces a thin continuous ribbon of alloy. The version used here involves a rapidly rotating copper saucer—the substrate—on to which a fine stream of molten alloy is projected. The investigators found that a small silicon addition was needed to produce a good ribbon: their alloy was of composition $\text{Nb}_{73}\text{Al}_{25.5}\text{Si}_{1.5}$. Hitherto, melt-spun ribbons have been used to examine mechanical or ferromagnetic behaviour: melt-spun superconductors are something new under the Sun.

The thickness of the ribbon and thus the effective quenching rate can be modified by altering the speed of rotation of the substrate: the thinnest ribbon was $25 \mu\text{m}$ in thickness and had grains $< 0.5 \mu\text{m}$ in diameter. The microstructure consisted of fragments of dendrites surrounded by transition layers presumed to be of variable composition. The crystal structure remained single phase A-15. To measure electrical characteristics, the samples had to be embedded in indium in order to provide good electrical and thermal connections. Even so, the contact resistance limited the feasible current density to $1.8 \times 10^6 \text{ A cm}^{-2}$. $25 \mu\text{m}$ ribbons were tested in fields up to 150 kG and the critical current density was found to be in excess of the experimentally enforced limit of $1.8 \times 10^6 \text{ A cm}^{-2}$ in all field strengths. When the ribbon was annealed at 750°C , T_c was raised from $\sim 16 \text{ K}$ to 18.4 K and the critical current density was still in excess of the experimental limit. (It is therefore not known whether heat treatment increased or decreased the critical current density). The highest critical current density in 105 kG for Nb_3Al (that is, the highest in any substance) reported in the past was $\sim 10^5 \text{ A cm}^{-2}$. Plainly an extreme refinement of microstructure is the key to an enhanced critical current density.

It is already clear, from these preliminary results with just one of the A-15 family of compounds, that a new generation of high-field super-

Robert W. Cahn is Professor of Materials Science at the University of Sussex.

conductors is in the offing. There will of course be problems—principally the need to incorporate superconducting ribbons into a thermally well-conducting matrix such as copper to provide insurance against a breakdown of superconductivity in service. A number of metallurgical techniques are available for this purpose (for example, Tsuei *J. appl. Phys.* **45**, 1385; 1974; Chen & Tsuei *ibid.* **47**, 715; 1976) but they generally depend on a precipitation of superconducting filaments *in situ*. Melt-spun ribbons will have to be incorporated in a matrix after they have been made: it may be possible to use a sintering technique with a low-melting metal such as aluminium. The fact that annealing does not damage the superconducting properties will simplify the task.

Rapid quenching from the melt has been used before in connection with superconduction, but in a different sense. Instead of using the technique to refine the structure of a stable compound, as the Harvard scientists have

done, workers at California Institute of Technology used it to create metastable structures. Johnson and Poon (*J. appl. Phys.* **45**, 3683; 1974) tested a whole range of metastable simple cubic inter-metallic superconductors (such as $\text{Te}_{70}\text{Au}_{30}$, $\text{Sb}_{75}\text{In}_{25}$) and established that T_c was systematically (and inversely) related to lattice parameter; $\text{Sb}_{75}\text{Au}_{25}$ had the highest T_c of ~ 6.5 K, and it seemed that the (meta) stability of the simple cubic lattice (one atom per unit cell) broke down for smaller lattices. Glassy alloys, made by melt-spinning, can also be superconducting: V. L. Johnson, S. J. Poon and P. Duwez (*Phys. Rev.* **B11**, 150; 1975) examined the superconductivity of $\text{Au}_{25}\text{La}_{100-x}$ glasses ($16 \leq x \leq 40$). Among other peculiarities, these glasses have ultra-short electronic mean free paths. For $\text{Au}_{25}\text{La}_{75}$, this path is 1.7 \AA , $T_c = 3.4$ K, the upper critical field ~ 10 kG. This category of material is a scientific curiosity but is not likely to threaten the technological superiority of Nb_3Al and its analogues. □

tion of heat sources is uncertain and must be assumed, albeit on the basis of reasoned geological and petrological arguments. In practice, Chapman and Pollack take an upper layer of 8 km with heat production A overlying a granulite facies lower crust of 32 km with heat production $0.25 \mu\text{W m}^{-3}$ overlying a depleted ultrabasic zone (80 km, $0.01 \mu\text{W m}^{-3}$) overlying a pyrolyte mantle ($0.084 \mu\text{W m}^{-3}$). In this context, A , the heat production in the upper crust, is the variable related to surface heat flow. By inserting different values of regional surface heat flow it is then possible to derive a series of geotherms for the continental lithosphere (making another assumption, again reasoned, on the variation of thermal conductivity with temperature).

The approach to the oceanic area, however, is rather different. Here the thermal regime is characterised by cooling following formation at a spreading ridge, although the oceanic lithosphere will also contain radioactive sources. Chapman and Pollack assume an initial temperature gradient of $1,300^\circ\text{C} + 1.5^\circ\text{C km}^{-1}$ and a heat production of $0.5 \mu\text{W m}^{-3}$ in the upper 10 km in deriving a family of oceanic geotherms in which the variable is again regional heat flow. And finally there is the question of the mantle solidus, for which assumptions are again necessary because of the uncertain role of volatiles in depressing solidus temperature. In fact, Chapman and Pollack go for a mixed volatile environment (chiefly H_2O and CO_2) which leads to a solidus intermediate between those applicable to volatile-free and purely hydrous melting.

Implicit in all this, of course, is the hope of an ultimate relationship between (regional) surface heat flow and lithospheric thickness which can be defined accurately and smoothly enough to enable the latter to be determined from the former. And such a relationship indeed emerges. From the intersections of the derived geotherms with the adopted solidus, Chapman and Pollack are able to obtain smooth curves (one each for continental and oceanic zones) of lithospheric thickness against heat flow. Moreover, where comparable direct data on lithospheric thickness are available (for example, for the Pacific and North America) the agreement between observation and the predicted curves is good, thus justifying the assumptions made in the first place. With this encouragement it is then but a short step to convert the world map of the 12th degree spherical harmonic analysis of global heat flow developed earlier by Chapman and Pollack (*Earth planet. Sci. Lett.* **28**, 23; 1975) into a parallel map of world lithospheric thickness.

Thickness of the lithosphere

from Peter J. Smith

FOR more than 50 years the Moho was regarded as the most important discontinuity in the upper Earth, and following its discovery by Andrija Mohorovičić in 1909 considerable effort was devoted to determining its depth throughout the world. The result is that worldwide contour maps, albeit imperfect ones, of crustal thickness have been around now for some time. But although the Moho is no less fundamental a boundary than it ever was, its significance has been overshadowed in recent years by that of the lithosphere–asthenosphere interface. The fact is that as far as plate tectonic processes are concerned, the crucial contrast is not the physicochemical one between the crust and mantle but the physical one between the lithosphere and asthenosphere.

So how thick is the lithosphere? A rapid and random survey of published opinions suggests that the top of the asthenosphere is most commonly thought to be 'about 70 km deep', although it is widely recognised that this is an average concealing variations. In this latter respect lithospheric thickness resembles crustal thickness; but the similarity ends there. The lithosphere–asthenosphere boundary appears to be less sharp than the Moho and thus more difficult to locate

accurately. Moreover, there are far fewer spot measurements of asthenospheric depth than of mantle depth because of the relatively recent origin of interest in the former. Certainly no one has yet suggested that there are sufficient direct data for the production of a worldwide contour map of lithospheric thickness.

Nevertheless, Chapman and Pollack (*Geology*, **5**, 265; 1977) have now produced just such a map. Of course, their approach has had to be indirect. Specifically, they have developed a thermal model of the lithosphere which has enabled them to derive a family of geotherms for continental and oceanic zones in which the principal independent variable is surface heat flow. The depth at which these geotherms intersect the mantle solidus is then taken as the surface of the asthenosphere. The point about all this is, of course, that surface heat flow and its variations throughout the world are now quite well known, so the problem of the primary data is largely solved.

On the other hand, there is a disadvantage in that, as with all indirect methods, it is necessary to make assumptions. For example, the continental model adopted by Chapman and Pollack not surprisingly involves steady state conduction through a lithosphere which contains radioactive heat sources and into which heat flows from below. But the vertical distribu-

What the new map shows is that the thickness of the lithosphere varies from a few tens of kilometres in young oceans and continental orogenic provinces to more than 300 km in shield areas. It is also evident from the analysis which led to the production of the map that beneath the continental shields the asthenosphere is very thin. Indeed, a strict interpretation would suggest that the asthenosphere does not exist there at all, for shield geotherms do not intersect the solidus at any depth, although shield lithosphere is presumably partially decoupled from the deeper interior at the depth at which the appropriate geotherm approaches the solidus most closely. In any event, there is a clear implication that the viscous drag at the base of shield lithosphere is much higher than elsewhere—a prediction which receives some support from observation.

Finally, it is clear that the thickness of the lithosphere, both continental and oceanic, increases (not linearly) with age. This implies that shield lithosphere was once much thinner than it is now and that the asthenosphere beneath it was thicker, thus facilitating continental drift during the Precambrian. As for the future, continental lithosphere will presumably continue to thicken, increasing the viscous drag at its base and thus gradually bringing drift to a halt. □

Communal nesting in birds

from John Krebs

THE Central and South American Groove-billed Ani (*Crotophaga sulcirostris*) lives in groups of one to four monogamous pairs. All the females in a group lay their eggs in a communal nest, and both members of each pair help to incubate and feed the young. The axiom of selfish genery warns one to expect that Ani groups are uneasy alliances in which each individual pursues its own reproductive ends. A study recently reported by S. L. Vehrencamp (*Science* **197**, 403; 1977) reveals that this is indeed the case. The most obvious and unequivocal sign of self interest is that the females in a group roll each other's eggs out of the communal nest. Females cannot, however, distinguish their own eggs from those of others so that an individual only engages in egg-rolling before she herself has started to lay. This means, of course, that the first female to start

laying eggs is more likely to suffer, and the last female to start loses no eggs. Although the first female(s) to lay to some extent compensate for egg loss by laying larger clutches, they still end up with fewer eggs than the last female at the time of incubation. In comparing nesting groups of different sizes, Vehrencamp found that the average number of eggs per female is positively correlated with group size, but the first female to lay in the group raises fewer young than a solitary pair, while the last female raises more. So far all the benefits seem to lie with the last female to lay and none with the first. There is potentially a cost to the late layer, because the first female starts to incubate as soon as she has completed her clutch, and the first young to hatch out survive better than later ones. The late female's answer to this is to lay her eggs more rapidly, at 1–2 day intervals compared with 2–3 days for early females, so that her young hatch out no later than average. All in all, the late females clearly do better than early layers, and they tend to be older (probably more dominant) individuals who are mated to larger than average males.

The late layers also gain when it comes to parental care: they contribute less to incubation and feeding the young than do the other females in a group even though most of the brood is theirs. This picture of more or less parasitic behaviour by late females is to some extent ameliorated by the fact that the males mated to late layers do more incubation and feeding of the young than any other group member. This implies that the male can tell that his mate has laid most of the successful eggs so that he is feeding his own children.

Clearly we need to know a lot more about the Ani system of communal nesting before a full cost benefit analysis for each group member can be done. At the moment it looks as though the late-laying female and her mate get more out of the system than the others, so further data are needed to work out why the communal nesting system is a stable, if selfish alliance.

In other communal nesting species which have been studied over several years, the members of a group are usually closely related to one another. However, these species, including babblers of the genus *Turdoides* and two American jays (*Aphelocoma* spp.), have a different system of communal nesting from that of the anis. Only one pair does all the reproducing, and the other group members are usually sub-adults, many of them offspring of the reproductive pair and hence siblings of the present nestlings. Although the helpers, as they are called, may in-

dators, and feed nestlings, there is some uncertainty about how much they actually increase the reproductive success of the breeding pair (Zahavi *Ibis* **116**, 84; 1974; Woolfenden *Auk* **92**, 1; 1975; Gaston, thesis, Oxford, Univ. 1976). One interpretation (Gaston *op. cit.*) is that the helpers are providing just enough aid to encourage the parents to allow them to stay in their territory. In both the babblers and jays, young birds have rather little chance of establishing their own territory away from home, because the suitable habitat is fully occupied by adults, who have a low mortality rate. The best bet for a young bird is to stay at home and try to take over the parental territory when one or both of the parents die.

In doing this, the young birds could reduce the future reproductive success of their parents by competing for food, attracting predators to the nest, and so on. In order to offset this, and hence make it profitable for the parents to allow them to stay, they provide a small amount of help in rearing new young.

This interpretation is speculative, but it emphasises that the apparently simple cooperation probably has a rich and fascinating underlying pattern of selfish behaviour. □



A hundred years ago

METEOROLOGY AND THE INDIAN FAMINE . . . Dr Hunter also found that six great scarcities of sufficient gravity to be officially returned as "famines" had occurred during the period 1880–77. Of these six famines five were caused by years of drought coincident with, or adjoining to, the periods of minimum sunspots. He further showed that the rainfall at Madras passed through an eleven year's cycle, corresponding with the cycle of sun-spots. That is to say, the rainfall reaches its minimum in the eleventh year, rises to its maximum about half-way through the cycle in the fifth year, and then declines again to its minimum in the eleventh year.

At the recent biennial meeting of the German Astronomical Society, which was held at Stockholm, the members received the news of the discovery of the two satellites of Mars with manifestations of grave doubts. The president at their request telegraphed to the Berlin Observatory, and in reply received a copy of the original telegram as it was sent from America.

From *Nature* **16**, 13 September, 425, 428; 1877.

Carbon fixation pathways

from Sandy Grimwade

A symposium on the C_3 and C_4 photosynthetic pathways was held by the Ecological Society of America at the Annual Meeting of the American Institute of Biological Science in East Lansing, Michigan in August 1977.

THE fixation of atmospheric carbon dioxide into metabolic intermediates by green plants can take place by three different pathways. In the majority of plants CO_2 is fixed by the classic Calvin cycle directly into ribulose diphosphate to form two molecules of phosphoglycerate—a three-carbon compound. In some plants, however, the initial carboxylation step results in the formation of C_4 compounds such as oxaloacetate, which are then decarboxylated in specialised bundle sheath cells where the released CO_2 is refixed by the usual Calvin cycle. The third, and most restricted type is found mostly in succulents of arid regions. Fixation of CO_2 in this case occurs into crassulacean acid.

The differences between the so-called C_3 and C_4 plants give rise to a wide variety of biochemical, ecological and climatological implications, some of which were discussed at the symposium.

B. N. Smith (Brigham Young University) outlined the biology of C_3 and C_4 plants and the resulting isotope fractionation effects. Atmospheric CO_2 contains both the ^{12}C and ^{13}C stable isotopes of carbon. Compared with a standard limestone sample, the relative concentration of the ^{13}C isotope in atmospheric CO_2 ($\delta^{13}C$) is -7% . The photosynthetic fixation of atmospheric CO_2 exhibits a substantial isotope effect, which differs markedly for C_3 and C_4 plants. As CO_2 fixation is the only reaction which shows such a substantial effect, the resulting isotope depletions are reflected throughout the plant tissues. In C_3 and C_4 plants, the $\delta^{13}C$ values are -26 and -12% respectively. In addition, these isotope depletions are maintained in the herbivores which eat the plants and so on up the food chain. As these differences are so marked, they provide a valuable tool in several fields. For instance, the relative concentration of C_3 and C_4 plants in fossil animals can be estimated. The adulteration of honey—mostly from C_3 plants—with

corn syrup, from a C_4 plant, could be detected and quantified.

Any consideration of C_3 and C_4 metabolism in plants gives rise to two related questions—why do plants use more than one pathway for CO_2 fixation and how did the pathways evolve. Answers to these questions must account for the fact that C_4 metabolism is found spread throughout many plant genera, and yet no genus is composed exclusively of C_4 plants. For instance, about 50% of grass species are C_4 plants as is a smaller proportion of Compositae. The only way to account for such a patchy distribution is to hypothesise that C_4 metabolism with its attendant array of specialised enzymes and cellular anatomy arose as many as 20 times in the course of plant evolution. The alternative proposition, that it arose once or a few times is almost impossible to reconcile with accepted schemes.

The question of the rationale for more than one pathway is easier to tackle. J. R. Ehleringer (Stanford University) presented data showing the advantages of C_4 metabolism in grasses in particular environmental circumstances. Measurements on whole plants of net carbon gain per unit weight show the following. At normal oxygen tension, C_3 and C_4 plants show similar efficiencies, but as O_2 tension decreases, the efficiency of C_4 metabolism increases whereas C_3 remains constant. Similarly, the yield of C_4 plants is constant with increasing temperature whereas the efficiency of C_3 metabolism decreases. C_4 plants also have the advantage at high light intensities and low humidity. Using these data, a computer simulation of the rate of carbon gain in a C_3 or C_4 grass canopy in the Great Plains of the United States in July shows that as latitude increases, so does the efficiency of C_3 grasses. At $45^\circ N$ the two types are about equal. A survey of the flora of the Great Plains reveals that in the south of Texas ($25^\circ N$) about 70% of the grass cover is C_4 , at $40^\circ N$ the two types are equal, and at $60^\circ N$ only C_3 types are found. Thus the relative physiological advantage of C_3 or C_4 metabolism is directly reflected in the flora of the region.

Similarly, a survey by C. T. Harrison (University of Wyoming) of the grass types along a transect of grasslands in Wyoming shows a decrease in the percentage of the biomass composed of C_4 grasses with increasing elevation, and hence decreasing average temperature. This point is nicely reflected in measurements of the $\delta^{13}C$ values of bison remains in a single burial pit in Wyoming. At the surface, the $\delta^{13}C$ values reflect a diet for the bison which contains a substantial proportion of C_4 grasses. With increasing depth,

and hence age, the proportion of C_4 in the diet decreases until the $\delta^{13}C$ value reflects a diet composed entirely of C_3 plants approximately 8,000 years ago. This is held to reflect the gradual warming of the climate, with the subsequent invasion of C_4 grasses from the south over the past few millennia. \square

Gravitation but no levitation

by Malcolm MacCallum

The Eighth International Conference on General Relativity and Gravitation was held at the University of Waterloo, Ontario, Canada on 7–12 August, 1977. The organising committees, headed by M. McKiernan (University of Waterloo) and W. Israel (University of Alberta, Edmonton), provided a most enjoyable and enlightening meeting.

'GR8' proclaimed the T-shirts: they were right in more ways than one. Up to nine parallel afternoon sessions were required to accommodate more than 400 talks contributed from among the 700 or so participants. The morning plenary sessions were devoted to invited talks, mainly reviews.

Informal discussions between V. Braginsky (Moscow), W. Unruh (University of British Columbia, Vancouver) and others evolved schemes to push the sensitivity of gravity wave detectors to the level where the instrument must be regarded as a quantum system. A factor of about one million improvement is required to measure the expected astrophysical sources, as described by K. S. Thorne (Caltech). The active groups developing the various possibilities were all represented, and there seemed to be considerable optimism about the feasibility of the new detectors and the likely technological spin-off from their development.

Experimental results by I. S. Shapiro (Massachusetts Institute of Technology) on time-delay data from the Viking missions to Mars, and by R. B. Partridge (Haverford College) on the isotropy of the cosmic microwave background contained no real surprises, apart from some unexplained and unsystematic deviations in Shapiro's data which he tentatively ascribed to procedural errors at the tracking stations. R. Newman (University of California,

Irvine) described new laboratory tests confirming the inverse square law at ranges around 4 cm in contradiction to some results of Long (*Nature* **260**, 417; 1976).

The ever-expanding capabilities of computers were much in evidence, and I suspect many relativists will have been converted to a new interest in the potential of the algebraic manipulation systems, most of which, such as the SHEEP system demonstrated by I. Frick (University of Stockholm), are designed for ease of casual use. Interesting numerical computations included two splendid films, by J. R. Wilson (Lawrence Livermore Laboratory) on collapse and by L. Smarr (Harvard University) and K. Eppley (University of Maryland) on the head-on collision of two equal mass black holes. Thorne suggested that generalisation of the latter to glancing incidence might increase the rather low gravitational radiation found by Smarr and Eppley.

The significance of black holes for astrophysics and cosmology was well reviewed by M. J. Rees (Institute of Astronomy, Cambridge) and I. D. Novikov (Moscow) respectively. Effects considered ranged from X rays through quasar models to the overall entropy of the Universe, and included the polarisation effects recently predicted by P. A. Connors and R. F. Stark of the University of Oxford (*Nature* **266**, 429; 1977). Among the other astrophysical contributions, J. L. Friedman (Wisconsin-Milwaukee) announced that all rotating stars are unstable, and C. M. Caves (Caltech) pointed out that gravitational Cerenkov radiation would cut off the cosmic ray spectrum in some alternative theories of gravity.

The singularities symposium consisted of many informal contributions, shorter even than their written abstracts, carefully organised by G. F. R. Ellis (University of Capetown) into an enlightening overview. The difficulties in defining singular boundary points and in formulating the 'cosmic censorship' hypothesis (roughly, that all singularities other than an initial big bang are hidden inside black holes) were well discussed. In the session on the initial-value problem Y. Choquet-Bruhat (Paris) reviewed her work with J. Marsden (University of California, Berkeley) proving that the energy of asymptotically flat spaces has a minimum, namely zero, at flat space, thus partially verifying the long-standing conjecture that such spaces have positive energy.

Complex variable techniques figured in various contexts. W. Kinnersley and D. M. Chitre (Montana State University) have developed a method for generating infinite families of solutions

of Einstein's equations. R. Penrose (University of Oxford), in his review, related his twistor programme to the soliton solutions of Yang-Mills theory.

The various schools of thought on quantum gravity were well represented. The most rapidly growing seems to be 'supergravity', in which gravity is related to a spin-3/2 field through a symmetry. This removes some divergences, has positive energy, and, as shown by R. Tabensky and C. Teitelboim (Princeton University), can be regarded as a 'square-root' of general relativity much as Dirac's equation is related to the Klein-Gordon equation. S. W. Hawking (University of Cambridge), speaking on path-integral formulations, pictured the vacuum as a sea of Planck mass black holes and warned 'Danger! Virtual black holes.' The gap between quantum and classical approaches was still apparent, as when S. Weinberg (Massachusetts Institute of Technology) told a questioner that geometry did not matter, and only belatedly added 'in this case'.

One interesting contribution that did not materialise was by L. Domash (Maharishi University) on levitation. Did his theory break down in mid-Atlantic? Anyway, only lesser adepts in meditation were present. □

Oocyte microinjection

from H. Chantrenne

A symposium on the use of *Xenopus* oocytes in the study of DNA transcription and mRNA translation was held on 16 July, 1977 at the Université Libre de Bruxelles as part of an EMBO course. It was organised by Dr G. Marbaix and Dr G. Huez, Laboratoire de Chimie Biologique, Département de Biologie Moléculaire, Université Libre de Bruxelles.

THE colloquium presented some recent results obtained with *Xenopus* oocytes. J. Brachet (Brussels) provided an introduction to oocyte structure and oogenesis.

A novel development is the use of oocytes to study the expression of injected DNA. J. B. Gurdon (MRC Laboratory of Molecular Biology, Cambridge) described techniques he has developed for studying the expression of purified genes by injecting the DNA into the oocyte germinal vesicle, where

it is transcribed. He also described recent experiments showing that the oocyte cytoplasm can 'reprogram' injected nuclei. Nuclei from fully-differentiated *Xenopus* cells injected into oocytes of the newt *Pleurodeles*, directed the synthesis of proteins characteristic of the *Pleurodeles* oocyte stage rather than proteins characteristic of the differentiated cell (see *Proc. natn. Acad. Sci. U.S.A.* **74**, 2470; 1977).

The oocyte provides an alternative to the more usual cell-free systems for studying the translation of mRNA. L. van Vloten-Doting (State University, Leiden) emphasised the advantage of the oocyte. Translation is correct, the oocyte is of course able to adjust the conditions for adequate operation of the complex protein-synthesising machinery, and translation continues for several days. Another remarkable feature is the oocyte's ability to bring about correct post-transcriptional modification of the newly-synthesised polypeptide.

An illustration of this was given by J. Ghysdael (Brussels) with the 35S RNA from avian myeloblastosis virus. When injected into oocytes this RNA directs the synthesis of a long polypeptide which is the precursor of the 'group antigens' of the virus. In the oocytes it is correctly split into four viral proteins (p27, p19, p15, p12) exactly as in infected chick fibroblasts, but much more slowly, allowing a clear analysis of the process by classical chasing methods. According to C. von der Helm (see *Proc. natn. Acad. Sci. U.S.A.* **74**, 911; 1977) protein p15, one of the final products of polypeptide cleavage, is involved in the cleavage. This raises an intriguing problem, for p15 is not initially present in the oocyte; no-one knows at present whether it can act enzymatically when it is still part of the large polypeptide and provided that the concentration of the latter is sufficient, or whether selective cleavage is brought about by oocyte enzymes.

G. Huez and G. Marbaix (Brussels) have been studying the role of poly(A) in the stability of the messenger. They showed that the addition of poly(A) to a histone mRNA lacking poly(A) increases its half-life in the oocyte from around 10 to more than 48 hours. G. Vassart (Brussels) showed how the translation of thyroglobulin mRNA in oocytes has resolved the controversy over the size of the protein subunit, which turns out to have a molecular weight of 300,000. It is only in the oocyte that this messenger is translated correctly. B. Lebleu (Brussels) showed that crude preparations of mRNA from cells induced to produce interferon directed the synthesis of biologically active interferon in the oocyte. □

H. Chantrenne is Professor of Biochemistry in the Faculty of Sciences, Université Libre de Bruxelles.

articles

Ultraviolet spectrum of quasi-stellar object 3C273

Arthur F. Davidsen, George F. Hartig & William G. Fastie

Department of Physics, The Johns Hopkins University, Baltimore, Maryland 21218

The first direct observation of the ultraviolet spectrum of a quasi-stellar object (QSO) has been made with a rocket-borne telescope. The emission line spectrum of 3C273 is similar to the spectra of high-redshift QSOs, but no absorption is observed. The results provide important new constraints on theoretical models of QSOs, place a severe limit on the density of neutral hydrogen in the intergalactic medium, and suggest a cosmological origin for much of the absorption seen in high-redshift QSOs. Comparison of the ultraviolet spectrophotometry of low- and high-redshift QSOs suggests that the universe is closed, with $q_0 \sim 1$.

It has long been recognised that ultraviolet observations of low-redshift quasi-stellar objects (QSOs) made with space-borne telescopes might reveal important clues concerning the nature of these objects. Among the problems for which such observations are relevant are the nature of the energy source, the physical conditions in the emission line envelopes, and the origin of the absorption lines seen in high-redshift QSOs. It now seems that these observations may also be of great significance for cosmology. Here we report the first observation of the ultraviolet spectrum of a QSO, made with a telescope carried above the Earth's atmosphere by a sounding rocket. The spectrum of 3C273 is presented and some of its implications are briefly discussed. Further details of the observation and analysis will be described elsewhere¹.

Faint object telescope

The observation was made with a new instrument², which we call the Faint Object Telescope (FOT), designed especially for moderate resolution (10–15 Å) ultraviolet spectrophotometry of faint extragalactic objects in the wavelength region between 1,200 and 1,700 Å. The FOT uses a 40-cm diameter $f/15$ telescope, whose 1,100 cm² collecting area makes it the largest optical telescope ever flown in a sounding rocket. A spectrum is formed by a concave grating which gives a dispersion of 20 Å mm⁻¹ at the detector focal plane. The spectrograph has two circular entrance apertures whose diameters, projected on the sky, are 2.2 arc min with a centre-to-centre spacing of 4.4 arc min. One aperture observes the target while the other accumulates simultaneous background data.

The FOT detector consists of two 25-mm diameter micro-channel plates mounted in a chevron configuration, followed by two one-dimensional resistive anode readout devices^{3–5} for accumulating separate spectra of the target and background. The detector has a magnesium fluoride window, and a caesium iodide photocathode is deposited directly on the front surface of the first microchannel plate. Pressure in the detector is maintained below 10⁻⁷ torr by an ion pump which is turned

off before flight. The spectrum observed in the target channel is displayed on the ground in real time by a multichannel analyser.

The overall effective collecting area of the FOT varies between 11 and 5 cm² over the range 1,250–1,700 Å. Pre-flight and post-flight calibrations agree within the errors of less than 5%. The absolute calibration, obtained by comparison with measurements made with an NBS-calibrated photodiode, is accurate to about 10%. The instrumental resolution is about 10 Å over most of the spectrum and degrades to about 15 Å at the shortest wavelengths.

A unique rocket pointing system has been developed for the FOT by the Sounding Rocket Division of NASA's Goddard Space Flight Center. Two star-trackers are mounted behind the telescope primary, with adjustable mirrors which enable them to view out through the side of the rocket. These star-trackers lock on to two bright stars which are chosen to be nearly orthogonal to the target direction and to each other. Signals from one tracker control yaw and roll jets in the attitude control system, while the second tracker controls pitch. With accurate alignment of the trackers and telescope, pointing within 1 arc min to any target can be accomplished.

In addition to this system, the FOT utilises an SIT vidicon television camera which is focused on the mirrored entrance plate of the spectrograph. Television pictures of a 15-arc min field surrounding the target and background apertures are transmitted to the ground, where the experimenter can verify the pointing of the telescope by reference to stars as faint as 10 mag. A remote command system can then be used to make fine adjustments to the pointing. The operation of the FOT is, therefore, somewhat similar to the operation of large ground-based telescopes.

Launch and data

The Faint Object Telescope was launched aboard a NASA Black Brant VC sounding rocket from White Sands Missile Range on 16 April 1977 at 0500 UT. The star-trackers acquired Vega and Capella and pointed the FOT so that 3C273 was within 50 arc s of the centre of the target aperture. A series of commands were then sent to centre the target in the spectrograph aperture. Pointing was automatically maintained throughout the rest of the flight with a total jitter of about ± 15 arc s. The payload reached a peak altitude of 218 km and obtained a total of 270 s of data at altitudes above 130 km.

Some 13,000 photons were detected from 3C273 during a 235-s observation after the target was centred in the spectrograph entrance aperture. The total background correction was $\sim 2,000$ counts, determined from simultaneous observations through the sky aperture as well as observations made with the target aperture before and after the 3C273 observation. About half of the background was due to dark counts distributed nearly uniformly over the whole spectrum. The other half was due to geocoronal Lyman α emission, for which the FOT has

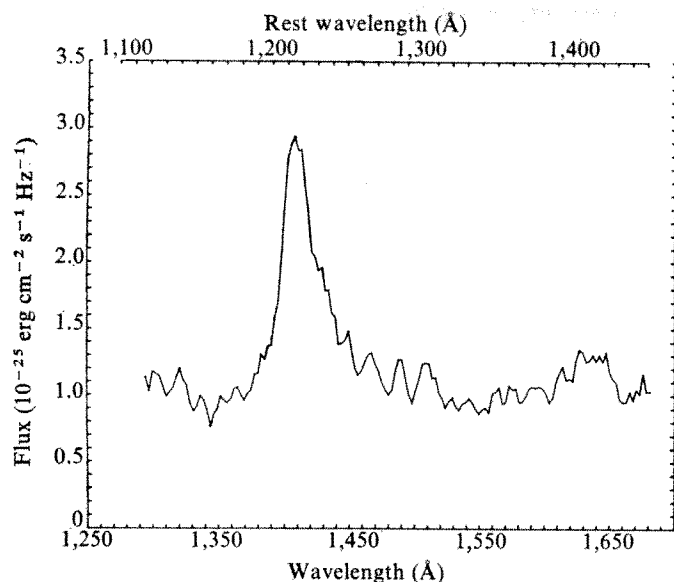


Fig. 1 The ultraviolet spectrum of the QSO 3C273. The strongest emission line is hydrogen Lyman α λ 1,216 with a redshift $z = 0.16$. The asymmetry of the line is attributed to N(v) λ 1,240 emission. The broad feature at λ_{rest} 1,400 Å is similar to that usually attributed to Si (iv) and O (iv) in high-redshift QSOs. There are also probable emission features which may be due to O (i) λ 1,304 and Si (ii) λ 1,265. The continuum is flat, with no evidence of strong absorption shortward of Lyman α .

a small residual sensitivity at detector positions corresponding to wavelengths shorter than 1,300 Å. The background corrected spectrum was smoothed with a sliding average of 3 wavelength bins (7.7 Å) to reduce the effects of slightly uneven bin widths in the analog-to-digital converter. The count-rate data were then reduced to absolute flux units by dividing by the response function of the FOT.

Emission line spectrum

The ultraviolet spectrum of 3C273 is shown in Fig. 1. As in the case of very high-redshift QSOs, the strongest emission feature is Lyman α . Its redshift, $z = 0.16$, is the same as the value obtained originally from the Balmer lines⁶. In addition there are emission features due to N(v) λ 1,240 (blended with L α), Si(iv) and O(iv) λ 1,400, and O(i) λ 1,304. There are also possible emission features at λ 1,265 and λ 1,286. The first of these can plausibly be identified with Si(ii). The equivalent width of the L α + N(v) feature is 67 Å in the rest frame. We estimate from the asymmetry of the feature that one quarter of this is due to N(v). The continuum is flat, with a flux of $9.5 \pm 1.0 \times 10^{-26}$ erg cm⁻² s⁻¹ Hz⁻¹. The flux in L α is, therefore, about 9×10^{-12} erg cm⁻² s⁻¹. Better estimates of the emission line intensities and their widths will be available when we have had the opportunity to compare the data with the results of synthetic FOT spectra (see ref. 1).

Several attempts to reproduce QSO emission line spectra have been made, using a model consisting of a power-law source of ionising radiation surrounded by clouds or filaments of gas⁷⁻¹⁰. Until now, however, there have been no observations of a single QSO covering both the ultraviolet and the optical emission lines. Theorists have had to resort to a 'composite QSO spectrum' pieced together from observations of different rest-wavelengths in many QSOs of varying redshifts. The usual assumption for normalising the ultraviolet and optical data has been to take the flux ratio $F(\text{L}\alpha)/F(\text{H}\beta) = 40$, corresponding to production of these lines primarily by radiative recombination, but with some enhancement of L α due to collisional excitation. Combining our measurement of the L α flux with the previously measured H β flux¹¹, we find $F(\text{L}\alpha)/F(\text{H}\beta) \approx 4$ in 3C273, an order of magnitude smaller than the canonical value. A similar result has been obtained in a very careful composite QSO study¹².

This remarkable departure of the observed Lyman/Balmer ratio from the expected one is likely to have a major impact on QSO models. The most obvious explanation, that the ultraviolet lines are attenuated by absorption by dust similar to that observed in the interstellar medium, seems untenable in view of observations of Paschen α that indicate the hydrogen lines are unreddened¹³. But, dust which is distributed within the line-emitting gas might destroy L α without having much effect on the Balmer and Paschen lines if the nebula has high optical depth to L α photons. An additional mechanism which might play a part in depleting L α in an optically thick nebula is photo-ionisation of He 2³S by trapped L α photons¹⁴. Some alternatives have been mentioned by Baldwin¹². Whatever mechanism is at work, an understanding of the reduced L α /H β ratio may lead to vastly improved knowledge of the physical conditions in QSO envelopes.

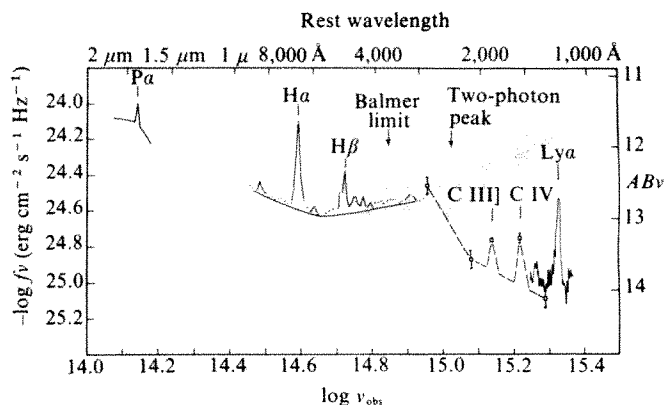
Non-thermal continuum

It is usually assumed that the optical continuum observed in QSOs and active galactic nuclei can be extrapolated to the far-ultraviolet with a power-law $F_{\nu} \propto \nu^{-\alpha}$ to provide the photo-ionising flux needed to explain the emission lines. Theoretical models of this type are sensitive to the flux at the Lyman limit as well as the spectral index α . This observation of 3C273 makes it the first QSO for which the continuum flux is well-determined over a broad wavelength range.

In Fig. 2 we have collected spectral data on 3C273 extending from the infrared to the far-ultraviolet. The data at 2 μ m are from Grasdalen¹³, the optical data are from Oke and Shields¹⁵, the near ultraviolet data are recent broad band measurements by the ANS satellite¹⁶, and the far-ultraviolet data are the measurements reported here. Two of the ANS bands fall at the expected wavelengths of the C(iv) λ 1,550 and C(III) λ 1,909 lines, which are normally strong in high-redshift QSOs.

From Pa to Ha the spectral index is $\alpha = 1.0$, but between Ha and L α the spectrum is flatter, with $\alpha = 0.6$. In addition there is a broad hump centred at $\sim 3,500$ Å. Such a feature has been noted in several QSOs by Baldwin¹¹, who has interpreted it as Balmer continuum emission. It is evident that the spectrum of 3C273 cannot be described by a single power-law, but because only a very small extrapolation is now required, the flux at the Lyman limit is reasonably well-determined. We estimate F_{ν} (Lyman limit) = 8×10^{-26} erg cm⁻² s⁻¹ Hz⁻¹, with an uncertainty of about 20%. This corresponds to a continuum luminosity $L_{\nu} = 7 \times 10^{30}$ erg cm⁻² s⁻¹ Hz⁻¹, assuming $H_0 = 50$ km s⁻¹ Mpc⁻¹ and $q_0 = +1$. The spectral index just below the Lyman limit is $0 \lesssim \alpha \lesssim 0.6$, but α must increase ($\alpha \gtrsim 1.2$) somewhere above the limit to agree with the X-ray observations¹⁷.

Fig. 2 The spectrum of 3C273 from the near-infrared to the far-ultraviolet. Infrared data¹³ are represented by a curve. Optical data are shown as measured fluxes and assumed continuum¹⁵. Five broad-band ultraviolet measurements by the ANS satellite¹⁶ are shown as squares, with dashed lines to indicate assumed continuum and lines. The far-ultraviolet data from Fig. 1 are also shown.



No corrections for reddening have been made to any of the data shown in Fig. 2. The chief evidence against reddening of the overall spectrum is the Pa/H β measurement¹³, which agrees with recombination theory. Galactic reddening is expected to be small or zero¹⁸ at the high galactic latitude ($b^{\text{II}} = 64^\circ$) of 3C273. An estimate based on the 21-cm hydrogen column density¹⁹ and the relation between N_{H} and reddening^{20, 21} gives $E_{\text{B-V}}$ (galactic) = 0.01, which would give extinction of 0.1 mag at Lyman α using the OAO extinction curve²².

Intergalactic matter

The absence of absorption shortward of the red-shifted La emission is another important result of this observation. Gunn and Peterson²³ showed that neutral hydrogen distributed smoothly in the intergalactic medium should produce an absorption trough extending from 1,216 Å to 1,216 (1+z) Å in the spectrum of a QSO, assuming its redshift is cosmological. The optical depth in the trough at a wavelength corresponding to redshift z is²⁴

$$\tau = 1.7 \times 10^{11} \frac{n_{\text{H}}(z)}{(1+z)^{3/2}}$$

in a Friedmann model universe with the critical density parameter $\Omega = 1$. Since no trough is observed in the 3C273 spectrum, we take $\tau < 0.2$ and find the upper limit

$$n_{\text{H}} < 1.5 \times 10^{-12} \text{ cm}^{-3}$$

for $z \sim 0.07$ –0.15. The contribution to the critical density due to neutral hydrogen is, therefore, $\Omega_{\text{H}} < 5 \times 10^{-7}$ at $z \simeq 0.1$. This is by far the strongest limit on neutral hydrogen in the intergalactic medium at a recent epoch, but a similar limit has previously been obtained at $z \simeq 2$ from observations of high-redshift QSOs (see ref. 25 for a review). These results indicate that if there is any intergalactic gas it has remained highly ionised over most of the history of the Universe.

QSO absorption lines

Absorption lines have been seen in the spectra of many high-redshift QSOs and most of these are found shortward of the La emission line²⁵. It is highly controversial whether all of these lines arise in material associated with the QSOs, or whether some of the absorption is produced in intervening clouds or galaxies. On the intervening matter hypothesis, the absorption should be absent in low-redshift QSOs²⁶.

Absorption lines are not readily detectable at the modest resolution of the present observations of 3C273. It is still possible to make an interesting comparison between 3C273 and high-redshift QSOs, however, because even at low resolution, these objects usually show a marked depression of the apparent continuum shortward of La due to the blending of many absorption lines. For example 10 QSOs with $2.5 \leq z \leq 3.1$ have been well observed both longward and shortward of La by Osmer and Smith²⁷. For these objects we have estimated the ratio of the flux density in the wavelength range 1,120–1,180 Å to that at 1,475 Å (tabulated by Osmer and Smith). A wavelength region slightly longer than La was not chosen because of possible contamination by emission lines. The mean value of this ratio is 0.66, with a range from 0.51 to 0.81. Our observation of 3C273 yields 0.98 ± 0.06 for this ratio, and suggests that absorption lines will not be detected when high resolution observations of this object are eventually made. These results are, therefore, consistent with the hypothesis that much of the absorption seen in high-redshift QSOs is due to matter at smaller cosmological redshifts.

One difficulty with this comparison is that the high redshift objects are more luminous than 3C273 by a factor between 3 and 10, and there could conceivably be more absorption in higher luminosity objects if, for example, material is expelled by radiation pressure. A scan of OH 471 (Baldwin, J. A., personal communication), which has $z = 3.4$ and a continuum luminosity very similar to that of 3C273 for $q_0 = +1$, has,

Table 1 QSOs of similar luminosity

Object	z	AB_{V} (1,450 Å)	$\log W_{\lambda}$ (La + N(v))	$\log L_{\text{V}}$ (1,450 Å)
4C25.05	2.34	18.7 ± 0.2	1.84	30.93 ± 0.08
2256+017	2.66	19.3 ± 0.4	1.79	30.76 ± 0.16
4C05.34	2.86	18.3 ± 0.2	1.79	31.20 ± 0.08
0830+115	2.97	19.2 ± 0.2	1.87	30.86 ± 0.08
OH 471	3.39	19.1 ± 0.2	1.86	30.97 ± 0.08
Average	2.84	18.92 ± 0.37	1.83 ± 0.03	30.94 ± 0.15
3C273	0.158	13.96 ± 0.10	1.83 ± 0.04	30.94 ± 0.04

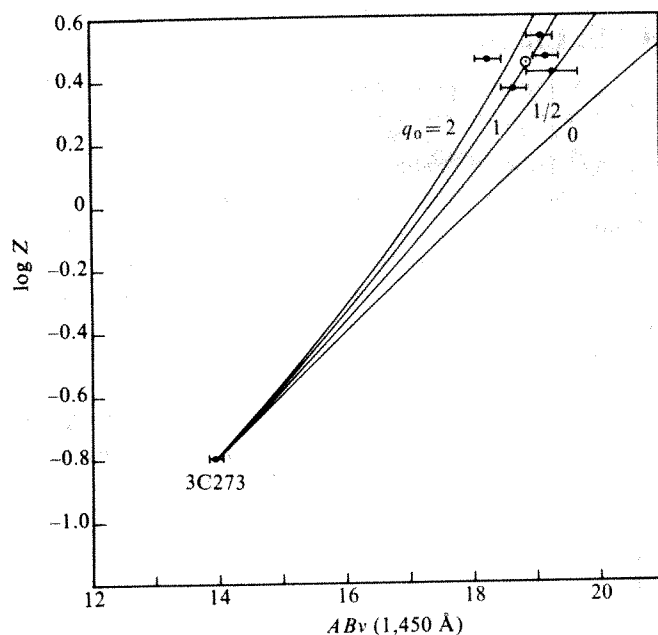
however, a continuum depression ratio of about 0.5, similar to that of the other high-redshift QSOs. Therefore, we tentatively conclude that the continuum depression ratio is correlated with redshift and that much of the absorption seen in high-redshift QSOs arises in matter at smaller, cosmological redshifts. It will be necessary to obtain further ultraviolet observations of intermediate-redshift QSOs, and eventually high resolution ultraviolet spectra, in order to confirm this conclusion.

Cosmology with QSOs

Quasars have been disappointing as cosmological probes, despite their high visibility at large redshifts, because if their redshifts are cosmological, they have a very large range of luminosities. It has recently been shown, however, that there is a good correlation between the continuum luminosity at a rest-wavelength of 1,450 Å, calculated under the assumption of cosmological redshifts, and the equivalent widths of the La+N(v) and C(IV) emission features in 20 high-redshift QSOs²⁸. Another study found a similar correlation with La (but not C(IV)) in 29 mostly radio-quiet QSOs²⁹. The relations are especially tight if the sample is restricted to only the flat spectrum radio sources²⁸, suggesting that these objects may be useful as standard candles.

In Table 1 we list the flat-spectrum radio QSOs from the study of Baldwin²⁸, which have equivalent widths W_{λ} (La+N(v)) within 10% of the value observed in 3C273. The continuum luminosities $L_{\text{V}}(1,450 \text{ Å})$ have been calculated under the assumption of cosmological redshifts with the Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and the deceleration parameter $q_0 = +1$. Also listed are the apparent magnitudes $AB_{\text{V}}(1,450 \text{ Å})$ on

Fig. 3. A redshift-magnitude diagram for the QSOs in Table 1. Only flat-spectrum radio sources with $W_{\lambda}(\text{La} + \text{N(v)})$ similar to that of 3C273 are included. The circled point is the average of the five high-redshift objects. The curves show expected relationships for various values of the deceleration parameter q_0 in Friedmann model universes.



the Oke system ($AB_V = -2.5 \log F_V - 48.60$). The average of the five high-redshift QSOs has the same equivalent width as 3C273 and the same luminosity if $q_0 = +1$. These objects can, therefore, be compared directly in a redshift-magnitude diagram. The result is shown in Fig. 3.

Also shown in Fig. 3 are the expected redshift-magnitude relationships for various values of q_0 in Friedmann model universes with zero cosmological constant³⁰. These curves are arbitrarily normalised to go through the point representing 3C273. This comparison of high- and low-redshift QSOs thought to have similar intrinsic luminosities favours a value of $q_0 \approx +1$. If q_0 were greater than 0.5 it would indicate that the universe is closed.

There are two important cautionary notes in connection with this result. First, the reality of the equivalent widths of the ultraviolet emission lines as a luminosity indicator is not yet firmly established. This is because one also obtains a good correlation between apparent luminosity and equivalent width¹⁸. This remains true with the addition of 3C273 to the sample if one considers observed equivalent widths rather than rest-frame values. Ultraviolet observations of several low-redshift QSOs and Seyfert galaxies with a wide range of luminosities are needed to decide whether the luminosity-equivalent width relationship is real.

A second uncertainty in the present result for q_0 is the reddening of 3C273. In Fig. 3, 3C273 is plotted with no correction for reddening the Galaxy. If $E_{B-V} = 0.01$ as estimated above, the correction at 1,450 Å is less than 0.1 mag, which is small compared with other uncertainties. If, however $E_{B-V} = 0.06$, the ultra-violet magnitude should be brighter by about 0.5, which would give $q_0 \approx 0.5$. A larger reddening correction would reverse the evidence favouring a closed universe.

While it would be premature to conclude that the universe is closed, it is encouraging to think that QSOs may turn out to be useful cosmological probes. Further ultraviolet observations

may be expected to contribute to the solution of this problem, as well as to our understanding of QSOs and active galactic nuclei.

This work was supported by NASA under grant NGR 21-001-001. A.F.D. acknowledges the support of the Alfred P. Sloan Foundation. We thank H. Weiser for help in the design and construction of the detector. The electronics were built by Spacom Electronics, Camarillo, California. EMR, Princeton, N. J. applied the photocathode to the microchannel plate. The rocket was prepared and launched by the Sounding Rocket Division of the Goddard Space Flight Center and the U.S. Navy team at the White Sands Missile Range.

Received 12 August; accepted 19 August 1977.

- ¹ Davidson, A. F., Hartig, G. F. & Fastie, W. G. *Astrophys J* (submitted).
- ² Davidson, A. F., Hartig, G. F. & Fastie, W. G. COSPAR/IAU Symp. New Instrumentation for Space Astronomy, June, 1977, (in the press).
- ³ Lampton, M. & Pearce, F. *Rev. scient. Instrum.* 45, 1098 (1974).
- ⁴ Lawrence, G. M. & Stone, E. J. *Rev. scient. Instrum.* 46, 432 (1975).
- ⁵ Weiser, H., Vitz, R. C., Moos, H. W. & Weinstein, A. *Appl. Optics*, 15, 3123 (1976).
- ⁶ Schmidt, M. *Nature* 197, 1040 (1963).
- ⁷ Bahcall, J. N. & Kozlovsky, B.-Z. *Astrophys. J.* 155, 1077 (1969).
- ⁸ Davidson, K. *Astrophys. J.* 171, 213 (1972).
- ⁹ MacAlpine, G. M. *Astrophys J* 175, 11 (1972).
- ¹⁰ Chan, Y.-W. T. & Burbidge, E. M. *Astrophys J.* 198, 45 (1975).
- ¹¹ Baldwin, J. A. *Astrophys J* 201, 26 (1975).
- ¹² Baldwin, J. A. *Mon. Not. R. astr. Soc.* 178, 67P (1977).
- ¹³ Grasdale, G. L. *Astrophys J* 208, L11 (1976).
- ¹⁴ MacAlpine, G. M. *Astrophys. J.* 204, 694 (1976).
- ¹⁵ Oke, J. B. & Shields, G. A. *Astrophys. J.* 207, 713 (1976).
- ¹⁶ Wu, C. C. *Bull. Am. astr. Soc.* 9, 296 (1977).
- ¹⁷ Sanford, P. W. paper presented at the 8th Texas Symp. Relativistic Astrophysics (1977).
- ¹⁸ Sandage, A. *Astrophys. J.* 183, 711 (1973).
- ¹⁹ Williams, D. R. W. in *Quasi-stellar Sources and Gravitational Collapse*, 213 (University of Chicago Press, Chicago, 1965).
- ²⁰ Gorenstein, P. *Astrophys J.* 198, 95 (1975).
- ²¹ Ryter, C., Cesarsky, C. J. & Adouze, J. *Astrophys J.* 198, 103 (1975).
- ²² Code, A. D., Davis, J., Bless, R. C. & Hanbury Brown, R. *Astrophys. J.* 203, 417 (1976).
- ²³ Gunn, J. E. & Peterson, B. A. *Astrophys. J.* 142, 1633 (1965).
- ²⁴ Field, G. B. A. *Rev. Astr. Astrophys.* 10, 227 (1972).
- ²⁵ Strittmatter, P. A. & Williams, R. E. A. *Rev. Astr. Astrophys.* 14, 307 (1976).
- ²⁶ Bahcall, J. N. *Astr. J.* 76, 283 (1971).
- ²⁷ Osmer, P. S. & Smith, M. G. *Astrophys J.* 210, 267 (1976).
- ²⁸ Baldwin, J. A. *Astrophys. J.* 214, 679 (1977).
- ²⁹ Osmer, P. S. *Astrophys J.* 214, 1 (1977).
- ³⁰ Sandage, A. *Astrophys J* 133, 255 (1961).

Future sea-level changes due to West Antarctic ice sheet fluctuations

J. A. Clark

Cooperative Institute for Research in Environmental Sciences and Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309

C. S. Lingle

Department of Geological Sciences, University of Maine at Orono, Orono, Maine 04473

Global sea-level changes which would result from an instantaneous uniform thinning of the possibly unstable West Antarctic ice sheet are calculated and found to be non-uniform. At locations distant from the ice sheet (Hawaii, New York, the North Sea), immediate submergence would be followed by gradual emergence. At New Zealand, immediate submergence would be followed by gradual additional submergence, then slow emergence would begin after 2,500 yr. At locations close to the ice sheet (Cape Horn, the Ross Ice Shelf), the sea level would fall for 1,100 yr. then rapid submergence would start resulting in a net sea-level rise after 10,000 yr equal to about 92% of the average global rise.

It is commonly believed that the rise in sea level due to a glacial melting episode is uniform everywhere. That is, the observed sea-level rise, S , due to the melting of a mass of ice M_i is $S = M_i / \rho_w A$ with ρ_w the density of water and A the area of the oceans. This is

wrong because the ocean floor deforms under the weight of the changing ice and water loads and the ocean surface (the geoid) distorts as mass is redistributed on the surface, as well as within, the Earth. The observed change in sea level is the change in separation between the geoid and the ocean floor and Farrell and Clark¹ show that, when glaciers melt, the change in separation between these dynamic surfaces is not constant. We use their method in this analysis to predict changes in sea level which would result from melting (or accretion) of a uniform amount of ice from the relatively unstable West Antarctic ice sheet.

Instability in the West Antarctic ice sheet

The West Antarctic ice sheet (WIS), that portion of the Antarctic ice sheet lying in the Western Hemisphere and mostly east of the Transantarctic Mountains, was shown to be grounded extensively below sea level by seismic studies completed before 1961 (ref. 2). Weertman³ has shown that such marine ice sheets are not inherently stable, and Hughes⁴ has discussed several lines of evidence which indicate that WIS has disintegrated extensively

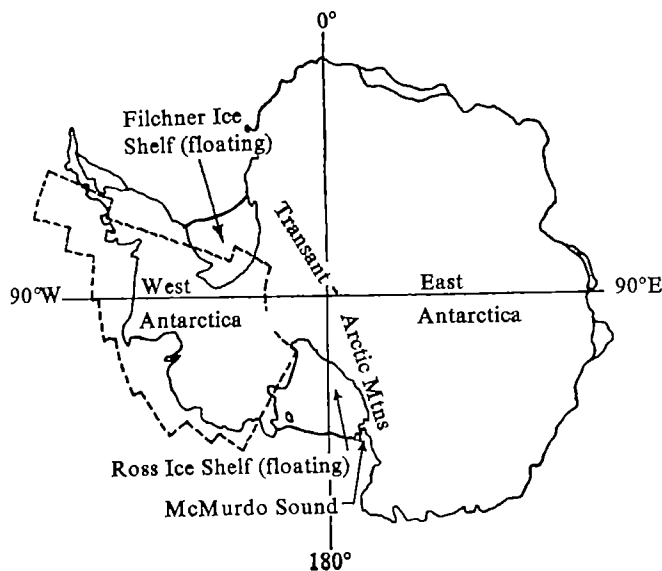


Fig. 1 The marine portion of the West Antarctic ice sheet (WIS) lies in the Western Hemisphere east of the Transantarctic Mountains. It is approximated for purposes of the numerical calculation by the area within the dashed line. The global sea-level changes which would result from a uniform thinning of the ice within this area are calculated.

since 10,000 yr BP, and has suggested that the process may be continuing.

Denton and Borns⁵ found that grounding of WIS in the vicinity of McMurdo Sound occurred before 47,000 yr BP. Following expansion, WIS remained grounded to the edge of the continental shelf until about 18,000 BP (ref. 5) (G. H. Denton, private communication) and then began retreating. WIS ungrounded once again in the McMurdo area by 6,000 yr BP (ref. 6). Thomas⁷ also suggests that accumulation rates near the West Antarctic ice divide imply continuous thickening of the WIS interior until 2,000 yr BP when thinning started, possibly as a delayed response to retreat of the grounding line.

Recent survey measurements made by Thomas⁷ on the Ross Ice Shelf indicate that part of the WIS grounding line may be advancing. If this is the case, the disintegration may be reversing, and WIS may now be expanding. The apparent inability of WIS to attain a state of equilibrium accords with the theory³ that predicts a marine ice sheet will be stable only after advancing to the edge of a continental shelf, or until water at the margin reaches a critical depth.

The model

The Earth is assumed to be a spherically symmetric, layered, self-gravitating, Maxwell viscoelastic solid. The elastic structure is that of a Gutenberg–Bullen Earth model with a uniform mantle viscosity of 10^{22} P and an inviscid core. Such a model has been suggested by others^{9,10} to closely approximate the real Earth.

The procedure, thoroughly described by Farrell and Clark¹ uses a Green function, $G(r-r_0, t-\tau)$, calculated by Peltier⁸ for the above Earth model. This Green function gives the effect of a 1-kg point load placed on the Earth's surface at position r and time τ upon relative sea level s at some other position r_0 and some later time t . To determine the relative sea-level change resulting from a distributed load that varies through time, consider the load to be a collection of point loads and add the effects of each. The relative sea-level change is then

$$s(r_0, t) = \int_{-\infty}^t \left(\iint_{\text{Load}} G(r-r_0, t-\tau) L(r, \tau) dr \right) d\tau \quad (1)$$

with L the surface load function. The load is comprised of a water load (L_w), and an ice load (L_i) so that

$$L = L_w + L_i = \rho_w s + \rho_i I \quad (2)$$

with ρ_w the density of water, ρ_i the density of ice, and I the ice thickness. Furthermore, the Green function can be separated into a time-independent elastic part (G_E) and a time-dependent viscous part (G_V)

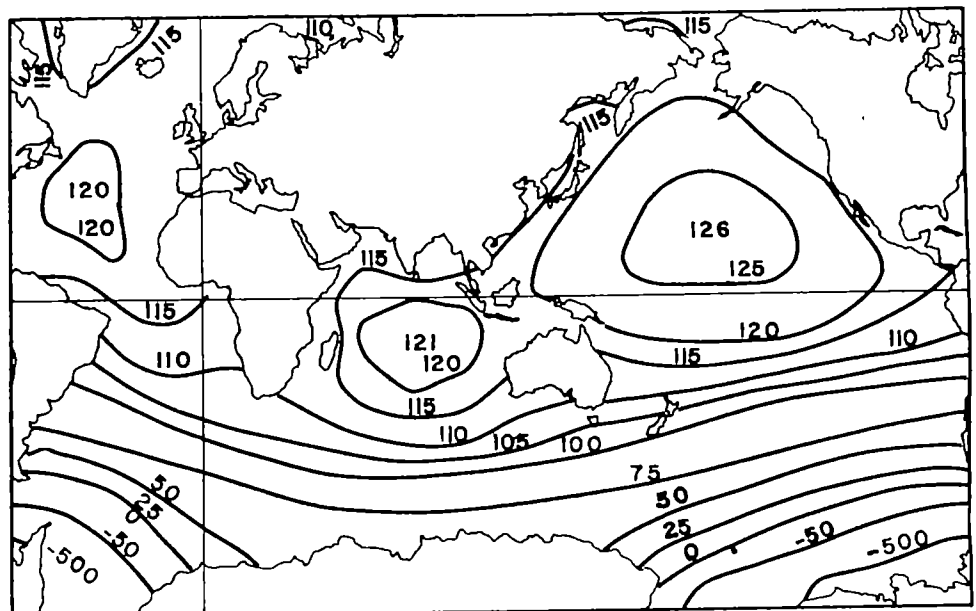
$$G = G_E + G_V \quad (3)$$

Substituting equations (2) and (3) into equation (1) gives

$$s(r_0, t) = \iint G_E(r-r_0) \rho_w s(r, t) dr + \iint_{\text{Ice}} G_E(r-r_0) \rho_i I(r, t) dr + \int_{-\infty}^t \left(\iint_{\text{Load}} G_V(r-r_0, t-\tau) L(r, \tau) d\tau \right) d\tau \quad (4)$$

The last term in equation (4) describes the time dependent viscous response of the Earth (the 'isostatic' response) to past ice and water load changes and the second term accounts for the immediate elastic response of the Earth to changing ice loads. The first term in equation (4) describes the Earth's elastic response to water load changes. Because s appears on both sides of equation (4), it is an integral equation describing a feedback process where meltwater

Fig. 2 Sea level changes everywhere immediately after a uniform thinning of WIS are shown as per cent of average global sea level rise (volume of water added to ocean-ocean area). The non-uniform distribution of sea level change is caused by the Earth's immediate elastic response to changed ice and water loading, plus the change in gravitational attraction exerted by WIS on the surrounding ocean. (The 100% contour corresponds to the 0.69 cm average oceanwide rise which would result from uniform thinning of WIS by 1 m.)



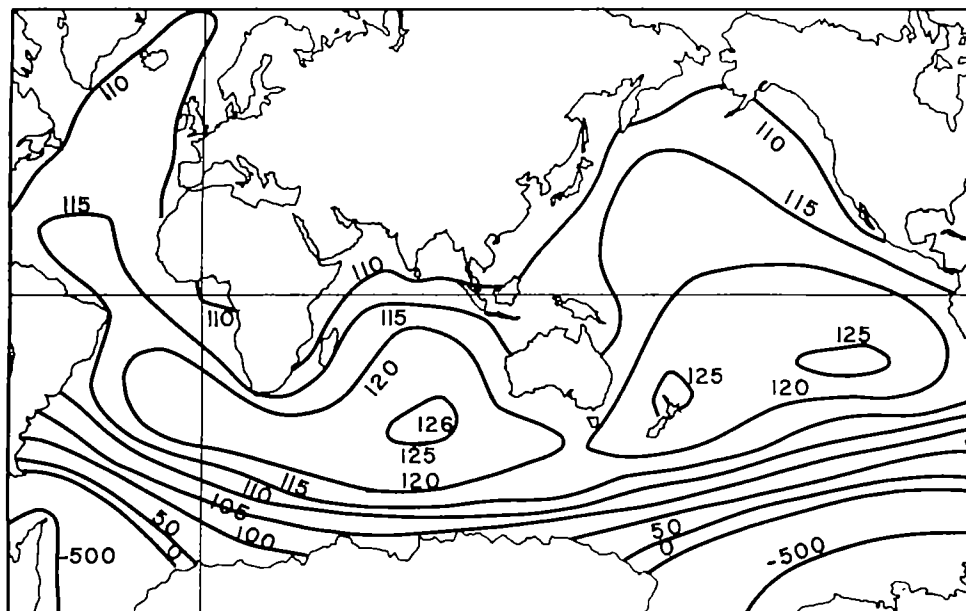
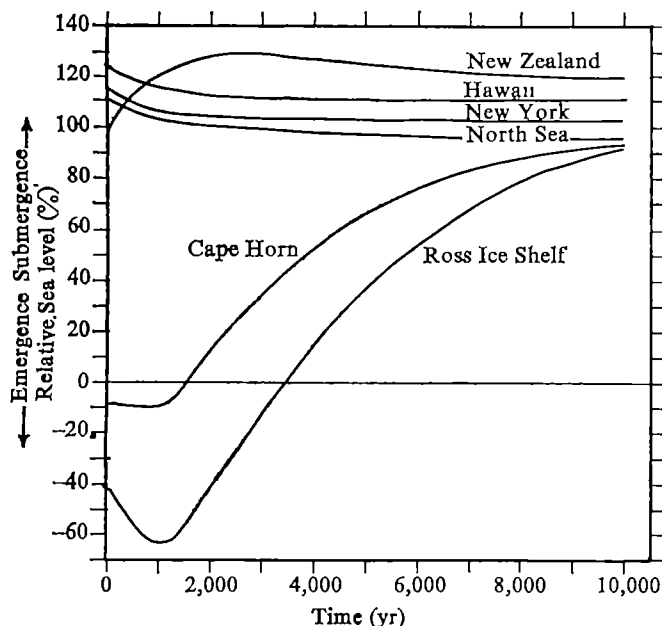


Fig. 3 Sea level changes everywhere 1,000 yr after the assumed uniform thinning of WIS, again shown as a percentage of average oceanwide rise. The new distribution of values results from gradual flow of material within the Earth's mantle.

deforms the Earth and then flows to fill the resulting depressions in the ocean surface. This changes the water load and the process is repeated until equilibrium is attained. Equation (4) can be solved numerically, with realistic ice and ocean configurations, for the relative sea level change at any location and at any time.

The ice thinning model is a simple one in which 1 m of ice is uniformly removed from the entire WIS (see Fig. 1). The oceanwide average sea level rise, S , is then 0.69 cm. The rheological model is linear and so a doubling, for instance, of our assumed amount of uniform thinning results in a doubling of the predicted sea level changes everywhere. Furthermore, if WIS should thicken by 1 m instead of thinning, the predicted sea level changes would be identical in magnitude but opposite in sign to those given below. Our results are, therefore, quite general.

Fig. 4 Sea level change against time at six selected locations, for 10,000 yr following the assumed uniform thinning of WIS. At New Zealand, immediate submergence nearly equal to the average global rise is followed by continued submergence for 2,500 yr. Slight emergence follows. 10,000 yr later, net submergence is 120% of average global rise. At Hawaii, New York and the North Sea, immediate submergence of 125% to 110% is followed by emergence of 15% to 13%. At Cape Horn and near the Ross Ice Shelf, the sea level actually falls by 8% and 40%, respectively. Emergence continues for about 1,200 yr, after which sea level begins to rise. After 10,000 yr, net submergence is about 92% of average global rise.



Results

The results are given as the changes in sea level which would be caused solely by thinning of WIS. Tectonic effects and effects due to changes in glaciers elsewhere are not included. All reported values are in per cent of S , the oceanwide average sea level change.

Figure 2 shows changes in sea level everywhere immediately after the ice has melted. On Hawaii, for example, sea level rises 125% of S ($1.25 \times 0.69 = 0.86$ cm for the 1 m thinning case), while near the Ross Ice Shelf, the negative values indicate that sea level actually falls by 40%. This fall in sea level occurs despite the fact that water was added to the ocean; it is caused by the combined effects of immediate elastic uplift of the ocean floor near the ice sheet¹¹ and reduced gravitational attraction of the ocean water by the ice mass^{12,13}. Only along the 100% contour does the observed sea level rise equal the oceanwide average rise, S .

After the Earth's immediate elastic response, viscous flow of material within the mantle slowly compensates for the new distribution of ice and water loads. Figure 3 shows the distribution of sea level changes (relative to present sea level) after 1,000 yr have elapsed. The sea level change at Hawaii is now only 116%, indicating that after the initial dramatic rise of 125% sea level slowly falls by 9%, resulting in slight emergence. In the extreme South Atlantic, however, where sea level rises only 50% initially (Fig. 2), Earth relaxation results in continual submergence so that after 1,000 yr sea level has risen to 100% of S . Figure 4 shows sea level change against time at six localities (New Zealand, Hawaii, New York, the North Sea, Cape Horn, and the Ross Ice Shelf) for 10,000 yr following the single ice-melting incident. For the North Sea, New York, and Hawaii, the sea level against time curves are similar in form, although slightly different in magnitude. Immediate submergence exceeding 100% of S is followed by slow emergence of only a few per cent of S . The predicted change in sea level on New Zealand is different because slow submergence, rather than emergence, occurs after the initial rapid rise of sea level. After 2,500 yr New Zealand also emerges slightly. Sea level falls at Cape Horn and the Ross Ice Shelf until 1,100 yr after the ice melting episode, then dramatic submergence occurs, drowning the present day beaches after 1,500 yr (at Cape Horn) and 3,400 yr (near the Ross Ice Shelf). Regions now under WIS will witness a continuing fall in sea level reaching magnitudes of up to -700% of S .

Conclusions

Sea level changes corresponding to future changes in the West Antarctic ice sheet will be affected by (1) changes in gravitational attraction exerted by the ice sheet on the surrounding ocean, (2) the

Earth's immediate elastic response to changed ice and water loads, and (3) the Earth's long-term response due to viscous flow within the mantle. If a uniform amount of ice was removed from WIS, the combined effect of these three processes would result in a non-uniform sea level change immediately afterwards ranging from +125% of the average global rise at Hawaii to -40% of the average rise near the Ross Ice Shelf, Antarctica.

Acknowledgment is made to the National Center for Atmospheric Research (NCAR), which is sponsored by the National Science Foundation, for computer time used in this research. The work was supported by the Division of Earth Sciences National Science Foundation, NSF Grants DES74-13047-A01 and EAR74-13047-A02. Thanks are extended to John T. Hollin for

critically reviewing the manuscript, and to W. Richard Peltier for supplying the necessary Green functions.

Received 4 May, accepted 30 June 1977

- ¹ Farrell, W. E. & Clark, J. A. *Geophys. J. R. astr. Soc.* **46**, 647-667 (1976)
- ² Bentley, C. R. & Ostenso, N. A. *J. Glaciol.* **3**, 882-912 (1961)
- ³ Weertman, J. *J. Glaciol.* **13**, 3-11 (1974)
- ⁴ Hughes, T. *J. geophys. Res.* **78**, 7884-7910 (1973), *Rev. geophys. Space Phys.* **13**, 502-526 (1975), *Rev. geophys. Space Phys.* **15**, 1-46 (1977)
- ⁵ Denton, G. H. & Borns, H. W. Jr. *Antarct. J.* **9**, 167 (1974)
- ⁶ Denton, G. H., Borns, H. W. Jr., Grosswald, M. G., Stuver, M. & Nichols, R. L. *Antarct. J.* **10**, 160-164 (1975)
- ⁷ Thomas, R. H. *Nature* **259**, 180-183 (1976)
- ⁸ Peltier, W. R. *Rev. geophys. Space Phys.* **12**, 649-669 (1974)
- ⁹ Cathies, L. M. *The Viscosity of the Earth's Mantle* 386 (Princeton University, Princeton, 1975)
- ¹⁰ Peltier, W. R. & Andrews, J. T. *Geophys. J. R. astr. Soc.* **46**, 605-646 (1976)
- ¹¹ Farrell, W. E. *Rev. geophys. Space Phys.* **10**, 761-797 (1972)
- ¹² Woodward, R. S. *U.S. geol. Survey Bull.* **48**, 87-170 (1888)
- ¹³ Clark, J. A. *Geology* **4**, 310-312 (1976)

Isotopic evidence for source of diagenetic carbonates formed during burial of organic-rich sediments

Hilary Irwin & Charles Curtis

Department of Geology, The University, Sheffield, UK

Max Coleman

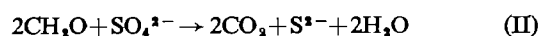
Institute of Geological Sciences, Geochemical Division, 64-78 Gray's Inn Road, London, UK

Organic matter is modified by several processes operating at different depths during burial diagenesis: (1) sulphate reduction; (2) fermentation; (3) thermally-induced decarboxylation, and so on. CO₂, one common product of each, can be distinguished by its carbon isotope composition: approximately (1) -25‰, (2) +15‰, (3) -20‰ relative to PDB. These values are preserved in diagenetic carbonates of the Upper Jurassic Kimmeridge Clay. Independent corroboration of the relative dominance of each process within specific depth intervals is given by the isotopic composition of incorporated oxygen which is temperature dependent (1) 0 to -2‰, (2) -1.5 to -5‰, (3) -3.5 to -7.0‰.

A BELATED recognition of the potentially disastrous supply/demand situation for liquid hydrocarbons has reawakened interest in organic matter maturation processes in sediments. Much emphasis is placed on thermally-induced reactions but little on several other processes which equally affect source rock potential. These are more important at shallower burial depths and also lead to diagenetic cementation which can affect both hydrocarbon migration and reservoir properties.

Curtis recently¹ proposed that several different depth-related zones could be recognised within burial sequences and that kinetic controls (notably rate of burial) dictated the relative importance of each zone in determining the extent and style of diagenetic modification.

Figure 1 represents an order-of-magnitude model for depth zonation with particular reference to the generation of carbon dioxide. Note the nonlinear depth scale which exaggerates the depth span of shallower zones. Bacterial processes dominate in zones I, II and III. Carbon dioxide is one product of organic matter degradation in each with the following equations indicating the nature of each overall reaction:



The boundary between zones I and II is determined by the extent of downward diffusion of dissolved molecular oxygen from overlying waters and the lower limit of zone II by downward diffusion of sulphate (we shall here restrict the discussion to fairly rapidly deposited marine mudstones containing abundant organic matter and within which sulphate reduction almost invariably occurs). Fermentation starts as soon as sulphate reduction stops (possibly because the bacteria cannot tolerate dissolved sulphides) and presumably continues until prevented by either high temperatures or exhaustion of suitable organic substrate. The rate of production of carbon dioxide within each zone decreases with depth due to diminishing oxygen, sulphate and substrate quality respectively.

Zone IV is less clearly defined with respect to depth or process. One well documented reaction is decarboxylation:



The rate of this (and similar) abiotic reaction presumably increases with rising temperature and then slows as suitable molecules are exhausted. Oxidation by detrital ferric compounds also must cause organic matter degradation: in Fig. 1 this is depicted to be relatively important at shallow depths where pyrite forms from reactive soil sesquioxides and again at greater depths where higher temperatures promote reduction of less reactive detrital phases.

The carbon dioxide produced by all these reactions dissolves readily in porewater to increase bicarbonate concentrations. Its carbon isotopic composition, however, must be anticipated to be very different from that of marine reservoir bicarbonate (~ 0‰PDB) since the source in each case is organic matter (~ -25‰PDB). Bacterial oxidation and sulphate reduction seem to impose little fractionation such that very light bicarbonate is to be anticipated. There is some evidence² for believing that decarboxylation produces somewhat heavier (but still light) carbon dioxide. We have found no information relating to fractionation during oxidation by ferric compounds and have assumed that none occurs. Fermentation, however, imposes an extremely large fractionation³ with bacterial meth-

are values of -75‰_{PDB} commonplace⁴. Consequently, carbon dioxide produced in this reaction must be heavy and, in the absence of accurate information, we estimate something of the order of $+15\text{‰}$; certainly positive.

As sediments are buried, therefore, they pass successively through different zones within which organic matter is being altered and carbon dioxide produced. Bicarbonate activities sufficient to cause carbonate super-saturation are unlikely to be reached in zone I because of upward diffusion into depositional waters, but below this zone these levels are probable. The interesting feature is that carbonate precipitated from zone II should

be very light. There should then be a rapid and dramatic change to very heavy carbonates in zone III and then a less dramatic return to lighter values as fermentation becomes less important and decarboxylation more important into zone IV. It should be noted that unstable primary carbonates ($\delta^{13}\text{C} \sim 0\text{‰}_{\text{PDB}}$) might dissolve and add carbon of intermediate isotopic composition to the porewater reservoir in which case the above trends might be blurred.

Kimmeridge Clay

The onset of Kimmeridge Clay sedimentation marks a period of

Table 1 Isotopic composition and inferred temperatures and depth of precipitation, Kimmeridge carbonates

Sample description	No.	$\delta^{18}\text{O}_{\text{PDB}}$	$\delta^{13}\text{C}_{\text{PDB}}$	$T(^{\circ}\text{C})^*$	$T_d(^{\circ}\text{C})^\dagger$	Depth (m)‡
'Primary' coccolithic limestones						
	846	-2.88	-0.35	24.3		—
	862	-2.92	-0.48	24.5		—
	875	-2.60	-0.56	23.0		—
	879	-3.52	-0.45	27.4		—
	881	-3.17	+0.28	25.7		—
	883	-3.28	-0.50	26.2		—
	887	-2.99	-0.57	24.8		—
Coccolith-rich carbonates	895	-3.45	-0.35	27.0		—
	896	-3.65	+0.08	28.0		—
	897	-3.73	+0.65	28.3		—
	898	-3.39	+0.39	26.7		—
	948	-3.47	-0.25	27.1		—
	953	-3.42	-0.60	26.8		—
	955	-3.14	+0.84	25.6		—
	997	-3.56	-2.21	27.5		—
	998	-3.85	-0.85	28.9		—
Diagenetic calcite nodules						
Rotunda nodule	1001	-0.74	-15.12	15.0		
	1002M	-1.46	-15.02	18.0		
Rotunda nodule	1002B	-0.17	-15.27	12.7		10‡
	817T	-0.80	-17.50	15.2		
Blackstone nodule	817M	-1.28	-15.74	17.2		
	817B	-2.91	-14.57	24.5	?	?
Mean 15.6 (excluding 817B)						
Dolomitic/ankeritic cementstones						
	915	-3.30	+0.32	44.3	23.4	290
	911	-4.05	-0.86	49.1	27.3	430
	909	-3.56	+0.52	46.0	24.7	335
	903	-3.59	-0.97	46.1	24.9	340
	897	-4.54	-2.16	52.3	29.9	520
	866	-3.32	-0.53	44.4	23.5	290
	865	-3.78	-1.08	47.4	25.9	380
	853	-3.35	+0.59	44.6	23.7	300
'Basalt Stone' band	852	-2.99	+1.32	42.4	21.9	235
	851	-4.22	-2.68	50.2	28.2	460
	749	-4.73	-3.02	53.6	30.9	555
'Grey Ledge' band	748	-3.80	+1.79	47.5	26.0	380
	747	-4.64	-3.53	53.0	30.4	540
	699	-2.17	+6.20	37.4	18.0	95
'Cattle Ledge' band	698	-2.87	+5.44	41.6	21.3	215
	697	-4.81	-4.21	54.2	31.4	575
	649	-3.38	+2.30	44.8	23.8	395
'Yellow Ledge' band	648	-1.64	+9.28	34.4	15.6	10
	647	-4.79	-3.59	54.0	31.2	565
	604	-5.70	-4.42	60.3	36.4	755
	603	-5.79	-5.54	61.0	36.9	770
	602	-5.35	-4.16	57.9	34.4	680
	599	-6.42	-5.46	65.5	40.7	905
'Nine Inch' band	598	-4.97	-6.38	55.3	32.2	605
	549	-6.42	-5.31	65.5	40.7	905
'Maple Ledge' band	548	-5.50	-3.03	58.9	35.2	710
	547	-6.28	-5.09	64.5	39.8	875
	499	-5.83	-2.06	61.3	37.2	780
'Washing Ledge' band	498	-6.46	-4.21	65.8	40.9	915
	497	-4.83	-0.88	54.3	31.5	580
	449	-2.66	+7.96	40.4	20.3	180
'Flat Stone' band	448	-3.13	+6.14	43.3	22.6	260
	447	-6.08	-4.47	63.1	38.6	830

* T calculated for water, $\delta^{18}\text{O} = -1.2\text{‰}$.

† T_d calculated for water, $\delta^{18}\text{O} = -4.9\text{‰}$.

‡Depth values calculated assuming: temperature at base of sulphate reduction zone 15.6°C . Depth of base of sulphate reduction zone 10 m. Thermal gradient 28°C per km. No values given for primary (surface water) precipitation carbonates.

marine transgression and water deepening over much of Western Europe. This has been linked with widespread tectonically-induced subsidence⁶. The sediments consist of uniform clays and shales, mostly organic rich, with occasional cementstones (dolomitic calcilitites) and siltstones. The Dorset Coast section is some 500 m thick and reflects a relatively deep basinal area within widespread epicontinental seas. The section is generally organic rich and a few horizons have been mined as oil shales. The Kimmeridge Clay is widely believed to be the principle source rock for North Sea oil.

Cementstone horizons of great lateral persistence are common in the Dorset Coast section, especially in its lower part. Many of these have been given local names⁶ which are included in Table 1, column 1. Discrete diagenetic carbonate nodules also occur and these too were sampled. In the upper part of the succession a third distinctive carbonate sediment is found as bands of impure (organic-rich) chalk, referred to as 'coccolithic limestones'. In all, 57 carbonate-rich samples were taken. Sample numbers (Table 1, column 2) reflect relative position within the sequence.

This sequence is entirely marine, relatively rapidly deposited, organic rich and contains much carbonate, some of which is undoubtedly of diagenetic origin. It also is sulphur rich (diagenetic pyrite and sulphur in organic compounds). It therefore should be a good place to test the reality of the zonal model outlined in Fig. 1.

Experimental procedures

Thin sections (very thin in the case of particularly fine-grained rocks) were prepared from each carbonate sample and 20–50 g crushed for analysis. Chemical (after Pearson⁷) and X-ray diffraction data were obtained for most samples and these results will be discussed in detail elsewhere.

The vast majority of samples fall into just three classes. The cementstones consist mostly of fine-grained mosaics of interlocking crystals euhedral to subhedral in outline. The carbonate phase ranges in composition between $(\text{Ca}_{0.848} \text{Mg}_{0.338} \text{Fe}_{0.117})\text{CO}_3$ and $(\text{Ca}_{0.554} \text{Mg}_{0.385} \text{Fe}_{0.062})\text{CO}_3$. Strictly, the iron-rich samples are ankerites, and the iron-poor samples ferroan dolomites. d_{104} varies between 2.898 Å and 2.909 Å. For simplicity this phase will be referred to as dolomite below.

The coccolithic limestones are all fairly pure calcites, ranging between $(\text{Ca}_{0.979} \text{Mg}_{0.002} \text{Fe}_{0.020})\text{CO}_3$ and $(\text{Ca}_{0.989} \text{Mg}_{0.004} \text{Fe}_{0.007})\text{CO}_3$ with d_{104} between 3.032 Å and 3.035 Å. In thin section, elongated lenses of coccoliths are separated by organic-rich laminae. Bioturbation and small scale cross lamination (ripples?) are not uncommon.

The nodules consist of fine-grained microsparite calcite (d_{104} 3.0206 Å to 3.0213 Å). Considerable Mg^{2+} and/or Fe^{2+} substitution is indicated. Pyrite is common in the Blackstone nodules which also show late calcite veins (817 V). Rotunda nodules often enclose ammonites.

For isotopic analysis, carbonate powder samples were washed with 1% sodium hypochlorite solution⁸ to remove organic compounds which can interfere with accurate isotope ratio determinations⁹. Carbon dioxide was prepared from about 10 mg of the treated sample by reaction with 100% phosphoric acid at 25.0 °C using a method similar to that described by McCrea¹⁰. Pure calcite samples had reacted completely in 2 h whereas dolomites required about 36 h.

Carbon dioxide was analysed with a Micromass 602-C mass spectrometer and the raw data corrected using the methods of Craig¹¹ and Deines¹². All samples were analysed with reference to carbon dioxide from a calcite standard prepared at the same time. Consequently, oxygen isotope ratios for dolomites were recalculated to compensate for a different fractionation factor for acid decomposition (assumed to be that for pure dolomite, 1.01110, ref. 13). The appropriate factor for calcite is 1.01025. Data for both oxygen and carbon are presented in the normal δ notation with reference to PDB standard in Table 1, columns 3 and 4.

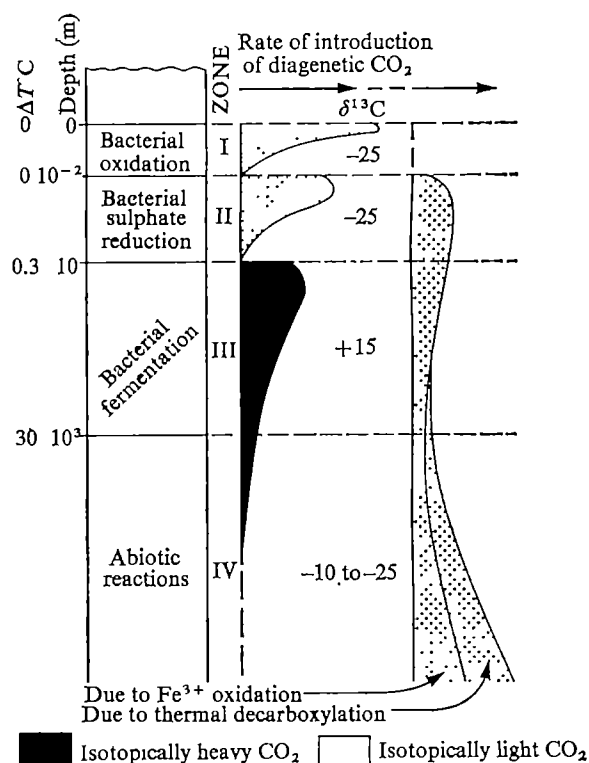


Fig. 1 Introduction of diagenetic CO_2 within different diagenetic zones.

The oxygen isotopic equilibrium fractionation between a carbonate and water is a function of temperature. For calcite the relationship is given by

$$T = 16.9 - 4.21 (\delta_c - \delta_w) + 0.14 (\delta_c - \delta_w)^2$$

(ref. 14) where $(\delta_c - \delta_w)$ is the measured difference in $\delta^{18}\text{O}$ between calcite and water and is used here in the same sense as originally defined by Epstein *et al.*¹⁵. But there is much controversy about the precise extent of the fractionation between dolomite and water. The extrapolation of high temperature experimental data^{16,17} to 25 °C suggests that dolomite should be 5–7‰ heavier than coexisting calcite. This contrasts with some measurements which show that coexisting sedimentary dolomites and calcites have similar isotopic values^{18,19}. Low temperature experimental precipitation of protodolomite¹⁹ shows that it should be approximately 3‰ heavier than coexisting calcite. This value is intermediate between other estimates and can be considered to correspond reasonably to the probable conditions of precipitation of Kimmeridge dolomites. The expression given below is calculated from the original data

$$T = 31.9 - 5.55 (\delta_d - \delta_w) + 0.17 (\delta_d - \delta_w)^2$$

No account has been taken of possible slight isotopic fractionations due to iron substitution in the dolomite lattice.

An isotopic composition of -1.20‰ , the value for pre-glacial oceans (Shackleton and Kennett²⁰) has been assumed for both seawater and porewater. The isotopic equilibrium temperature, T , for each sample is listed in Table 1, column 5.

Discussion

Figure 2 is a plot of $\delta^{18}\text{O}_{\text{PDB}}$ against $\delta^{13}\text{C}_{\text{PDB}}$. Two precipitation temperature scales are given, one for calcite and one for dolomite (cementstones). The isotopic composition of the coccolith limestones is very much that to be expected for precipitation from marine reservoir bicarbonate in warm

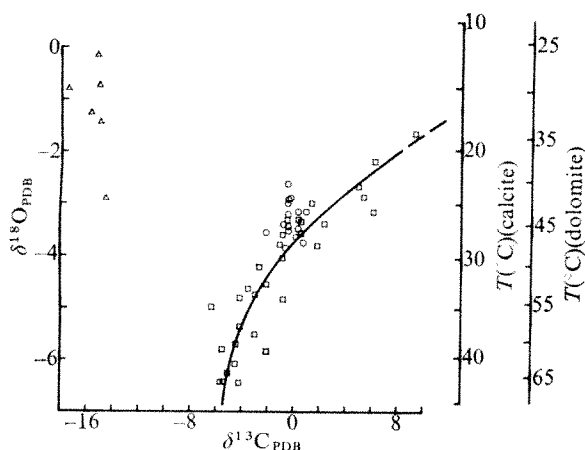


Fig. 2 Plot of oxygen isotope ratio against carbon isotope ratio for all samples. Triangles represent diagenetic calcites, squares diagenetic dolomites and circles coccolithic calcites. The two temperature scales relate to different fractionations of dolomite and calcite with water of $\delta^{18}\text{O} = -1.2\text{‰}$.

surface waters ($\delta^{13}\text{C} \sim \text{zero}$, $\delta^{18}\text{O} \sim -3.3\text{‰}$ corresponding to approximately 26°C). Bearing in mind that some of the samples contain minor diagenetic carbonate, the range is small.

The nodular calcites show very different isotope ratios with $\delta^{13}\text{C} \sim -16\text{‰}$ and $\delta^{18}\text{O} \sim -0.8\text{‰}$ corresponding to a much lower precipitation temperature $T \sim 15^\circ\text{C}$ (exception sample 817B). These values lie well outside the range of all other samples. It seems reasonable to suggest that they precipitated not far below the sediment/water interface largely from carbon dioxide produced by sulphate-reducing bacteria (zone II, Fig. 1). The involvement of these bacteria during concretion development has been discussed by Raiswell²¹. The thermal gradient within marine depositional waters is in the reverse direction to that within buried sediments: the sediment/water interface must lie close to the temperature minimum within the total water plus sediment column. The presence of pyrite in these samples has already been noted.

The cementstones are remarkable for their wide range of both carbon and oxygen isotopic ratios. Carbon ranges from very heavy to light and oxygen isotope ratios indicate precipitation temperatures from 34°C to 66°C . Most remarkable of all is the very strong correlation between the two sets of data: heaviest carbonates being precipitated at the lowest temperatures. The obvious first interpretation is that these carbonates started to precipitate at some depth below the sediment/water interface from porewaters supersaturated with ^{13}C -rich bicarbonate and that precipitation continued to much greater burial depths where porewater bicarbonate was very much poorer in ^{13}C , in fact enriched in ^{12}C relative to marine reservoir bicarbonate. All these data can be matched easily with the zonal model in Fig. 1. The lower temperature dolomite samples precipitated near the top of zone III where fermentation processes introduced ^{13}C -rich bicarbonate from organic matter. The gradual and systematic changeover to ^{12}C -rich carbonates with depth of precipitation reflects the increasing importance of abiotic oxidation and decarboxylation reactions relative to fermentation downwards through zone III into zone IV. Although some cementstones contain minor calcite and it is possible that some primary calcite dissolved to contribute bicarbonate to the porewater pool, the observed trend is so well developed that we are forced to conclude that bicarbonate derived from organic matter was the principle source for both cementstones and nodular calcites.

From this discussion we feel that the isotope data obtained strongly support the view that distinctive depth-related zones of diagenesis, as shown in Fig. 1, persisted within the sediment column during Kimmeridge times. This work underlines the importance of diagenetic reactions being responsible for

significant modification of organic matter and the production of massive amounts of carbonate cement. There remains the intriguing and possibly important prospect of being able to determine the depth of precipitation assuming a particular thermal gradient.

The precipitation temperature data (Table 1, column 5 with the exception of Sample 817B) indicate a considerable break between zones II and III. For a thermal gradient of about 30°C per km this implies that bacterial fermentation first becomes important at depths of the order of 700 m. This is at variance with the proposition that the onset of fermentation is simply precluded by the activities of sulphate-reducing bacteria. There are, however, at least two reasons for doubting the reality of this large break between zones II and III.

As mentioned above, there is some uncertainty in the calibration of dolomite/water fractionation as a function of temperature (especially as the effect of iron substitution is unknown). If, for example, the value for dolomite were the same as that for calcite, as suggested by some evidence^{18,19} then the break would not exist. Using experimentally-derived calibrations^{16,17} would, of course, increase its magnitude. We do not think, however, that calibration uncertainties alone could account for the scale of the temperature/depth break.

There is a more compelling argument for doubting the reality of the break which also reconciles experimental and geological evidence. The major isotopic effect of diagenesis on porewater is depletion of ^{18}O associated with formation of diagenetic minerals enriched in that isotope. This applies to clay minerals as well as carbonates. The main accompanying physical effect is reduction of pore space and upward expression of water. At depth one would expect diagenetic minerals to be formed in equilibrium with isotopically light modified connate water. Measurements on porewater in samples recovered from the Deep Sea Drilling Project^{22,23} show a systematic reduction of $\delta^{18}\text{O}$ with depth to a maximum of 3‰ at 300 m. This was attributed to diagenetic reactions. This depletion should prevail at all depths below a mixing zone open to the effectively infinite seawater reservoir. The Kimmeridge sediments were probably deposited much more rapidly than those referred to above such that the 'mixing zone' was relatively limited in extent and effective for a shorter time with respect to any individual sediment unit. It should be noted that enrichment of ^{18}O in formation waters by exchange with isotopically heavy rocks²⁴ is a quite different effect from that produced by precipitation of diagenetic minerals.

A useful if very simple model which incorporates these effects can be constructed if certain assumptions are allowed. The downward limit of sulphate reduction is controlled by diffusion (see above): this limit thus crudely approximates to the boundary between open access to depositional water above and a closed system below. If we assume that the lowest temperature (shallowest) dolomite sample (No. 648) formed immediately below the sulphate reduction zone, then it would have precipitated at a similar temperature to nodular calcite (average 15.6°C). The $\delta^{18}\text{O}$ value of water in equilibrium with dolomite at this temperature is -4.9‰ . This figure can be thought of as the net depletion, caused by diagenetic processes in the burial column. To simplify the calculation we assume that the expressed water maintained this value throughout the uppermost 1 km of the sediment column and then changed abruptly to -1.2‰ at the sulphate-reduction zone.

Precipitation temperatures for the deeper diagenetic carbonates were calculated on this basis. These values, T_d , are given in Table 1, column 6. This approach, of course, is a gross oversimplification since there will be variation in $\delta^{18}\text{O}$ with depth below the mixing zone boundary. It nevertheless represents a reasonable first approximation. By assuming the temperature of the base of the sulphate-reduction zone is 15.6°C , its depth to be 10 m and a thermal gradient of 28°C per km (a value used in ref. 1), precipitation depths can be calculated and these are listed in Table 1, column 7. In Fig. 3, the various carbonate samples are plotted as $\delta^{13}\text{C}$ against precipitation temperature:

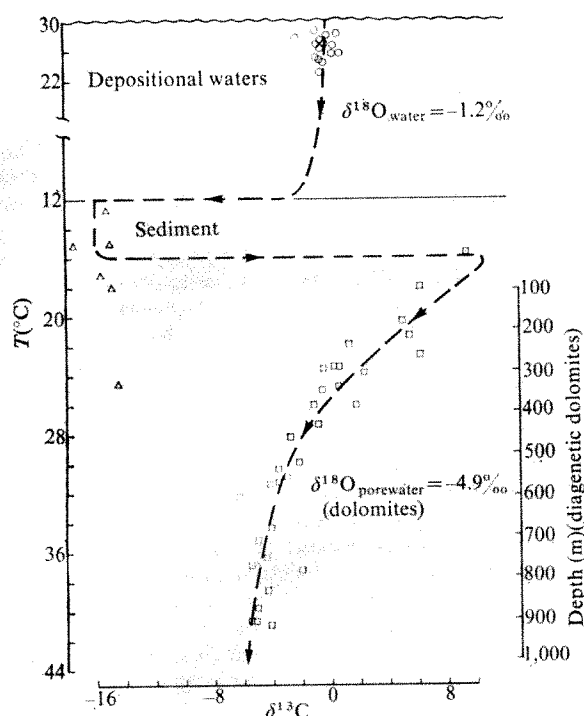


Fig. 3 Plot of calculated precipitation temperatures for Kimmeridge carbonates against carbon isotope ratio. 'Primary' carbonates are shown associated with depositional waters, diagenetic carbonates with sediment porewaters. Precipitation pathway is indicated showing passage through zone II (sulphate reduction) to zone III (fermentation-heavy C) to zone IV at the base where light carbon isotope ratios are again encountered. For discussion of porewater isotope composition and precipitation depth estimates see text. Symbols as for Fig. 2.

T for calcites, T_d for dolomites. Coccolithic limestone samples are plotted separately since they formed within the depositional environment.

Estimates of burial depths for precipitation of diagenetic dolomites suggest values ranging to 1 km. This means that all these carbonates precipitated relatively early during diagenesis and almost certainly predated liquid hydrocarbon formation or migration. The trend depicted in Fig. 3 represents a time

sequence for precipitation of carbonates at any particular sediment horizon. Carbonates precipitated from depositional waters obviously constitute the first component. Shortly after burial sulphate reduction may promote precipitation of early diagenetic carbonate, very rich in ^{12}C and reflecting low temperatures. The next carbonates to form will do so at similar temperatures but will have extremely different carbon isotope ratios with ^{13}C enrichment in consequence of fermentation reactions. Thereafter, successively later carbonates will reflect higher precipitation temperatures and the increasing influence of abiotic and decarboxylation reactions (^{12}C -rich bicarbonate input).

Work in hand involves extension of the investigation to later (deeper) precipitated carbonates in sandstones as well as in mudstone sequences. It is hoped to improve the calibration of depth/temperature scales as better models and new data become available.

We thank Professor C. Downie for help and advice, and Dr J. D. Hudson (Leicester University) for constructive criticism. Isotopic measurements were made in the Institute of Geological Sciences. The Natural Environment Research Council is supporting this work through a Research Studentship (H.L.) and Research Grant GR3-3086 (C.D.C.).

Received 17 March; accepted 20 June 1977.

- 1 Curtis, C. D. *Phil. Trans. R. Soc.* (in the press).
- 2 Abelson, P. & Hoering, T. C. *Proc. natn. Acad. Sci. U.S.A.* **47**, 623 (1961).
- 3 Rosenfeld, W. & Silverman, S. *Science* **130**, 1658 (1959).
- 4 Claypool, G., Presley, B. J. & Kaplan, I. R. in *Initial Reports of the Deep Sea Drilling Project* **19**, 879 (US Government Printing Office, Washington, D.C., 1973).
- 5 Hallam, A. & Sellwood, B. *J. Geol.* **84**, 301 (1976).
- 6 Arkell, W. J. *The Jurassic System of Great Britain* (Oxford University Press, Oxford, 1933).
- 7 Pearson, M. J. *Miner. Mag.* **39**, 696 (1974).
- 8 Forester, R. H., Sandberg, P. A. & Anderson, T. F. in *Living and Fossil Bryozoa* (ed. Larwood, G. P.) (Academic, London and New York, 1973).
- 9 Weber, J. N., Deines, P., Weber, P. H. & Baker, P. A. *Geochim. cosmochim. Acta* **40**, 31 (1976).
- 10 McCrea, J. M. *J. chem. Phys.* **18**, 849 (1950).
- 11 Craig, H. *Geochim. cosmochim. Acta* **12**, 133 (1957).
- 12 Deines, P. *Int. J. Mass Spectrom. Ion Phys.* **4**, 283 (1970).
- 13 Sharma, T. & Clayton, R. N. *Geochim. cosmochim. Acta* **29**, 1317 (1965).
- 14 Craig, H. *Proc. Spoleto Conf. on Stable Isotopes in Oceanographic Studies and Paleotemperatures*, 3 (1965).
- 15 Epstein, S., Buchsbaum, H. A., Lowenstam, H. A. & Urey, H. C. *Bull. geol. Soc. Am.* **64**, 1315 (1953).
- 16 Northrop, D. A. & Clayton, R. N. *J. Geol.* **74**, 174 (1966).
- 17 O'Neil, J. R. & Epstein, S. *Science* **152**, 198 (1966).
- 18 Degens, E. T. & Epstein, S. *Geochim. cosmochim. Acta* **28**, 23 (1964).
- 19 Fritz, P. & Smith, D. G. W. *Geochim. cosmochim. Acta* **34**, 1161 (1970).
- 20 Shackleton, N. J. & Kennett, J. P. in *Initial Reports of the Deep Sea Drilling Project* **29**, 743 (US Government Printing Office, Washington, D.C., 1975).
- 21 Raiswell, R. W. *Chem. Geol.* **18**, 227 (1976).
- 22 Lawrence, J. R., Gieskes, J. M. & Broecker, W. S. *Earth planet. Sci. Lett.* **27**, 1 (1975).
- 23 Perry, E. A., Jr., Gieskes, J. M. & Lawrence, J. R. *Geochim. cosmochim. Acta* **40**, 413 (1976).
- 24 Clayton, R. N. *J. geophys. Res.* **71**, 389 (1966).

X-ray structures of two oxidation states of a flavin-nicotinamide biscoenzyme and models for flavin—nicotinamide interactions

David J. T. Porter* & Harold J. Bright

Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Donald Voet†

Department of Chemistry and Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104

The flavin nicotinamide biscoenzymes $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$ assume extended conformations in the solid state. In both derivatives the nicotinamide and flavin groups associate through hydrogen bonding. The bending angle of the reduced flavin moiety is less than half that in any previously reported 1,5-dihydroflavin structure. This effect is apparently due to ring stacking interactions.

REDUCTION of a flavoenzyme by NADH is the primary step in many important biological oxidoreduction processes. Interactions between flavin and nicotinamide species of differing oxidation states have been described in several such flavoenzyme catalysed

*Present address: Novo Laboratories, 59 Danbury Road, Wilton, Connecticut.

†To whom correspondence should be addressed.

reactions¹. Complexes composed of reduced flavin and oxidised nicotinamide have been characterised as charge-transfer complexes by spectral criteria² as well as by the correlation of the energies of their long wavelength absorptions with the calculated lowest vacant orbital energies of their nicotinamide components³. Similarly, kinetic and spectral data have indicated that a kinetically important charge-transfer complex between reduced nicotinamide and oxidised flavin occurs before the reduction of flavins⁴⁻⁸. The geometry of this complex has been proposed to be one in which the two ring systems are face-to-face such that the nicotinamide C(4) atom is opposite the flavin N(5) atom⁵. Flavin-nicotinamide oxidoreduction is then postulated to occur by hydride ion transfer from the nicotinamide C(4) position to the flavin N(5) position⁵. Alternately, it has been proposed that such reactions occur through a one electron transfer followed by the migration of a hydrogen atom⁹. Chemical evidence supporting these mechanisms is provided by the demonstration of direct hydrogen transfer from dihydronicotinamide to the C(5) position of N(5)-deazaflavin, both in a model system¹⁰ and enzymatically¹¹.

This study was undertaken in order to visualise the associations between nicotinamide and flavin in their various oxidation states. In doing so, we have elucidated the structures of two oxidation states of a propyl linked flavin-nicotinamide biscoenzyme, $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$. $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: 10-[3-(3-carbamoyl-1-pyridinium)-propyl]-7,8-dimethylisalloxazine. $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: The anion of $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ generated by ionisation of the flavin N(3) position. $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$: 10-[3-(3-carbamoyl-1-pyridinium)-propyl]-1,5-dihydro-7,8-dimethylisalloxazine. $\text{HFl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$: The anion of $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$ generated by the ionisation of the flavin N(1) position. These compounds, which were originally synthesised for model studies of the 1,4-dihydronicotinamide reduction of flavins², exhibit intermolecular associations that are of potential biological significance.

Experimental methods

$\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+ \cdot 7\text{H}_2\text{O}$ synthesis: $(\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ was synthesised as previously described^{2,5}. Needle shaped yellow crystals were grown by the vapour equilibration of a 10 mM aqueous NH_3 solution with a 5 mM $(\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ solution. Thin layer chromatography of these crystals followed by staining with AgNO_3 failed to reveal the presence of bromide ion. Hence the

crystals consist of the zwitterionic species $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$. A crystal measuring $0.16 \times 0.13 \times 0.62$ mm was sealed inside a thin walled glass capillary tube to prevent the observed disintegration of the crystals on exposure to air. Oscillation and Weissenberg photographs revealed the space group to be $\text{P}2_1/\text{c}$. The unit cell parameters are $a = 7.740(3)$, $b = 23.800(12)$, $c = 13.958(5)$ Å and $\beta = 101.93(3)^\circ$. The crystal density of 1.392 g cm^{-3} , as determined by flotation in a CCl_4 -cyclohexane mixture, indicates that the contents of the asymmetric unit are $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+ \cdot 7\text{H}_2\text{O}$.

$(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^- \cdot 4\text{H}_2\text{O}$ synthesis: The flavin moiety in an aqueous solution of $(\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ at pH 8.0 was selectively reduced by dithiothreitol in anaerobic conditions. This caused the solution to change colour from the yellow characteristic of oxidised flavins to dark blue-black. After a day the zwitterionic species $\text{HFl}_{\text{red}}^- - \text{C}_3 - \text{Nic}^+$ had precipitated as black spherical clumps of crystallites that were unsuitable for X-ray study. These were dissolved at about 50°C in a minimal amount of 50 mM HNO_3 to form a dark brown solution. After several days, red plate-like crystals appeared. These crystals are stable in air for several minutes but longer exposures result in their disintegration and the return of the yellow colour of oxidised flavins.

A crystal measuring $0.75 \times 0.08 \times 0.12$ mm was sealed inside a thin walled glass capillary tube together with a small amount of 0.1 M $\text{Na}_2\text{S}_2\text{O}_4$ to act as an oxygen scavenger. Oscillation, Weissenberg and precession photographs showed the crystal to have triclinic lattice symmetry. The statistical distribution of the subsequently measured diffraction data indicated that the space group of the crystal was $\text{P}\bar{1}$. The unit cell parameters are $a = 8.068(3)$, $b = 11.369(3)$, $c = 14.190(7)$ Å, $\alpha = 77.89(3)^\circ$, $\beta = 88.99(4)^\circ$ and $\gamma = 89.12(3)^\circ$. The crystal density of 1.405 g cm^{-3} , as determined by flotation in a cyclohexane- CCl_4 mixture, indicated the stoichiometry of the asymmetric unit to be $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^- \cdot 4\text{H}_2\text{O}$.

A detailed description of the techniques used in intensity measurement, data treatment and structure determination for both of these compounds will appear elsewhere¹². For both crystals, reflection intensities were measured by a Picker FACS-I diffractometer using graphite monochromatised $\text{CuK}\alpha$ radiation. A total of 3,806 and 4,076 reflections, respectively, for $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$ were measured to the limit $2\theta = 125^\circ$. Both structures were solved by the straightforward application of the direct methods program MULTAN¹³. In both cases all 30 non-hydrogen atoms of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ skeleton appeared as the highest peaks in the E-map. Subsequent Fourier maps revealed the positions of the non-hydrogen atoms of seven water molecules in the case of $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and those of the nitrate ion and four water molecules for $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$.

The structures were refined by least-squares methods. Hydrogen atoms were located in difference Fourier maps and were subsequently also refined. Seven solvent hydrogen atoms could not be located in the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ structure and, similarly, four methyl hydrogens and two water hydrogens were not found in that of $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$. The refinements converged at the final agreement factors of $R = 0.064$ based on 3,318 observed reflections for $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $R = 0.069$ based on 2,963 observed reflections for $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$.

Molecular structures

$\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: The final atomic parameters of the structures together with their covalent distances and angles will be presented elsewhere¹². Figure 1 illustrates the conformation of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion and presents the atomic numbering scheme. The covalent bonding parameters are mostly in excellent agreement with published values of corresponding distances and angles in similar molecules¹⁴⁻¹⁹. The only significant exceptions to this are the predictable distortions of the flavin pyrimidinoid ring in the region of the ionised N(3) atom^{14,20}. The flavin residue is nearly planar as has been found for other oxidised flavins². The root mean square deviation of its 18 atoms from coplanarity is 0.026 Å. That of

Fig. 1 A perspective drawing of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion. Atoms are shown as thermal ellipsoids.

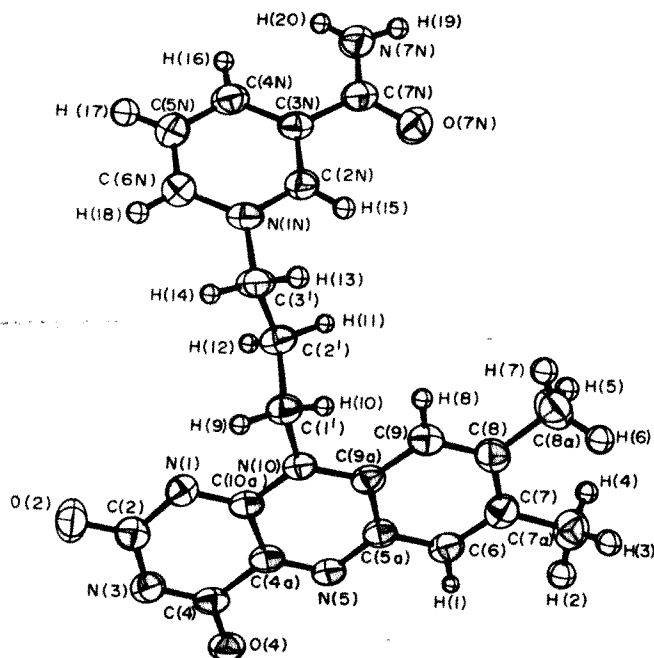
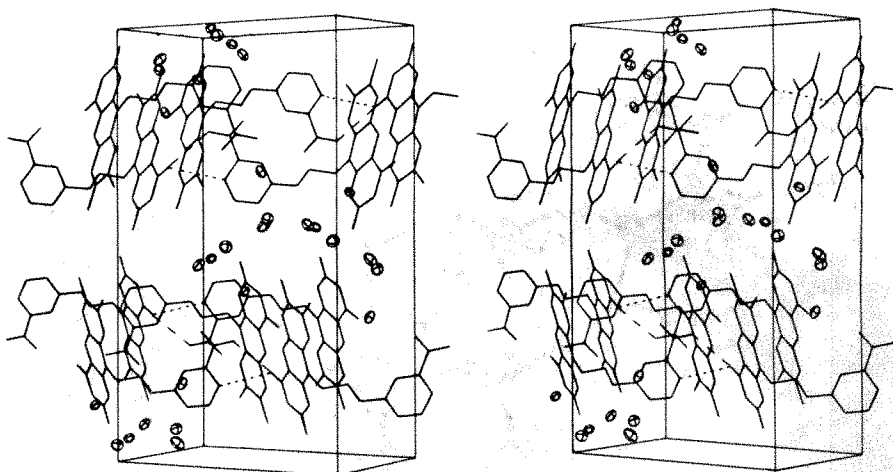


Fig. 2 A stereo diagram illustrating the packing of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ unit cell. The view is roughly along the a axis with the b and c axes extending vertically and horizontally, respectively. Water oxygen atoms are illustrated as thermal ellipsoids. Hydrogen bonds and $\text{C}-\text{H} \cdots \text{O}$ hydrogen bond-like interactions are shown as dashed and dotted lines, respectively. Hydrogen atoms have been omitted for clarity.



the 6 atoms of the nicotinamide ring is 0.005 Å, so this ring is planar to within experimental error. The amide group is oriented such that the $\text{C}(7\text{N})-\text{N}(7\text{N})$ bond is *trans* to the $\text{N}(1\text{N})-\text{C}(3')$ bond with the planes of the amide group and the nicotinamide ring forming a dihedral angle of 1.1° . This nearly coplanar conformation is found in a majority of the known nicotinamide structures^{16-19,22}.

The propyl group is in the all *trans*, fully extended conformation. The least-squares planes through the nicotinamide and flavin rings form a dihedral angle of 59.5° .

Figure 2 is a stereo diagram showing the packing of the structure in the unit cell. The crystal structure largely consists of broad bands, endlessly extending parallel to the (010) plane, that contain the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterions. Within these bands the flavin residues associate by forming endless stacks of overlapping parallel ring molecules. Here neighbouring molecules are related by centres of symmetry. The distances between the least-squares planes of the parallel flavin residues are 3.460 and 3.385 Å, respectively, for the two different stacking interactions. There is no interatomic contact in either interaction that is less than its corresponding van der Waals distance. Hence these associations are normal stacking interactions.

The nicotinamide residues extend alternately to opposite sides of the flavin stacks. The nicotinamide rings within a given band of heterocycles are parallel due to symmetry considerations. As can be seen in Fig. 2, however, neighbouring nicotinamide residues do not overlap.

The only hydrogen atoms of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion that are capable of forming normal hydrogen bonds are those of the amide group. Of these, only one participates in a hydrogen bond between the nicotinamide and flavin moieties of the structure. This is the intermolecular $\text{N}(7\text{N})-\text{H}(20) \cdots \text{O}(4)$ hydrogen bond illustrated in Figs 2 and 3. This interaction is flanked by an apparently strong intermolecular $\text{C}(4\text{N})-\text{H}(16) \cdots \text{O}(4)$ hydrogen bond-like interaction. Both of these interactions are unusual in that the nicotinamide and flavin planes are more nearly perpendicular than parallel to each other. Flavin atom $\text{N}(5)$, a potential hydrogen bond acceptor, is 2.86 Å from amide hydrogen $\text{H}(20)$. This is, however, too large a distance to be considered a hydrogen bond.

The bands containing the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterions are interspersed by parallel bands containing the solvent molecules. These are arranged in a complex hydrogen bonded network which also involves the hydrophilic groups of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion.

$\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$: Figure 4 illustrates the conformation of the $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$ ion and the atomic numbering scheme. The covalent bonding parameters are in reasonable agreement with the corresponding values reported for other 1,5-dihydroisalloxazine derivatives.

The eight atoms of the pyrimidinoid group and the six atoms of the benzenoid ring of the flavin moiety deviate from their respective least-squares planes by root mean square distances of 0.006 and 0.012 Å. Hence these rings are both highly planar. The reduced flavin nucleus assumes the expected 'butterfly' confor-

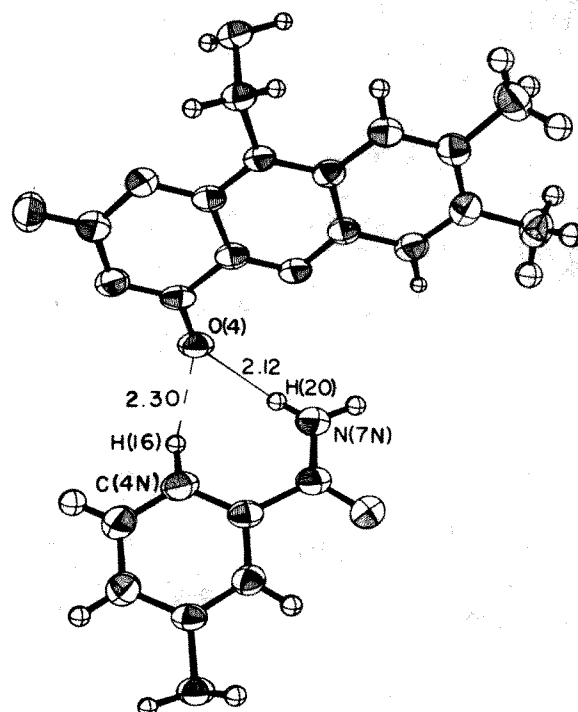
mation²³ with a 12.7° dihedral angle between the foregoing planes. The corresponding angles in the four previously reported 1,5-dihydroflavin structures range from 28.8 to 35.5° (refs 21, 24)—all more than twice that found in the present structure. The pyrazoid ring is in the usual boat conformation.

The nicotinamide ring is also highly planar. Its six atoms deviate from their least-squares plane by a root mean square distance of 0.008 Å. The amide group in this ion is roughly *trans* to the $\text{N}(1\text{N})-\text{C}(3')$ bond but is twisted about the $\text{C}(3\text{N})-\text{C}(7\text{N})$ bond such that the $\text{C}(2\text{N})-\text{C}(3\text{N})-\text{C}(7\text{N})-\text{O}(7\text{N})$ torsion angle is 29.4° .

The propyl group is folded such that the conformation about its $\text{C}(2')-\text{C}(3')$ bond is *gauche* and that about its $\text{C}(1')-\text{C}(2')$ bond is *trans*. The dihedral angle between the least-squares planes through the nicotinamide ring and the entire flavin residue is 73.4° .

Figure 5 is a stereo diagram illustrating the packing of the structure in the unit cell. It can be seen that the flavins associate,

Fig. 3 A perspective drawing illustrating the hydrogen bonding interactions between the flavin and nicotinamide residues of neighbouring molecules in the structure of $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$. Atoms are shown as thermal ellipsoids. Hydrogen bonds and $\text{C}-\text{H} \cdots \text{O}$ hydrogen bond-like interactions are represented by thin and dashed lines, respectively, and are accompanied by their corresponding lengths (Å).



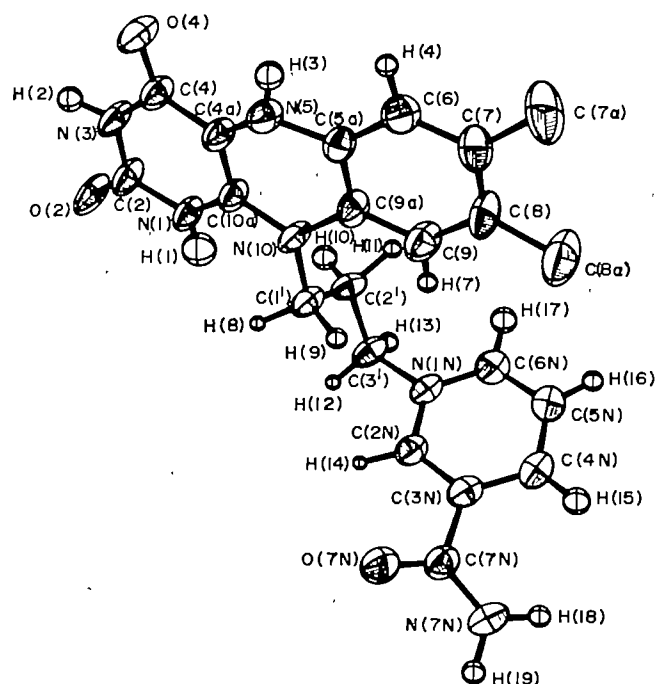


Fig. 4 A perspective drawing of the $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ion. Atoms are shown as thermal ellipsoids

through stacking and hydrogen bonding interactions, into bands of ring molecules endlessly extending parallel to the (001) plane. Neighbouring heterocycles within the columns of stacked flavins are related by centres of symmetry. Their butterfly conformation thereby causes them to alternately fold towards and away from each other. There are no interatomic contacts closer than van der Waals distances within these stacks.

Neighbouring flavins base pair across centres of symmetry in a manner by which flavins have often been observed to associate²⁵. The $\text{N}(3)\text{-H}(2)\cdots\text{O}(2)$ hydrogen bond length of 2.796 Å is within the normal range for such interactions¹⁴.

The nicotinamide residues alternately extend to opposite sides of the flavin stacks and are oriented such that they are nearly parallel to the flavin stacking axis. The nicotinamide groups are too far removed from one another to associate in any manner.

The nicotinamide amide oxygen $\text{O}(7\text{N})$ accepts a tenuous hydrogen bond from the flavin $\text{N}(5)\text{-H}(3)$ group of a neighbouring $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ion. This association, illustrated in Figs 5 and 6, seems to be quite strained. The $\text{N}(5)\cdots\text{O}(7\text{N})$ and the

$\text{H}(3)\cdots\text{O}(7\text{N})$ distances of 3.184 and 2.53 Å, respectively, are very long for $\text{N-H}\cdots\text{O}$ hydrogen bonds. The $\text{N}(5)\text{-H}(3)\cdots\text{O}(7)$ angle of 152° deviates significantly from the expected near linearity for such interactions. Furthermore, although an amide group is normally expected to be essentially coplanar with an N-H group to which it is hydrogen bonded, flavin atoms $\text{N}(5)$ and $\text{H}(3)$ deviate 2.399 and 1.98 Å, respectively, from the plane of the amide group with which they are associated. In fact, without the large $\text{C}(2\text{N})\text{-C}(3\text{N})\text{-C}(7\text{N})\text{-O}(7\text{N})$ torsional angle, the foregoing hydrogen bond could not be formed.

The regions in the crystal structure between the associating $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ions are occupied by the nitrate ions and the solvent molecules. These associate through hydrogen bonded networks that involve the hydrophilic groups of the heterocycles. All potential hydrogen bonds in the structure seem to have been formed.

Discussion

Studies of the nitroalkane reduction of the FAD requiring enzyme D-amino acid oxidase have proven that, at least in certain classes of enzymatic reactions, the flavin $\text{N}(5)$ atom is the position through which electrons are transferred²⁶. This is further corroborated by the reduction of $\text{N}(5)$ -deazaflavin by direct hydrogen transfer to its $\text{C}(5)$ position^{10,11}. Thus it is of interest that the flavin $\text{N}(5)\text{-H}(3)$ group is hydrogen bonded to nicotinamide atom $\text{O}(7\text{N})$ in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$.

The $\text{C}(4)$ atom of nicotinamide is known to be the position through which electrons are transferred in the biological oxidation-reduction reactions of pyridine nucleotides. It is therefore of note that in $\text{Fl}_{\text{ox}}\text{-C}_3\text{-Nic}^+$ the $\text{C}(4\text{N})\text{-H}(16)$ group forms a hydrogen bond-like association with flavin atom $\text{O}(4)$.

The nicotinamide amide group has been shown not to be an essential component of NAD^+ in the reactions mediated by certain dehydrogenases²⁷. Rather, it seems that a major requirement for the activity of these enzymes is that the substituent to the pyridinium 3-position be a carbon atom that is double bonded to an electronegative atom, such as O, N or S²⁷. Nevertheless, it seems likely that the amide group of nicotinamide serves a specific function in some vital biological process for without such a function it is expected that evolutionary processes would have chemically altered the amide group in some manner in at least some organisms. The similarities of the nicotinamide-flavin associations in both structures presented here suggest that the function of the nicotinamide amide group during enzyme mediated reduction of flavin by 1,4-dihydronicotinamide may be to bind the reactive portions of flavin and nicotinamide together to facilitate electron transfer.

The observed 12.7° bending angle of the 1,5-dihydroflavin

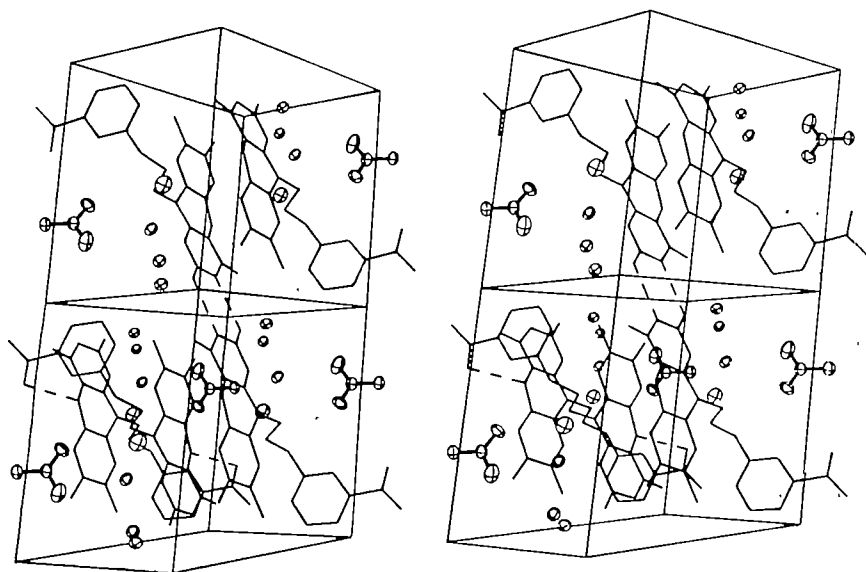


Fig. 5 A stereo drawing illustrating the crystal structure of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3^-\cdot 4\text{H}_2\text{O}$ in relation to the unit cell boundaries. The view is roughly along the c axis with the a and b axes extending horizontally and vertically, respectively. Water oxygen atoms and the atoms of the nitrate ion are shown as thermal ellipsoids. Hydrogen bonds between heterocycles are shown as dashed lines. Hydrogen atoms have been omitted for clarity.

moiety of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is considerably less than the 32° average of this angle in the previously reported 1,5-dihydroflavin structures^{21,24}. All of these latter compounds have more and bulkier substituents than does the flavin residue in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ and hence it might seem that their large bending angles are due to steric interference among these substituents. Molecular models of these compounds, however, reveal that such overcrowding is relieved at bending angles of the order of 10° . Furthermore, a similarly substituted fully oxidised flavin is observed to be nearly planar²⁸

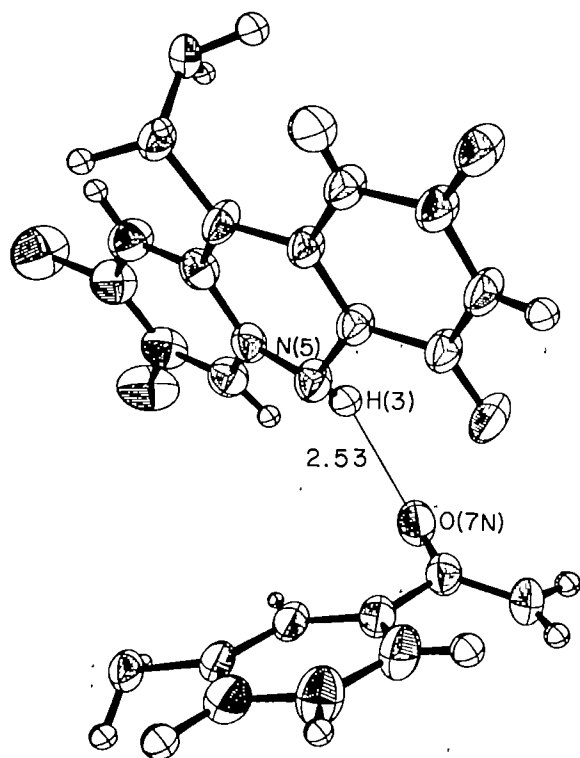


Fig. 6 A perspective drawing illustrating the hydrogen bonding association of the flavin and nicotinamide residues of neighbouring molecules in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$. Atoms are shown as thermal ellipsoids. The hydrogen bond is represented by a thin line accompanied by its length (Å).

Self-consistent field molecular orbital calculations incorporating the PRDDO (partial retention of diatomic differential overlap) approximation indicate that the barrier for ring inversion in 1,5-dihydroflumiflavin has a surprisingly low value of nearly 4 kcal mol^{-1} (Lindner, D. L., Branchaud, B., Dixon, D. A., and Lipscomb, W. N., to be submitted). Nuclear magnetic resonance (NMR) measurements on several reduced flavin derivatives yield a larger but still relatively low value of 10 kcal mol^{-1} for this barrier²⁹. Therefore the observation that there is considerable flavin/flavin stacking in the crystal structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ but very little ring overlap in the structures of other reduced flavins^{21,24} suggests that the small flavin bending angle in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is due to the influence of stacking interactions.

X-ray studies of flavodoxin from *Clostridium MP* have shown, as expected, that the flavin moiety of the bound FMN cofactor is planar in both the oxidised^{30,31} and the semiquinone^{31,32} forms of the enzyme. The flavin residue is also nearly planar in reduced *Clostridium* flavodoxin; its bending angle is estimated to be 8.6° (ref 31). This is corroborated by the essential identity of the NMR spectra of oxidised and reduced *Clostridium* flavodoxins³³.

It has been suggested that the constraint of a protein bound reduced flavin to the planar configuration enhances its reducing power by modifying its redox potential^{29,31,34}. The nearly planar conformation of the flavin moiety in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ cor-

roborates this hypothesis by demonstrating the flexibility of the flavin bending angle.

The red colour of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3^-$ crystals, in contrast to the yellow colour of other crystalline reduced flavins^{21,24} might reasonably be attributed to either a charge-transfer complex or a spectral shift in the reduced flavin caused by its flattening²³. Charge-transfer interactions have been observed between stacked oxidised flavin molecules in the crystalline complex lumiflavin-2,6-diamino-9-ethyladenine³⁵ but have not been previously observed between reduced flavin molecules. The most likely site for such an interaction in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is within the stacks of reduced flavins. However, the fact that all interatomic contacts between reduced flavin residues are greater than van der Waals distances indicates that any charge transfer forces present would be weak at best. Likewise, the relatively small changes in the visible absorption spectrum of the nearly planar flavin molecule of reduced flavodoxin from *Clostridium MP*, in comparison with that of free reduced FMN³⁶, do not account for the red colour of crystals of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3^-$. Hence the origin of this colour remains obscure.

Our study provides no evidence supporting the face-to-face charge-transfer complex model of flavin/nicotinamide interactions postulated on the basis of solution studies²⁻⁵. The absence of such associations in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ corroborates the observation that these interactions are relatively weak⁵. This is in agreement with the assertion that the characteristics of charge-transfer complexes are highly dependent on the relative orientations of the donor and the acceptor and on the contribution of other weak binding forces to the total potential energy of the complex³⁷. Thus it seems likely that the formation of any face-to-face flavin/nicotinamide complex in aqueous solutions would require stabilisation by hydrophobic forces similar to those promoting the stacking of nucleic acid bases in aqueous solutions³⁸.

We thank W. N. Lipscomb for providing the results of the molecular orbital calculations on flavins before publication. This work was supported, in part, by grants from the US NIH (GM 11040) and the MRL program of the NSF (DMR76-00678).

Received 28 March, accepted 27 June 1977

- ¹ Massey, V. & Ghisla, S. *Ann N Y Acad Sci* **227**, 446-465 (1974)
- ² Proffitt, R. T., Ingraham, L. L. & Blankenhorn, G. *Biochem biophys Acta* **362**, 534-548 (1974)
- ³ Sakurai, T. & Hosoya, H. *Biochem biophys Acta* **112**, 459-468 (1966)
- ⁴ Porter, D. J. T., Blankenhorn, G. & Ingraham, L. L. *Biochem biophys Res Commun* **52**, 447-452 (1973)
- ⁵ Blankenhorn, G. *Eur J Biochem* **50**, 351-356 (1975), **67**, 67-80 (1976) *Biochemistry* **14**, 3172-3176 (1975), *Flavins and Flavoproteins*, Vol. V (ed. Singer, T. P.), 261-267 (Elsevier, Amsterdam, 1976)
- ⁶ Bruce, T. C., Main, L., Smith, S. & Bruce, P. Y. *J Am chem Soc* **93**, 7325-7328 (1971)
- ⁷ Gumbley, S. J. & Main, L. *Tetrahedron Lett.* 3209-3212 (1976)
- ⁸ Steffens, J. J. & Chipman, D. M. *J Am chem Soc* **93**, 6694-6696 (1971)
- ⁹ Bruce, T. C. *Prog bioorg Chem* **4**, 1-87 (1976)
- ¹⁰ Brustlein, M. & Bruce, T. C. *J Am chem Soc* **94**, 6548-6549 (1972)
- ¹¹ Fisher, J. & Walsh, C. *J Am chem Soc* **96**, 4345-4346 (1974)
- ¹² Porter, D. J. T. & Voet, D. submitted to *Acta crystallogr*
- ¹³ Main, P., Woolfson, M. M. & Germain, G. *MULTAN: A Computer Program for the Automatic Solution of Crystal Structures* (Department of Physics, University of York, York, UK, 1971)
- ¹⁴ Voet, D. & Rich, A. *Prog Nucl Acid Res molec Biol* **10**, 183-265 (1970)
- ¹⁵ Wang, M. & Fritche, C. J. *Acta crystallogr B29*, 2040-2045 (1973)
- ¹⁶ Voet, D. *J Am chem Soc* **95**, 3763-3770 (1973)
- ¹⁷ Johnson, P. L., Maier, C. A. & Paul, I. C. *J Am chem Soc* **95**, 5370-5377 (1973)
- ¹⁸ Freeman, G. R. & Bugg, C. E. *Acta crystallogr B30*, 431-443 (1974)
- ¹⁹ Herriott, J. R., Cammerman, A. & Deranleau, D. R. *J Am chem Soc* **96**, 1585-1589 (1974)
- ²⁰ Burnett, R. M. *et al J Biol Chem* **249**, 4383-4392 (1974)
- ²¹ Kierkegaard, P. *et al in Flavins and Flavoproteins*, Vol. III (ed. Kamen, H.) 1-22 (University Park, Baltimore, 1971)
- ²² Wright, W. B. & King, G. S. D. *Acta crystallogr* **7**, 283-288 (1954)
- ²³ Dudley, K. H., Ehrenberg, A., Hemmerich, P. & Müller, F. *Helv Chim Acta* **47**, 1354-1383 (1964)
- ²⁴ Leyonmarch, M. & Werner, P. E. *Acta chem scand* **25**, 2273-2290 (1971)
- ²⁵ Wang, M. & Fritche, C. L. Jr. *Acta crystallogr B29*, 2040-2045 (1973)
- ²⁶ Porter, D. J. T., Voet, J. G. & Bright, H. J. *J Biol Chem* **248**, 4400-4416 (1973)
- ²⁷ Anderson, B. M. & Kaplan, N. O. *J Biol Chem* **234**, 1226-1232 (1959)
- ²⁸ von Glehn, M. & Norrestam, R. *Acta chem scand* **26**, 1490-1492 (1972)
- ²⁹ Tauscher, L., Ghisla, S. & Hemmerich, P. *Helv chim Acta* **56**, 630-644 (1973)
- ³⁰ Burnett, R. M. *et al J Biol Chem* **249**, 4383-4392 (1974)
- ³¹ Ludwig, M. L. *et al in Flavins and Flavoproteins*, Vol. V (ed. Singer, T. P.) 393-404 (Elsevier, Amsterdam, 1976)
- ³² Anderson, R. D. *et al Proc natn Acad Sci U S A* **69**, 3189-3191 (1972)
- ³³ James, T. L., Ludwig, M. L. & Cohn, M. *Proc natn Acad Sci U S A* **70**, 3293-3295 (1973)
- ³⁴ Ludwig, M. L. *et al Cold Spring Harb Symp quant Biol* **36**, 369-380 (1971)
- ³⁵ Scarbrough, F. E., Shieh, H.-S. & Voet, D. *Proc natn Acad Sci U S A* **73**, 3807-3811 (1976), *Acta crystallogr B33*, (in the press)
- ³⁶ Ghisla, S., Massey, V., Lhoste, J. M. & Mayhew, S. G. *Biochemistry* **13**, 589-597 (1974)
- ³⁷ Shiklin, M. A. *Physical-Chemical Properties of Nucleic Acids* **1**, (ed. Duchesne, J.), 67-98 (Academic, New York, 1973)
- ³⁸ Ts'o, P. O. P. *Fine Structure of Proteins and Nucleic Acids* (eds Fasman, G. D. & Timasheff, S. N.) 49-190 (Marcel Dekker, New York, 1970)

Possible regulatory function of acetylcholine receptor in maintenance of retinotectal synapses

John A. Freeman

Vanderbilt University Medical School, Nashville, Tennessee 37232 and National Institute for Medical Research, London NW7, UK

α -Neurotoxins bind to cholinergic receptor, block transmission, and induce sprouting of retinal terminals in the toad tectum. New connections retain an orderliness that suggests a selective affinity between presynaptic terminals. The results suggest that postsynaptic cells exert a control, associated with receptors, on the growth of presynaptic terminals and on the maintenance of their synaptic connections.

THE effects of the presynaptic element of a synapse on the physiological and metabolic properties of its postsynaptic component have been extensively studied in several systems. But little is known about the influences which the postsynaptic element of a synapse might exert on the selection, maintenance, or metabolic control of its presynaptic component. Such information might provide important insights into the molecular mechanisms underlying growth, repair, and the response to injury in the central nervous system (CNS). The amphibian retinotectal system provides a useful model. The retina projects in an orderly visuotopic map on to the tectum, where the terminals from different classes of ganglion cells form synapses in separate layers^{1,2}. Moreover, the optic nerve will regenerate³, and in doing so re-establish a nearly identical pattern of connections^{4,5}.

Functional connections require a matching between neurotransmitter and postsynaptic receptor protein. Evidence has recently been obtained that acetylcholine (ACh) is an excitatory neurotransmitter in the amphibian optic tectum, and might be utilised by at least two different classes (I and IV) (ref. 1) of retinal ganglion cells. For example, (1) the retinotectal synaptic layers contain high levels of ACh, choline acetyltransferase, and ACh-esterase⁶; (2) tectal cells are excited by iontophoretically-applied ACh and synaptic responses are blocked by nicotinic antagonists⁷; (3) the reversal potentials for both iontophoretically applied ACh and the native transmitter are the same, indicating similar permeability mechanisms (manuscript in preparation); and (4) optic nerve terminal-associated synaptosomes possess a high affinity uptake system for choline, and also bind snake venom α -neurotoxins (J. A. F. and R. Oswald, in preparation). In addition, α -bungarotoxin (α -BTX) and related polypeptide α -neurotoxins, which selectively bind to nicotinic acetylcholine receptor protein (ACh-R) at neuromuscular junction^{8,9}, also bind to synapses in the tectal neuropil^{10,11}, and produce a long-lasting abolition of retinotectal transmission^{7,10}. Light autoradiographs using tritiated α -BTX reveal a distinct trilaminar distribution of receptor sites^{10,11} corresponding to the distribution of retinal terminals.

These experiments were designed to determine whether receptor-mediated changes in transmission affecting the postsynaptic cell influence the growth of presynaptic terminals. I report here that dynamic changes in the patterns of retinotectal connections occur after application of α -neurotoxins to tectal neurones in the South American toad *Bufo marinus*. The results, obtained by the use of a new mapping technique, suggest that the maintenance of synapses requires functional interaction between transmitter and receptor.

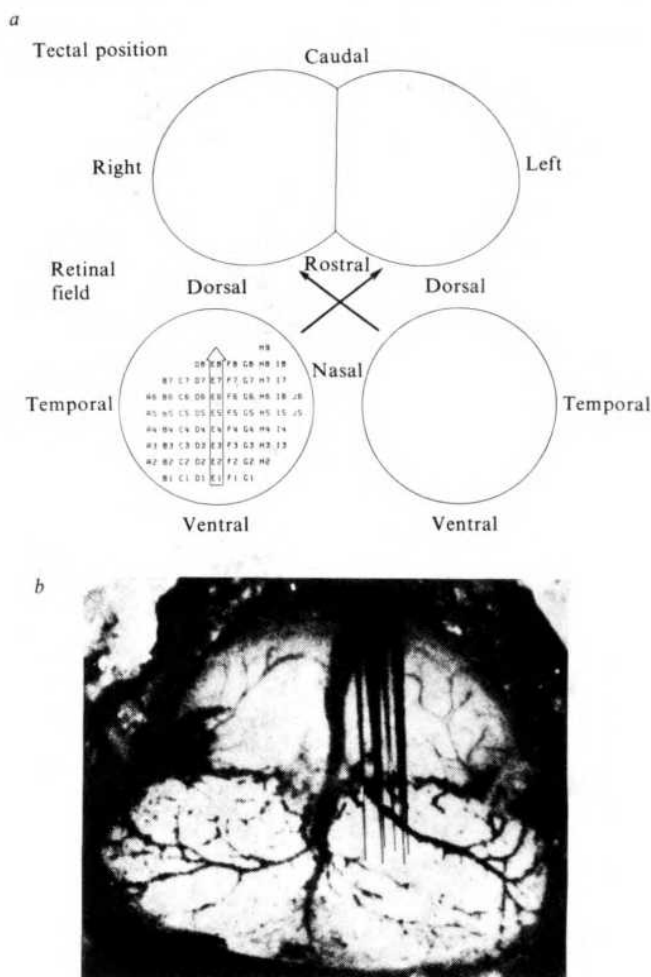


Fig. 1 Retinotectal mapping technique. Animals were immobilised either by spinal epidural anaesthesia (0.3 ml of 0.5% lidocaine in 2% sucrose) or by cooling the trunk and limbs in ice, and the tecta exposed, using a local anaesthetic. Spontaneous eye movements were eliminated by the use of a small ring held against the sclera. The optical axis of the eye, determined ophthalmoscopically, was centred on a Tektronix 611 CRT screen on which were presented electronically-generated patterns whose shape, position and brightness were controlled by a PDP-12 computer. The size and position of the equivalent retina image was computed from the translation-refraction matrix of the reduced eye. A standardised grid of retinal loci (a) was activated covering 140° of solid visual angle, and for each retinal locus the corresponding area of representation in the contralateral tectum was determined by measuring the laminar field potentials with an array of six microelectrodes (b), consisting of glass micropipettes filled with toad Ringer's, whose tips (bevelled for easier penetration¹²) were positioned 100 μ m apart in a 2 \times 3 grid. Computer-averaged field potentials were combined with previously determined extracellular resistivities to compute the three-dimensional current source density (CSD), thereby providing a clear separation of pre- and postsynaptic responses, facilitating a direct comparison of maps to be made between different animals, or in the same animal mapped at different times.

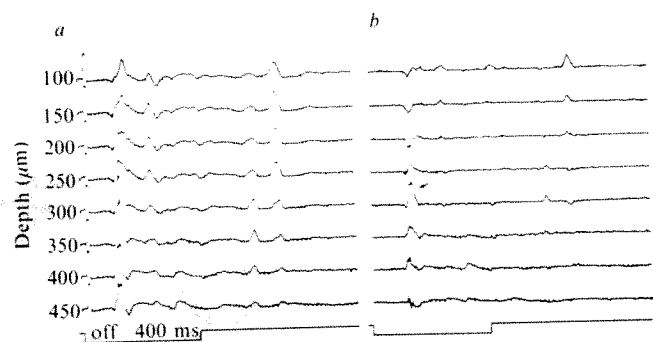


Fig. 2 Laminar field potentials (a) and corresponding CSDs (b) generated in optic tectum at indicated depths beneath the surface by the turning off of a 2° spot of light. 'Off' fibres produce a prominent sink at a depth of 300 μm , the postsynaptic component of which (arrow) is associated with a set of superficial (100–200 μm) and deep (450 μm) sources drawn from radially oriented tectal dendrites. The more superficial (100–150 μm) longer latency class II fibres are also clearly localised in (b). Calibration pulses at beginning of field potential records: 500 μV , negativity upwards. Same amplitude on CSD records corresponds to 10 mA cm^{-2} , sink upwards.

Mapping retinotectal projections

To detect subtle changes that might occur in the positions of retinal terminals, a new mapping technique was devised, in which the retina was stimulated visually at regularly spaced loci (Fig. 1). The actual tectal location of the retinal terminals thus activated was determined by searching the tectum with an array of six microelectrodes and computing the extracellular current source density (CSD) (refs 13–15). The CSD, obtained by computing the divergence of the field potential weighted by the extracellular conductivity, represents the net transmembrane flux in a specified volume of tissue¹⁴. For measurements obtained at an interelectrode spacing of N , the CSD localises activity originating in an interval of $N/2$ (or 50 μm in the present study)¹⁵.

It has recently been shown¹⁶ that an extracellular microelectrode selectively records the activity of retinal terminals (which branch extensively, thereby providing a greatly increased extracellular current) rather than of fibres of passage, although it records postsynaptic activity as well. An additional advantage of the CSD technique is that it readily distinguishes between pre- and postsynaptic activity^{2,13,15}.

The following results deal primarily with class IV retinal terminals¹ ('off' fibres), which respond selectively to a sudden decrease of illumination of their receptive fields. These terminals, which synapse on dendrites of tectal neurones whose somas lie in the central grey strata¹⁷, occupy a compact lamina 300 μm below the tectal surface. Their location is shown in the CSD records of Fig. 2b.

Separation of visually evoked potentials into pre- and postsynaptic components by CSD analysis is shown more clearly in Fig. 3, which also demonstrates the nicotinic cholinergic nature of class IV retinotectal synapses. In Fig. 3a, a 10^{-9} M solution of *d*-tubocurarine was applied to the tectal surface. At time 'zero' (control), the CSD waveform computed from responses to visual stimuli consists of two components: an early presynaptic sink, followed by a postsynaptic sink produced by dendritic depolarisation. This second component is gradually abolished over the next 15 min, and returns to near control values after 60 min. Figure 3b shows the CSD responses obtained at the same location in response to electrical stimulation of the optic nerve, after application to the tectal surface of a $1 \cdot 10^{-9}$ M solution of α -TBX. Initially (time zero) the early presynaptic sink is followed by a prominent postsynaptic sink, which, however, is irreversibly abolished after 180 min. The toxin produced no detectable change in the presynaptic response.

Experiments were performed in eight animals to determine the normal sequence of regeneration of retinal fibres after a lesion of the optic nerve. The projections of the intact optic nerve were first determined by CSD mapping. One optic nerve was then gently crushed at a distance of approximately 2.5 mm from the optic chiasm, and each animal, maintained at 20–24 °C, was remapped at various times thereafter. The earliest evidence of re-entry of regenerating fibres into the tectum occurred at 10–14 d. The earliest patterns of presynaptic terminals were highly variable with little if any organisation. Over the ensuing 2–3 weeks considerable organisation occurred, and by 6–8 weeks after the lesion the pattern of retinal projections differed very little from the original pattern.

Effect of toxin application on retinotectal projections

α -BTX was applied to a discrete region of tectum by placing a small square of Millipore filter soaked in toxin (5×10^{-9} M)

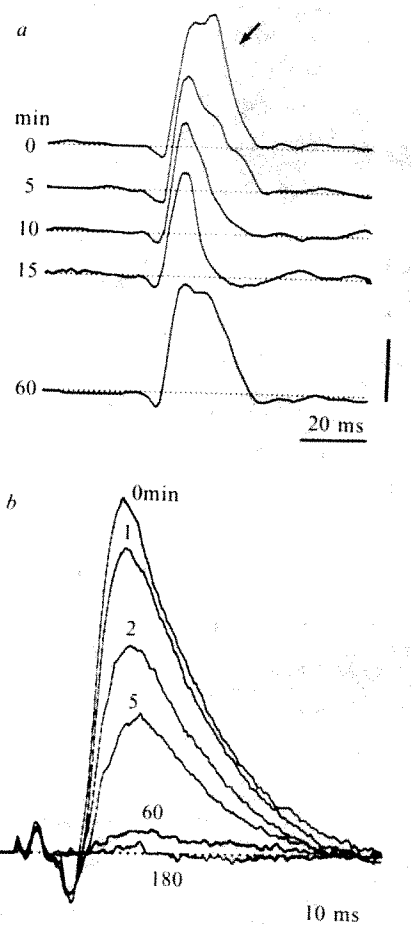


Fig. 3 Abolition of postsynaptic response to 'off' fibres by nicotinic antagonists. The lyophilised venom of *Bungarus multicinctus* was purified by chromatography on SP-Sephadex (C-25). The peak corresponding to α -BTX was collected and shown to be homogeneous on polyacrylamide gel electrophoresis using the technique of Reisfeld *et al.*¹⁸. An *in vivo* assay of toxicity was performed on toad sartorius muscle, using intracellular recording. A dose of $1 \cdot 10^{-9}$ M caused complete loss of synaptic transmission within 30 min, with no discernable loss of presynaptic activity recorded simultaneously with a second extracellular microelectrode. In (a), a 10^{-9} M solution of *d*-tubocurarine caused reversible block of transmission in which the postsynaptic component (arrow) is gradually abolished in 15 min, but returns to near control value after 60 min. CSD records computed at 300 μm depth, following turning off of a 3° light spot at beginning of each record. In (b), $1 \cdot 10^{-9}$ M α -BTX caused irreversible block of postsynaptic response to electric stimulation of optic nerve (arrow). Stimulus artefact was electronically suppressed. Average of 50 responses in (a), and 5 responses in (b). Calibration: 5 mA cm^{-2} in (b), sink upwards.

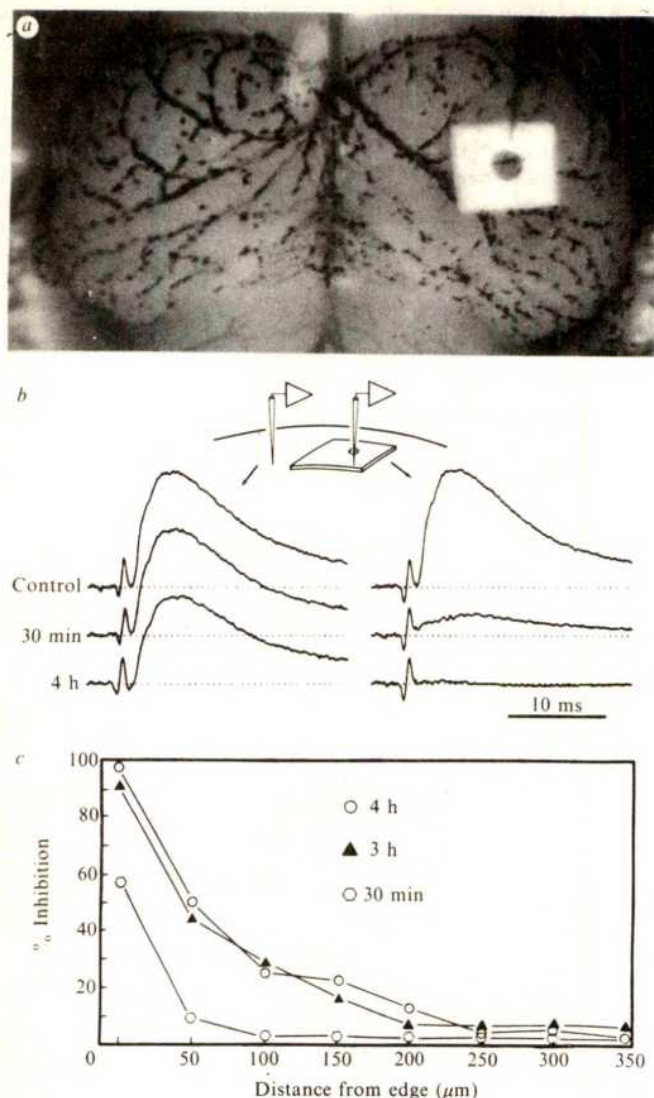


Fig. 4 Application of α -BTX to discrete region of tectum. A small square (approximately $700 \times 700 \mu\text{m}$) of α -BTX-soaked (5×10^{-9} M) Millipore filter was placed on the tectal surface (a). Two microelectrodes, one (dark shadow) inserted through a central hole, and the other inserted at various distances from the edge of the filter, were used to compute current source densities from responses to class IV fibres activated by shocking optic nerve. Postsynaptic responses were blocked immediately underneath filter (b, right side) but were relatively unaffected 250 μm from edge of filter (b, left side) over a 4-h period. (c) Inhibition (relative to control value before toxin application) of postsynaptic responses at indicated times and positions with respect to one edge of filter. Lack of synaptic blockade beginning approximately 250 μm outside of toxin-treated zone at 4 h (open circles) shows that little toxin diffused away from region of application.

on the tectal surface. To determine the extent of spread of toxin applied in this manner, a small square of filter was prepared with a hole cut in its centre, through which a microelectrode was lowered as shown in Fig. 4a. In Fig. 4b, the postsynaptic responses to optic nerve stimulation were progressively abolished underneath the filter over a period of 4 h, but were not significantly reduced outside of a region beginning approximately 250 μm from a side, presumably due to low diffusional mobility of toxin coupled with a high affinity of toxin-binding sites located under the region of application. The spatial and temporal spread of postsynaptic blockage produced by locally applied α -BTX is shown in Fig. 4c.

In seven animals, a small square of Millipore filter soaked in 5×10^{-9} M α -BTX was applied to the tectal surface two weeks after lesioning the contralateral optic nerve, when the re-

generating retinal fibres were beginning to re-enter the tectum. The filter was left in place for 4–5 h, its position photographed for later comparison, and then removed, and the animals were subsequently remapped at various times thereafter. Figure 5 shows the results of one such experiment. The map illustrated in Fig. 5b, obtained 20 d post-lesion (6 d after toxin application), shows the presence of presynaptic terminals within the toxin-treated zone, but otherwise has little organisation. By contrast, in the map obtained 14 d later (Fig. 5c), the toxin-treated zone lacked any evidence of presynaptic terminals, which instead seem to be clustered and compressed about its margins. Similar results were obtained from five other animals. On the other hand, three control animals (in which the filter was soaked in toad Ringer's rather than in α -BTX) mapped at this time, as well as another group of four animals mapped 20–30 weeks after toxin application, had an essentially normal pattern of projection with no evidence of compression.

One explanation for these effects might be that toxin killed the cells in the zone where it was applied, but that they subsequently regenerated. This seems unlikely, inasmuch as neurones remained normally responsive to other (for example, diencephalic¹⁹) inputs after toxin application, as shown by CSD analysis and single unit recording performed both during the early period (3–8 d) when toxin might be expected to produce a maximum effect, as well as at later times (2–6 weeks). A second possibility is that toxin prevented the formation of synapses. Similar experiments^{20,22} with chronic α -BTX application at the myoneuronal junction suggest that nerve-muscle synapses can in fact form when ACh-R is bound with toxin (although the possibility that they form only after bound toxin has been displaced from the receptor has not been conclusively demonstrated). A third possibility is that synapses, initially formed between regenerating terminals and α -BTX-treated tectal neurones, failed to be maintained, and that the presynaptic element continued its growth in search of a surface on which it could form an enduring synapse.

To test the effect of α -BTX on the maintenance of retino-tectal synapses, toxin was applied to the intact tectum in six animals, and the retinotectal projections were subsequently mapped at intervals of 7 d thereafter. The results from one animal are shown in Fig. 6, and are similar to those obtained from the others. One week after toxin application little if any presynaptic activity could be elicited from the toxin-treated zone (Fig. 6b) and the presynaptic terminals seemed to have shifted position and to have compressed into the borders surrounding it, displacing terminals previously located there (Fig. 6a). As shown by CSD analysis, some aberrant terminals evoked postsynaptic responses, signifying the formation of functional synapses, although the postsynaptic response amplitude was quite variable and was not easily quantitated. Maps made two weeks later (Fig. 6c) have essentially returned to normal, again suggesting that a dynamic, neuroplastic re-ordering of terminals has occurred.

These results show that the loci from which retinal terminals can be recorded change in a characteristic way after application of α -BTX. To account for these results, possible causes for the apparent sprouting, displacement, and subsequent reordering of retinal terminals should be considered.

Sprouting of retinal terminals

Several possibilities could account for the apparent shift in position of retinal terminals following the application of α -BTX shown in Figs 5 and 6, including (1), migration of postsynaptic cells²³ and their inputs; (2), unmasking of 'silent' terminals^{24,25} normally present and (3), active growth (that is, sprouting) of terminals. The first possibility is unlikely, since cells in toxin-treated regions which normally receive convergent inputs from both retinal and non-retinal (for example, diencephalic) afferents continued to respond to the latter, and were thus not likely to have migrated. The second possibility is also unlikely, since any 'silent' retinal terminals would have de-

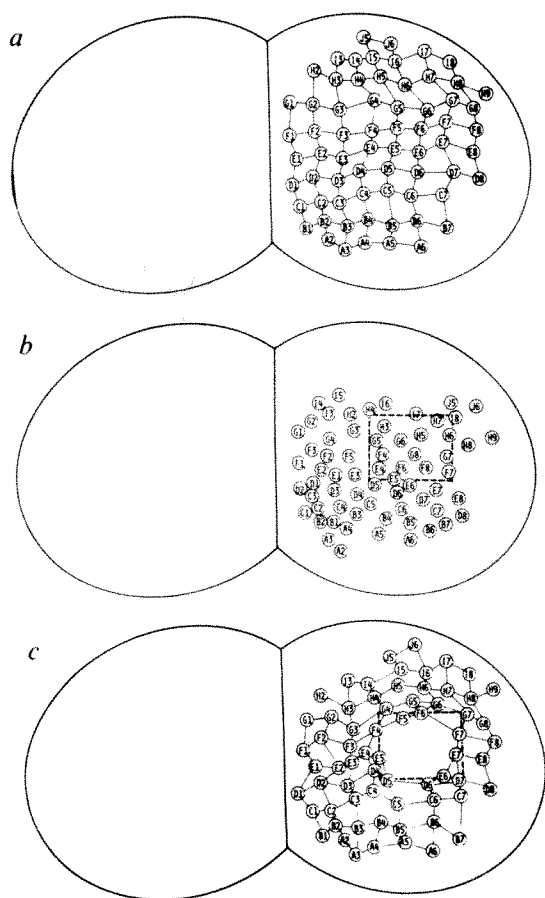


Fig. 5 Effect of localised application of α -BTX on pattern of regenerating retinal terminals. Control map shown in (a), representing positions (computed by CSD analysis) of class IV retinal terminals activated by stimulation of contralateral retinal loci shown in Fig. 1(a). Corresponding pattern of regenerating terminals shown in (b), 20 d after lesion of contralateral optic nerve, and 6 d after application of α -BTX to region indicated by dashed lines. Note the presence of terminals within the toxin-treated zone, and the general lack of organisation of the regenerating terminals. Considerable order has been restored in (c), made two weeks later. At this time, however, the toxin-treated zone is almost completely devoid of terminals, which appear clustered about its borders.

generated following optic nerve lesions. It is likely, therefore, that the results represent active growth of retinal terminals in response to toxin application, which as shown in the acute experiments of Figs 3b and 4b blocked synaptic transmission. Chronic block of neuromuscular transmission with tetanus²⁶ or with botulinum²⁷ toxin has been shown to result in sprouting of presynaptic terminals, which is suppressed by implantation of an extra nerve²⁸. Together, these results suggest that the postsynaptic element of a synapse might exert a trophic influence, dependent on the status of functional transmission, on the presynaptic element.

Similarly, the absence of detectable presynaptic activity in Figs 5c and 6b could also be accounted for by terminals growing away from the toxin-treated zones. On the other hand, some of the original terminals may have remained in the zone, but failed to produce sufficient extracellular current to be detected by the CSD technique if either the number or surface area was substantially decreased, or if chronically applied toxin interfered with the generation of action potentials (for example by binding to presynaptic ACh-R (ref. 29) or some other membrane component and altering membrane permeabilities or molecular structure). Chronic application of α -BTX for 10 d did not, however, produce any detectable decrement in action potentials in either optic nerve or sciatic nerve. Alternatively, terminals

may have degenerated, due to some primary effect of α -BTX, trace amounts of β -BTX (ref. 30) (not detectable by chromatography or gel electrophoresis), or a secondary loss of some required trophic factor. A recent³¹ electron microscopic examination of rat neuromuscular junction chronically treated with α -BTX showed no evidence of degeneration of nerve terminals, and I found no evidence of terminal degeneration in preliminary light microscopic examinations of toxin-treated regions, but detailed EM examination has not yet been done.

Displacement of retinal terminals

Maps made one week after toxin application (Fig. 6b) reveal an apparent displacement of terminals normally occupying the borders of the toxin-treated zone. Some terminals in these border regions seemed to have been displaced by as much as 250 μ m whereas terminals normally located within the toxin-treated zone seemed to have been displaced by as much as 600–700 μ m. Since the accuracy with which the eye's optical

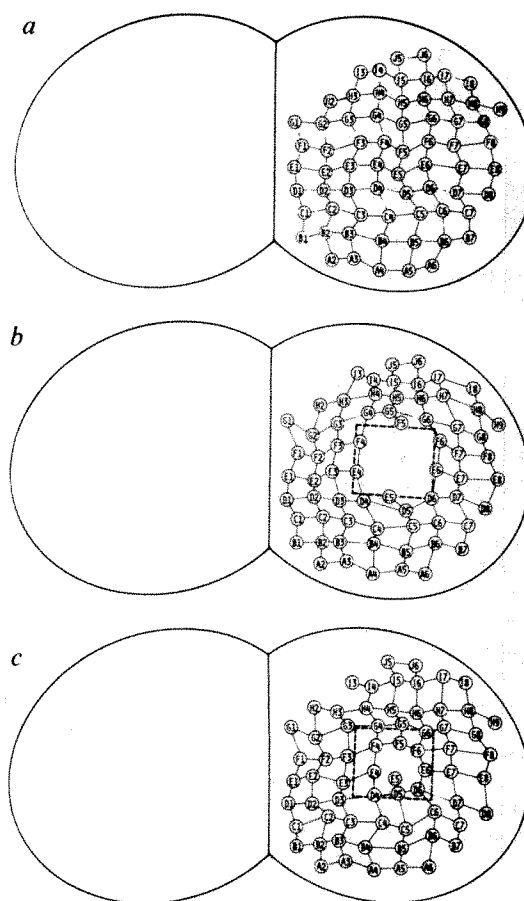


Fig. 6 Plastic changes in positions of class IV terminals following localised application of α -BTX to normally innervated tectum. Control map shown in (a). In (b), obtained one week later after applying square of Millipore filter soaked in α -BTX to region indicated by dashed lines, terminals previously located there (for example, E4-E6, F4-F6, G5) have shifted position and compressed into regions near borders, displacing terminals previously located there. In (c), obtained two weeks later, terminals have essentially returned to their original positions. Quantitative measurements of the amount of compression and order in the projections of displaced terminals in (b) were made by computing the mean distance between each recorded terminal site and its eight nearest neighbours, as well as its eight 'appropriate' neighbours. The results showed that a displaced terminal had a higher probability of retaining the same relative relationships with its displaced neighbours as with its original neighbours than any other relationship. This suggests that some selective interaction exists between terminals of neighbouring retinal ganglion cells.

axis can be precisely realigned is limited, the absolute displacement is less certain than is the relative displacement. This may represent a change in dendritic morphology of tectal neurones, or a true shift in the alignment of pre- and postsynaptic elements. An effort to differentiate between these two possibilities was made by mapping the lateral extent of antidromic field potentials generated by dendrites^{15,32} in response to focal microstimulation of tectal neuronal cell bodies in the central grey strata, by a stimulating microelectrode. The mean diameter of tectal dendritic fields estimated in this way was $110 \pm 20 \mu\text{m}$. No significant variation of dendritic field size was found within or surrounding the toxin-treated zone. This suggests that the displacement in the position of terminals in the border zones represents a true change in alignment of pre- and postsynaptic elements. As seen in Figs 5c and 6b, displaced terminals tended to retain their original orientation with respect to each other rather than to distribute randomly. The retention of a high degree of order between both invading as well as displaced terminals suggests the existence of a selective interaction between neighbouring terminals.

Reordering of retinal terminals

If the preceding interpretations are correct, both the original displacement and the subsequent reordering of retinal terminals seen in Fig. 6c represent sprouting. One would expect the sprouting terminals to ramify in the vicinity of the receptor-blocked cells, and to form contacts there. The apparent failure for this to occur implies that sites capable of receiving synapses did not exist on the receptor-blocked cells, and that the sprouting terminals did not induce their formation. In muscle, synapses occur at sites of high receptor density³³⁻³⁵, although membrane components other than the receptor are probably required for synapse formation³⁶ and recognition³⁷, as well. The proliferation of extra-junctional ACh-R that occurs in muscle after transmission block with α -BTX is significantly less than that which occurs following denervation³⁸, and is primarily related to disuse³⁸⁻⁴⁰. Since, in the present experiments, tectal cells treated with α -BTX were still driven by other inputs, there may have been relatively little stimulus of new receptor synthesis⁴¹. If synapse formation in CNS neurones requires a region of high receptor density, these results suggest that the proliferation of new ACh-R on receptor-blocked cells is slow with respect to the dynamics of the sprouting mechanism. Although the latter seems likely to involve a change in the sequence of gene activation in the ganglion cell nucleus, the expression of which will be delayed by the time required to transport materials between the cell body and its axonal terminals⁴², sprouting is known to occur rapidly in other systems in response to denervation⁴³.

It is possible that the stimulus for the apparent growth of terminals back into the toxin-treated zone occurred as a result of factors independent of the cells there (for example, crowding, non-optimum matching of molecules on pre- and postsynaptic elements, functional mismatch of information carried by displaced terminals, or a spontaneous tendency to sprout). Conversely, the question arises as to whether the re-establishment of the original pattern of terminals after toxin application is associated with the appearance of unbound receptors. The latter would be brought about by loss of toxin due to dissociation and proteolysis, and by receptor turnover, in addition to any stimulated increase in synthesis. Kouvelas and Greene⁴⁴ measured a half life of 5.1 h for α -BTX-ACh-R complex in synaptosomal fractions of chick optic tectum. A half-life of 26 h was obtained in synaptosomal fractions of toad optic tectum, using similar techniques (R. Oswald and J.A.F., in preparation). Toxin-receptor dissociation in subcellular fractions may differ significantly from that in the intact membrane, due to possible alterations of the normal molecular interactions affecting receptor conformation⁴⁵. Light autoradiographs made one week after ^3H - α -BTX application in toad tectum still showed a distinct trilaminar pattern of silver

grains (J.A.F. and W. A. Lutin, in preparation) corresponding to the distribution of retinal terminals⁴⁶. This would suggest that toxin-receptor complex is more stable *in situ* than in membrane fractions, and that its removal is slow. The turnover rate of ACh-R in brain has not yet been measured, although Lajtha *et al.*⁴⁷ have shown that 94% of membrane proteins (presumably including ACh-R) labelled with ^{14}C -tyrosine had a mean half life of 10 d. The above observations suggest that the effects of α -BTX are likely to persist for several days.

If re-ordering of retinal terminals in Fig. 6c is associated with the molecular dynamics of ACh-R, how is information about the state of surface receptors communicated to the displaced terminals? Either some terminals must remain in the toxin-treated zone allowing for direct communication, or the cells there must liberate (or fail to liberate) some factor, sensed by the displaced terminals, which affects their growth. It is possible that some trophic factor, metabolically linked to the synthesis of ACh-R, is involved in the re-ordering of synapses.

If these ideas are correct then the receptor protein might have a role in determining the postsynaptic cell's own innervation. Although the acetylcholine receptor apparently does not have a critical role in the molecular recognition or initial formation of synaptic sites, it does seem to have such a role in their maintenance, and might participate in structuring the patterns of synaptic connections.

This work was supported by NIH Career Development Award EY70240 and National Eye Institute grant EY01117. I thank W. Lutin, R. Oswald, R. Brady, J. Norden, and members of the Department of Physiology, NIMR, Mill Hill, London, for their help.

Received 10 February; accepted 8 July 1977.

- Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. & Pitts, W. H. *J. gen. Physiol.* **43**, suppl. 129-175 (1960).
- Chung, S. H., Bliss, T. V. P. & Keating, M. J. *Proc. R. Soc. B* **187**, 421-447 (1974).
- Sperry, R. W. *J. Neurophysiol.* **7**, 57-70 (1944).
- Gaze, R. M. *J. exp. Physiol.* **43**, 209-214 (1959).
- Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. & Pitts, W. H. *Science* **130**, 1709-1710 (1959).
- Gruberg, E. R. & Freeman, J. A. *M.I.T. Prog. Rep.* **116**, 266-273 (1975).
- Freeman, J. A., Lutin, W. A. & Brady, R. N. *Trans. Neurosci. Soc.* **5**, 138 (1975).
- Chang, C. C. & Lee, C. Y. *Archs int. Pharmacodyn. Ther.* **144**, 241-257 (1963).
- Changeux, J. P., Kasai, M. & Lee, C. Y. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1241-1247 (1970).
- Freeman, J. A., Lutin, W. A. & Brady, R. N. *Trans. Am. Neurochem. Soc.* **6**, 143 (1975).
- Lutin, W. A., Brady, R. N., Jensen, C. F., Skene, P. & Freeman, J. A. *Trans. Neurosci. Soc.* **5**, 630 (1975).
- Brown, K. T. & Flaming, D. G. *Brain Res.* **86**, 172-180 (1975).
- Freeman, J. A. & Stone, J. in *Neurobiology of Cerebellar Evolution and Development* (ed. Llinas, R.) 421-430 (A.M.A. Press, Chicago, 1967).
- Nicholson, C. & Freeman, J. A. *J. Neurophysiol.* **38**, 356-368 (1975).
- Freeman, J. A. & Nicholson, C. *J. Neurophysiol.* **38**, 369-382 (1975).
- George, S. A. & Marks, W. B. *Exptl Neurol.* **42**, 467-482 (1974).
- Szekely, G. in *Handbook of Sensory Physiology VII/3* (ed. Jung, R.) ch. 13 (Springer, Berlin, 1975).
- Reisfeld, R. A., Lewis, U. J. & Williams, D. E. *Nature* **195**, 281-283 (1962).
- Trachtenberg, M. C. & Ingle, D. *Brain Res.* **79**, 419-430 (1974).
- Steinbach, J. H., Harris, A. J., Patrick, J., Shubert, D. & Heinemann, S. J. *Gen. Physiol.* **62**, 255-270 (1973).
- Harris, A. J. in *Synaptic Transmission and Neuronal Interaction* (ed. Bennett, M. V. L.) 315-337 (Raven, New York, 1974).
- Jansen, J. K. S. & van Essen, D. C. *J. Physiol., Lond.* **250**, 651-667 (1975).
- Gaze, R. M. & Watson, W. E. in *Growth of the Nervous System* (eds Wolstenholme, G. E. W. & O'Connor, M.) 53-66 (Churchill, London, 1968).
- Marotte, L. R. & Mark, R. F. *Brain Res.* **19**, 41-62 (1970).
- Merrill, E. G. & Wall, P. D. *J. Physiol., Lond.* **226**, 825-846 (1972).
- Duchen, L. W. *J. Neurol. Sci.* **19**, 155-167 (1975).
- Duchen, L. W. *J. Neurol. Neurosurg. Psychiat.* **33**, 40-54 (1970).
- Duchen, L. W., Rogers, M., Stolkin, C. & Tonge, D. A. *J. Physiol., Lond.* **241**, 1P (1975).
- Lentz, T. L., Rosenthal, J. & Mazurkiewicz, J. E. *Trans. Neurosci. Soc.* **5**, 976 (1975).
- Kelly, R. S. & Brown, F. R. *J. Neurobiol.* **5**, 135-150 (1976).
- Duchen, L. W., Heilbronn, E. & Tonge, D. A. *J. Physiol., Lond.* **250**, 26-27P (1975).
- Llinas, R., Bloedel, J. R. & Roberts, W. J. *Neurophysiol.* **32**, 881-891 (1969).
- Fischbach, G. D., Berg, D. K., Colsen, S. A. & Frank, E. *Cold Spring Harb. Symp. quant. Biol.* **40**, 347-358 (1976).
- Fambrough, D. M. & Rash, J. E. *Dev. Biol.* **26**, 55-68 (1971).
- Sytkowski, A. J., Vogel, Z. & Nirenberg, M. W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 270-274 (1973).
- Grinnell, A. D., Rheuben, M. B. & Letinsky, M. S. *Nature* **265**, 368-370 (1977).
- Appel, S. H. *Exptl Neurol.* **48**, 52-74 (1975).
- Pestrouk, A., Drachman, D. B. & Griffin, J. W. *Nature* **260**, 352-353 (1976).
- Chang, C. C., Chuang, S.-T. & Huang, M. C. *J. Physiol., Lond.* **250**, 161-173 (1975).
- Lomo, T. & Westgaard, R. H. *Cold Spring Harb. Symp. quant. Biol.* **40**, 263-274 (1976).
- Roper, S., Purves, D. & McMahan, U. J. *Cold Spring Harb. Symp. quant. Biol.* **40**, 283-296 (1976).
- Grafstein, B. *Exptl Neurol.* **48**, 32-51 (1975).
- Cotman, C. W. & Lynch, G. S. in *Neuronal Recognition* (ed. Barondes, S. H.) 69-108 (Chapman and Hill, London, 1976).
- Kouvelas, E. D. & Greene, L. A. *Brain Res.* **113**, 111-126 (1976).
- Changeux, J. P. *et al. Cold Spring Harb. Symp. quant. Biol.* **40**, 211-230 (1976).
- Gruberg, E. R. & Freeman, J. A. *M.I.T. Prog. Rep.* **116**, 263-265 (1975).
- Lajtha, A., Latzkovits, L. & Toth, J. *Biochim. biophys. Acta* **425**, 511-520 (1976).

letters to nature

Observations of OE323(4C34.13), MO758+120, and OS210(4C28.40) with the 5-km telescope

FOUR extended radio sources identified with quasars of large redshift have been observed¹, they are important in defining the relationship between mean radio angular diameter and redshift and thus limiting the permissible range of q_0 and cosmological evolution of the radio source population. A difficulty with such analyses is that of allowing for the variation of the physical sizes of individual sources with time, particularly if there are different types of source present in the sample. The ideal would be to restrict the analysis to a single type with a comparatively small range of the relevant properties. We have, therefore, mapped three of the four sources—those accessible to the 5-km telescope—in order to study their structures with greater angular resolution and a better beam shape. We have also re-measured the optical positions of the associated quasars from the prints of the Palomar Sky Survey. Our results remove the positional discrepancy for MO758+120 (ref. 1) and confirm the association of an optical quasar with each of the radio sources. The suggestion that the optical and radio positions of MO758+120 (ref. 3) had been accidentally interchanged seems to be

incorrect, since our optical position agrees with that given by Baldwin *et al.*³ to within 0.25 arc s.

Our observing frequency was 2.7 GHz, as used by Gearhart and Pacht¹, and the beam size (FWHP) $3.7 \times 3.7 \cos \delta$ arc s; in one case, OS210, the source had previously been mapped at 5 GHz (ref. 2) with a beam size 2×4.1 arc s.

Figure 1 shows the 2.7-GHz maps in which the declination scales have been compressed so that the beams seem circular; one can then more easily detect the presence of marginally resolved components. The peak positions and flux densities of the components are given in Table 1. In the case of OS210 no central nuclear component was found, and our observations at 5.0 and 2.7 GHz may be combined with measurements of the total flux density at other frequencies to obtain a mean spectral index for the two outer components. The other two sources are found to be triples, in one of which the nucleus provides most of the flux at 2.7 GHz. To estimate the spectra of the outer components of each, an attempt was made to correct the measurements of total flux density, which are available over a wide range of frequency, by subtracting the flux density attributable to the 2.7-GHz nucleus on the assumption that its spectrum is flat. Details for the individual sources follow.

A weak central radio component lies within about 1 arc s of the optical quasar OE323, confirming the identification. The

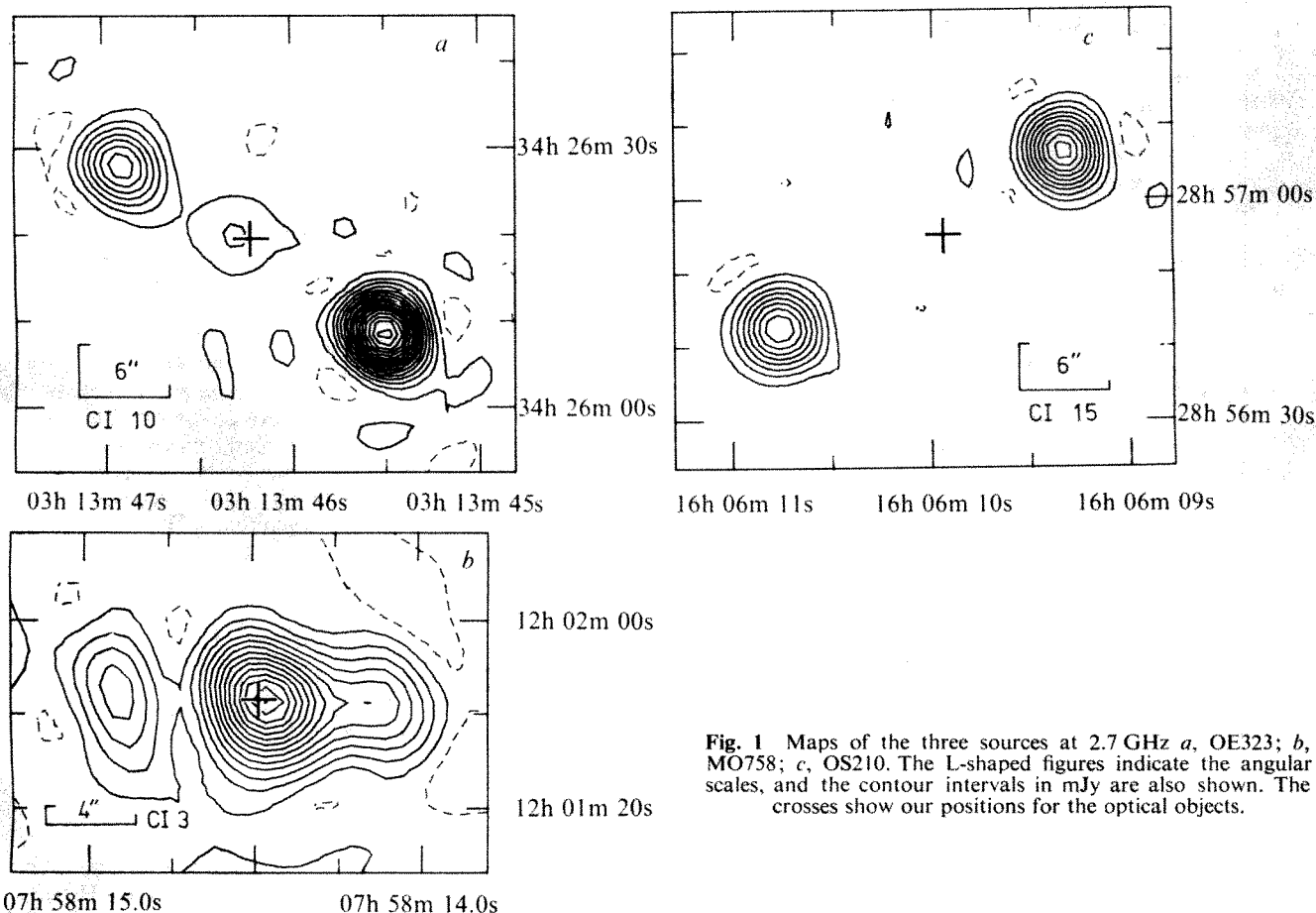


Fig. 1 Maps of the three sources at 2.7 GHz a, OE323; b, MO758; c, OS210. The L-shaped figures indicate the angular scales, and the contour intervals in mJy are also shown. The crosses show our positions for the optical objects.

Table 1 Peak positions and flux densities

Table 1. Position and flux densities of the sources											
Source	Component	α (1950.0)				δ (1950.0)				$S_{2.7}(\text{mJy})$	\pm
		h	m	s	\pm s	°	'	"	\pm''		
OE323	Nf	03	13	46.92	0.02	34	26	27.8	0.3	76	15
	nucleus	—	—	46.31	0.04	—	—	19.7	0.6	14	5
	optical	—	—	46.22	0.07	—	—	19.5	0.7	—	—
	Sp	—	—	45.50	0.02	—	—	08.4	0.3	192	20
MO758+120	Nf	07	58	14.92	0.03	12	01	44.2	0.6	11	5
	nucleus	—	—	14.46	0.02	—	—	42.5	0.3	39	15
	optical	—	—	14.48	0.05	—	—	43.1	0.5	—	—
	Sp	—	—	14.16	0.03	—	—	42.5	0.6	16	5
OS210	Np	16	06	09.34	0.02	28	57	06.9	0.4	166	20
	optical	—	—	09.95	0.06	—	56	55.1	0.7	—	—
	Sf	—	—	10.77	0.02	—	—	42.2	0.5	154	25

Table 2 Physical extent, total intrinsic luminosity and the relative luminosity of the nucleus to the total

Source	z	θ (arc s)	$S_{2.7}$ (mJy)	D (kpc)	$\Omega = 0$		$\Omega = 1$		$\frac{P_N}{P_{\text{Total}}}$	α_{outer}
					$(10^{26} \text{ W Hz}^{-1} \text{ sr}^{-1})$	P_5 ($10^{26} \text{ W Hz}^{-1} \text{ sr}^{-1}$)	D (kpc)	P_5 ($10^{26} \text{ W Hz}^{-1} \text{ sr}^{-1}$)		
OE323	1.156	26.2	282	299	1.8	225	1.0	0.04	0.96	
MO758+120	2.66	11.3	66	152	4.1	86	1.3	0.34	1.43	
OS210	1.989	31.1	320	401	9.0	255	3.7	<0.01	0.92	

spectral index of the unresolved outer components over the range 178 to 2,695 MHz is $\alpha = 0.96 \pm 0.05$ (defined by $S \propto \nu^{-\alpha}$).

The source MO758+120 is a well-defined triple with a very powerful central component; our optical position for the quasar lies within about 0.7 arc s of the central component. Gearhart and Pacht's observation did not resolve the source, and indicated a positional difference of some 16 arc s between the radio centroid and the optical identification. The spectrum of the outer components can be derived from our 2.7-GHz results and low-frequency observations for which the correction for the nucleus is small. Over the range 38–2,695 MHz the spectrum is very steep with $\alpha = 1.43 \pm 0.02$.

OS210 is a double with unresolved components, our optical position lying within 0.8 arc s of the line joining the components. The earlier 5-GHz map shows both components to have angular sizes less than 2 arc s. Over the range 38–2,695 MHz, $\alpha = 0.94 \pm 0.02$.

In order to compare the physical characteristics of these sources, the physical extent, the total intrinsic luminosity (P) and the relative luminosity of the nucleus to the total for each were computed for the Einstein–de Sitter ($\Omega = 1$), and Milne ($\Omega = 0$) models (Table 2). To reduce the uncertainty arising from the spectrum of the nucleus, these values are extrapolated to 5 GHz, which corresponds more closely to the mean frequency of emission than to that of observation of 2.7 GHz.

It can be seen that even amongst these three sources there is a wide spread in projected diameter, luminosity and morphological type and, in attempting to distinguish between different cosmological models (including the type of source evolution involved), it will be necessary to take account of more source parameters than have been considered hitherto. The results emphasise the importance of high resolution and high sensitivity in the study of these problems.

We thank the director of the Institute of Astronomy for the use of the x-y measuring machine. M.A.C.P. thanks the SRC for a maintenance award.

M. A. C. PERRYMAN

M. RYLE

Cavendish Laboratory,
Madingley Road,
Cambridge, UK

Received 13 July; accepted 2 August 1977.

¹ Gearhart, M. R. & Pacht, E. *Nature* **266**, 819–822 (1977).

² Riley, J. M. & Pooley, G. G. *Mem. R. astr. Soc.* **80**, 105–137 (1975).

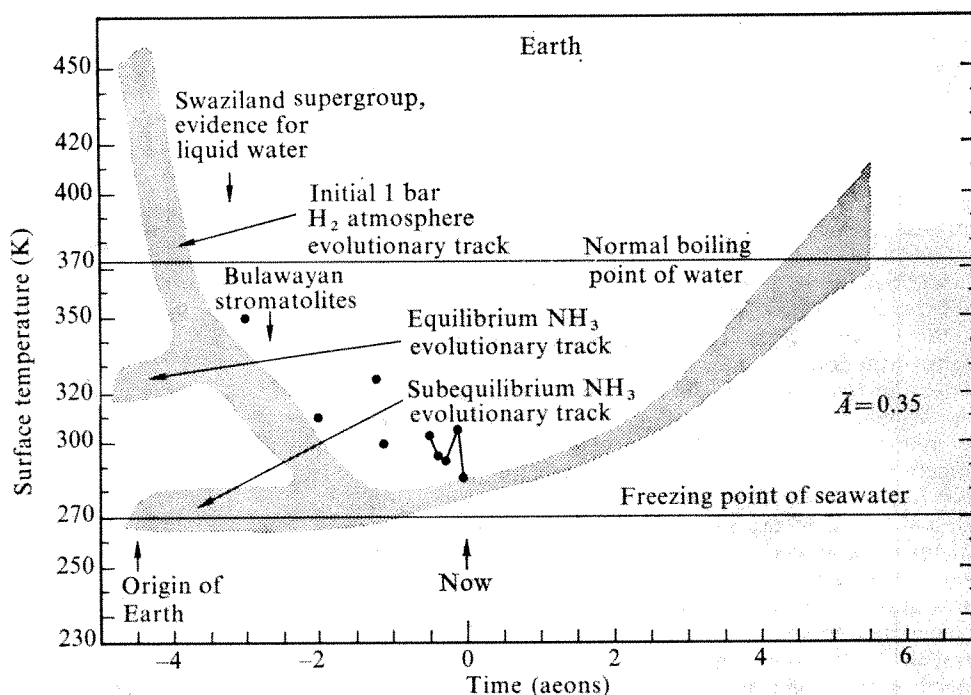
³ Baldwin, J. A. *et al. Astrophys. J.* **206**, L83–L86 (1976).

Reducing greenhouses and the temperature history of Earth and Mars

THE modern theory of stellar evolution implies that the Sun has increased in brightness by several tens of per cent over geological time. Were all other global parameters held constant, this would imply that the mean temperature of the Earth was below the freezing point of seawater about 2×10^9 yr ago¹. There is, however, excellent geological and palaeontological evidence that there were extensive bodies of liquid water on the Earth between 3 and 4×10^9 yr ago. A possible solution to this puzzle, first postulated by Sagan and Mullen¹, is that the Earth's primitive atmosphere contained small quantities of NH_3 and other reducing gases which significantly enhanced the global greenhouse effect. NH_3 is especially effective in the 8–13 μm window in a CO_2 – H_2O atmosphere. It was argued that plausible changes in other global parameters, such as the infrared emissivity or the Russell–Bond albedo, would have been ineffective. The increase in solar brightness over geological time seems model-invariant even under extreme model perturbation for the solar interior¹, as has recently been reconsidered in a straightforward stellar structure scaling argument². Cosmochemical considerations point strongly to a higher abundance of reduced constituents in the primitive than in the contemporary terrestrial atmosphere; and reduced atmospheric components such as NH_3 and CH_4 are required to understand the accumulation of prebiological organic compounds necessary for the origin of life in this same period, between 3 and 4×10^9 yr ago³.

The calculations of Sagan and Mullen used a very simple square-wave opacity model, and radiative equilibrium. The model predicts the correct value of the present terrestrial global temperature (about 288 K). Calculations were carried out for three primitive atmospheres, as shown in Fig. 1: (1) a high-density hydrogen-rich primitive atmosphere where the opacity was provided primarily by pressure-induced dipole and forbidden quadrupole transitions of H_2 ; (2) a hydrogen-poor, but still reducing, primitive atmosphere in

Fig. 1 Evolutionary tracks showing the time evolution of global surface temperatures of the Earth for three different atmospheric chemistries¹. The arrows for Swaziland and Bulawayan rocks indicate two of several lines of evidence for extensive liquid water in the early history of the Earth. The points show recent measurements of surface temperatures from isotopic thermometric techniques⁴. The bolometric Russell-Bond albedo is taken as 0.35. Time is measured in aeons (1 aeon = 10^9 yr).



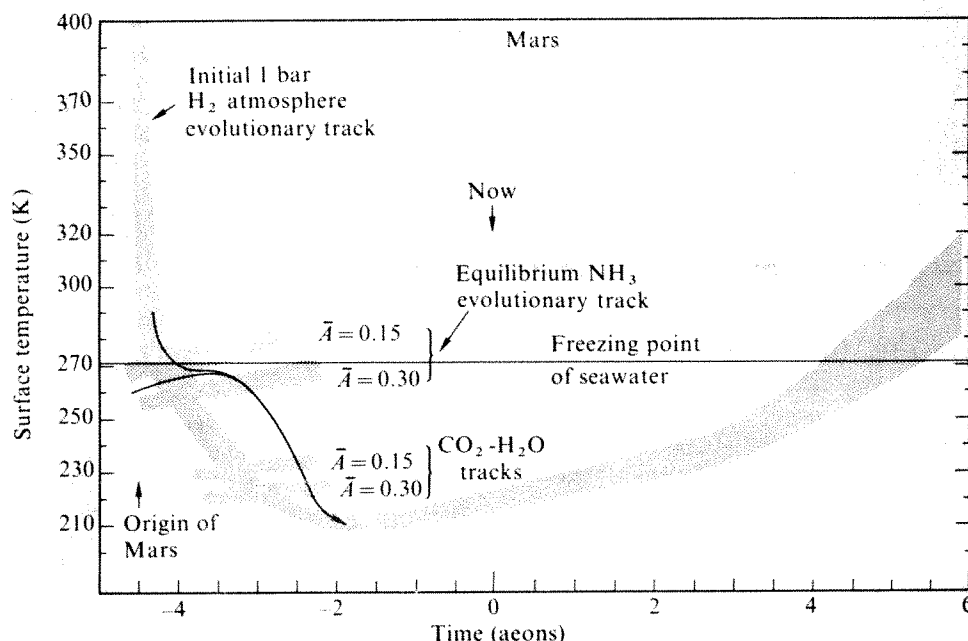
which the opacity was provided primarily by the chemical equilibrium abundance of ammonia ($\sim 10^{-4.5}$ volume mixing ratio); and (3) a hydrogen-depleted atmosphere in which the ammonia abundance was subequilibrium, due primarily to the photodissociation of NH_3 . The time dependence of these opacities was determined crudely, allowing for Jeans escape of hydrogen from the exosphere and the subsequent oxidation of reducing compounds, with a characteristic period $\sim 3 \times 10^8$ yr.

Knauth and Epstein⁴ have obtained, through $\text{D}/^{18}\text{O}$ isotopic archeothermometry, data on mean global temperatures from the present back to about 3×10^9 yr ago. These points are also shown in Fig. 1 and roughly parallel the high opacity evolutionary tracks (either 1 bar H_2 and equilibrium NH_3 , or equilibrium NH_3 alone), and provide some support for the idea of a substantial primitive greenhouse effect described in (1) above. Since the observed temperatures seem a little higher than the tracks calculated, it seems useful to point out that octopole transitions of methane, neglected in (1), have been shown by Pollack³ to

be significant for the greenhouse of Titan. If a primitive terrestrial atmosphere with total pressure ~ 1 bar had more than ~ 0.01 CH_4 , the CH_4 opacity would have made an important contribution to the greenhouse effect. Even if the NH_3 opacity were overestimated in (1), CH_4 and NH_3 together as minor constituents in the primitive terrestrial atmosphere seem capable of explaining in a very natural way what otherwise would be a serious discrepancy between astrophysical theory and geological observation.

The same computational protocol has been applied to the evolution of surface temperature on the planet Mars. Results are shown in Fig. 2, for two possible bolometric Russell-Bond albedos of Mars, 0.15 and 0.30, and for three conceivable early compositions: (1) a 1 bar H_2 atmosphere; (2) a 1 bar H_2 -depleted early atmosphere with NH_3 as a minor constituent; and (3) a low-pressure early atmosphere, comparable to the present martian atmosphere, in which CO_2 and small quantities of water vapour provide the principal sources of infrared opacity. Three separate Viking instruments have determined the abundances of trace

Fig. 2 Evolutionary tracks for the time dependence of surface temperature for Mars for three early compositions and two different bolometric Russell-Bond albedos.



isotopic constituents in the contemporary martian atmosphere, and have been interpreted, by a set of independent arguments, to indicate a possible higher pressure atmosphere in early martian history⁶⁻⁸. The most frequently cited estimates of total pressure are about 0.1 bar, but estimates range as high as 1 bar. Because of the low exosphere temperature, H₂ does not escape from Mars today significantly faster than it does from Earth, despite the larger gravitational acceleration of the latter.

The results indicate that if primitive Mars, like primitive Earth, had even a mildly reducing atmosphere, martian global temperatures a few times 10⁹ yr ago may have been in the vicinity of the freezing point of seawater and, in many latitudes, the mean temperatures may have been above the freezing point. This result is of possible interest for the martian sinuous channels, many of which seem to be produced by aqueous fluvial erosion and which have been crudely dated by cratering statistics techniques as having been formed 1–4 × 10⁹ yr ago⁹⁻¹⁵. Although the Viking imaging and microbiology experiments have so far yielded no unambiguous evidence for martian biology¹⁶⁻²⁰, the implication of this work—that in earlier martian history, temperatures and pressures may have been substantially higher in a reducing atmosphere and extensive bodies of liquid water present—certainly enhances significantly the *a priori* likelihood of the origin of life on early Mars. Of course, even if life arose, there is no guarantee that it survived subsequent inclemencies of martian history. Our results also imply, because of the increasing solar luminosity, a very clement martian epoch a few times 10⁹ yr in the future (Fig. 2), when conditions on Earth will become—due to the inexorable nature of stellar evolution—less than ideal (Fig. 1).

This research was supported by the NSF and the NASA. I thank Samuel Epstein and James Pollack for useful discussions, and George Mullen for assistance with the calculations.

CARL SAGAN

Laboratory for Planetary Studies,
Cornell University,
Ithaca, New York 14853

Received 28 March; accepted 13 July 1977.

- 1 Sagan, C. & Mullen, G. *Science* **177**, 52–56 (1972).
- 2 Newman, M. J. & Rood, R. T. *Nature* (in the press).
- 3 Sagan, C. *Origins of Life* **5**, 497–505 (1974).
- 4 Knauth, L. T. & Epstein, S. *Geochim. cosmochim. Acta* **40**, 1095–1108 (1976).
- 5 Pollack, J. B. *Icarus* **19**, 195–201 (1973).
- 6 McElroy, M. B., Yung, Y. L. & Nier, A. O. *Science* **194**, 70–72 (1976).
- 7 Nier, A. O., McElroy, M. B. & Yung, Y. L. *Science* **194**, 68–70 (1976).
- 8 Biemann, K., Owen, T., Rushneck, D., La Fleur, A. & Howarth, D. W. *Science* **194**, 76–78 (1976).
- 9 Sagan, C., Toon, O. B. & Gierasch, P. J. *Science* **181**, 1045–1049 (1973).
- 10 Milton, D. J. *J. geophys. Res.* **78**, 4009–4030 (1973).
- 11 Baker, V. R. & Milton, D. J. *Icarus* **23**, 27–41 (1974).
- 12 Milton, D. J. *Science* **183**, 654–656 (1974).
- 13 Sharp, R. P. & Malin, M. C. *Geol. Soc. Am. Bull.* **86**, 593–609 (1975).
- 14 Malin, M. C. *J. geophys. Res.* **81**, 4825–4845 (1976).
- 15 Pieri, D. *Icarus* **27**, 25–49 (1976).
- 16 Mutch, T. A. *et al. Science* **193**, 791–801 (1976).
- 17 Levinthal, E. C. *et al. J. geophys. Res.* (in the press).
- 18 Klein, H. P. *et al. Science* **194**, 99–105 (1976).
- 19 Horowitz, N. H., Hobby, G. L. & Hubbard, J. S. *Science* **194**, 1321–1322 (1976).
- 20 Levin, G. V. & Straat, P. A. *Science* **194**, 1322–1328 (1976).

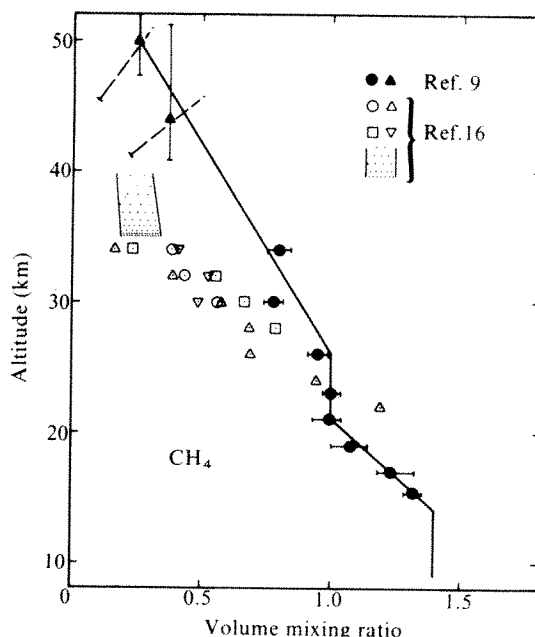
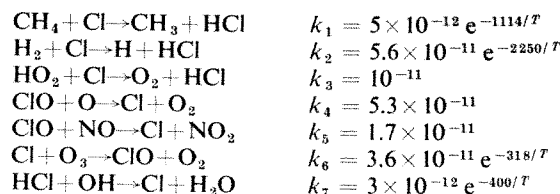


Fig. 1 Vertical distribution of the volume mixing ratio of methane in parts per million. The *in situ* sampling results⁹ are shown with the values determined¹⁶ by infrared spectrometry of the P₃ to P₈ multiplets of the 3.3-μm band. The solid curve represents the values used in model calculations⁴. The envelope above 35 km corresponds the methane absorptions at Zenith angle, χ , smaller than 90°.

namely HCl (ref. 5) and ClO (ref. 6). The ratio of their abundances can be expressed by the relation³

$$n\text{HCl}/n\text{ClO} = (k_1 n\text{CH}_4 + k_2 n\text{H}_2 + k_3 n\text{HO}_2) \times (k_4 n\text{O} + k_5 n\text{NO}) / (k_6 n\text{O}_3 + k_7 n\text{OH}) \quad (1)$$

where nA represents the number density of species A and values of k expressed below in cm³ mol⁻¹ s⁻¹, represent the rate constants⁷ of the reactions



The H₂O₂ + Cl → HCl + HO₂ reaction has been neglected due to its low rate constant⁸.

All species in very low stratospheric concentration (except HO₂ which has a minor role) intervening in relationship (1) have been measured—H₂ (ref. 9), NO (refs 10, 11), O (refs 12, 13) and OH (ref. 14). The abundance of ozone is known¹⁵ and most studies have relied on CH₄ data obtained in the stratosphere by *in situ* sampling⁹.

In the altitude range 32–36 km where data are available for computation the $n\text{HCl}/n\text{ClO}$ ratio is observed to be about 0.8 while values ranging from 4.5 to 3.5 result from model calculations⁴.

We have performed a new determination¹⁶ of CH₄ using infrared absorption spectrometry from a balloon platform to give methane mixing ratios over 25 km lower than currently used in stratospheric models. The results, shown in Fig. 1, were derived from spectra of the 3.3-μm CH₄ band obtained simultaneously with HCl absorption spectra¹⁷. They have been used in conjunction with all other measurements to evaluate the $n\text{HCl}/n\text{ClO}$ ratio from equation (1) in order to deduce the effect of these new data on the chlorine–ozone cycle. The

Stratospheric CH₄, HCl and ClO and the chlorine–ozone cycle

SINCE it was suggested¹ that chlorine may influence stratospheric ozone balance, and that halocarbons can contribute significantly to the stratospheric chlorine content²⁻⁴ much attention has been paid to the subject. We report here a new determination of atmospheric methane levels which gives a value lower than that often assumed by other workers. In model calculations this suggests that chlorine has a greater effect on the ozone balance than previously suspected.

In addition to atomic chlorine two main species of the chlorine–ozone cycle have been measured in the stratosphere—

Table 1 Data used in the evaluation of $n\text{HCl}/n\text{ClO}$ and results

	Altitude (km)		
	32	34	36
$n\text{O}_3$ (ref. 15)	2.5×10^{12}	1.9×10^{12}	1.4×10^{12}
$n\text{CH}_4$ (ref. 16)	1.2×10^{11}	7.0×10^{10}	4.2×10^{10}
$n\text{H}_2$ (ref. 9)	2.0×10^{11}	1.4×10^{11}	1.0×10^{11}
$n\text{HO}_2$ (ref. 3)	2.2×10^7	1.9×10^7	1.5×10^7
$n\text{OH}$ (ref. 4)	1.1×10^7	1.1×10^7	1.5×10^7
$n\text{O}$ (ref. 13)	1.3×10^8	2.6×10^8	3.9×10^8
$n\text{NO}$ (ref. 11)	1.3×10^9	1.1×10^9	7.0×10^8
$T(\text{K})$ (ref. 18)	228	234	239
$n\text{HCl}$ (observed) ¹⁷	4.2×10^8	3.0×10^8	2.4×10^8
$n\text{ClO}$ (observed) ⁶	5×10^8	4×10^8	3×10^8
$n\text{HCl}/n\text{ClO}$ (equation (1))	1.2	1.2	0.8
$n\text{HCl}/n\text{ClO}$ (observed)	0.84	0.75	0.8
$n\text{HCl}/n\text{ClO}$ (model) ⁴	4.5	3.9	3.5

number densities used in the computation are listed in Table 1 with the results. The computed values of the $n\text{HCl}/n\text{ClO}$ ratio seem to be in good agreement with observed values while the model calculated ratios are, especially at the lowest altitude, in marked disagreement. Part of this disagreement is due to the use in models of high methane concentrations which have already been considered as being surprisingly large by Wofsy and McElroy¹⁹. A similar opinion has been expressed by Rowland and Molina³ who have considered the possibility of lower methane concentrations leading to an enhancement by a factor of 2 or 3 of the chlorine atom chain.

Thus introduction of high methane concentrations⁹ into model calculations⁴ has lead to a computed $n\text{HCl}/n\text{ClO}$ ratio, for the case considered here, 2.3 times higher than that obtained when using the lower level of methane reported here.

M. ACKERMAN

D. FRIMOUT

C. MULLER

*Institut d'Aeronomie Spatiale
de Belgique,
3, avenue Circulaire,
B-1180—Brussels, Belgium*

Received 16 June; accepted 12 July 1977.

- Stolarski, R. S. & Cicerone, R. J. *Can. J. Chem.* **52**, 1610–1615 (1974).
- Molina, M. J. & Rowland, F. S. *Nature* **249**, 810–812 (1974).
- Rowland, F. S. & Molina, M. J. *Rev. Geophys. Sp. Phys.* **13**, 1–35 (1975).
- Halocarbons: Effects on Stratospheric Ozone* (National Academy of Sciences, Washington, DC, 1976).
- Eyre, J. R. & Roscoe, H. K. *Nature* **266**, 243–244 (1977).
- Anderson, J. G. *NASA Conference on the Stratosphere and Related Problems*, Logan, Utah (September, 1976).
- Nicolet, M. *Rev. Geophys. Sp. Phys.* **13**, 593–636 (1975).
- Hampson, R. F. & Garvin, D. *Chemical Kinetic and Photochemical Data for Modelling Atmospheric Chemistry*, Addenda of June 1975, NBS Technical Note 866.
- Ehhalt, D. H., Heidt, L. E., Lueb, R. H. & Pollack, W. *Pageoph* **113**, 389–402 (1975).
- Ackerman, M. *et al. Nature* **245**, 205–206 (1973).
- Ackerman, M. *J. Atmos. Sci.* **32**, 1649–1657 (1975).
- Anderson, J. G. *Geophys. Res. Lett.* **2**, 231–234 (1975).
- Anderson, J. G. in *Halocarbons: Effects on Stratospheric Ozone* (National Academy of Sciences, Washington, DC, 1976).
- Anderson, J. G. *Geophys. Res. Lett.* **3**, 165–168 (1976).
- Krueger, A. J. *Pageoph* **106–108**, 1272–1280 (1973).
- Ackerman, M., Frimout, D. & Muller, C. *Aeronomica Acta* **180** (1977).
- Ackerman, M., Frimout, D., Girard, A., Gottignies, M. & Muller, C. *Geophys. Res. Lett.* **3**, 13–16 (1976).
- US Standard Atmosphere Suppl.* (US Government Printing Office, Washington, DC, 1966).
- Wofsy, S. C. & McElroy, M. B. *J. geophys. Res.* **78**, 2619–2624 (1973).

Defects in natural type IB diamond

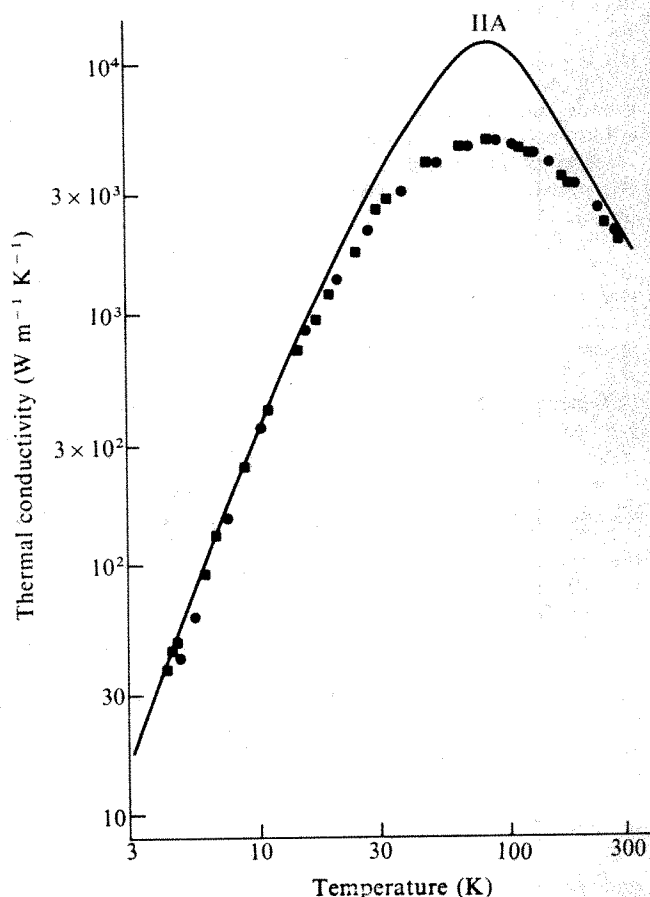
NATURAL type IB diamond has an anomalously low thermal conductivity. To investigate this we have carried out birefringence, infrared and ultraviolet absorption spectroscopy, electron spin resonance and transmission electron

microscopy (TEM) experiments. We suggest here that the low IB thermal conductivity is due to the presence of previously unknown defects in this type of diamond. It is also suggested that these defects are related to the more common 'platelets' or planar faults which are found in type IA diamond.

The thermal conductivity of IB diamond (specimens C8 and C9 in Fig. 1) was first measured in 1972¹ and subsequent experiments^{2,3} have produced similar results. It was found that the strength of the phonon scattering mechanisms which significantly reduced the IB conductivity as compared to purer IIA diamond, could not be explained solely by the presence of paramagnetic singly substitutional nitrogen atoms^{1,4}. This has now been confirmed to be the case for six IB specimens. A satisfactory theoretical explanation of the original measurements on the basis of Rayleigh-type phonon scattering, could not be found⁴ although the most recent results can be explained if the non-paramagnetic nitrogen (which in this case was more highly concentrated than is usual for type IB) was assumed to be in clusters of several atoms⁵.

We chose for our investigations a transparent amber coloured African diamond (no. 2) from a batch provided by the Diamond Research Laboratory on the basis of extremely low birefringence. Electron spin resonance experiments were performed on this specimen using a Varian E-line spectrometer and a characteristic IB response was observed. Singly substitutional nitrogen atoms are responsible for this behaviour⁵. No attempt was made to calculate the spin density rigorously but it was estimated to be $\sim 10^{23} \text{ m}^{-3}$. Infrared and ultraviolet absorption spectroscopy confirmed that the specimen was a typical IB

Fig. 1 The thermal conductivity of two type IB diamonds, ■, C8 and ●, C9. It is impossible to compute the experimental IB thermal conductivity solely by the addition of a phonon point defect scattering term to that used to compute IIA conductivities.



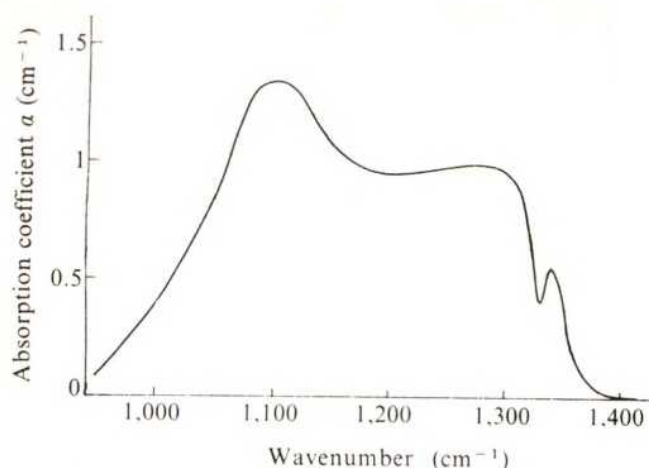


Fig. 2 The infrared absorption spectrum of diamond no. 2.

diamond⁶ with an average nitrogen concentration of $\approx 6 \times 10^{24} \text{ m}^{-3}$ depending on what fraction of the $1,280 \text{ cm}^{-1}$ absorption was due to A and B centres (Fig. 2). A thin (100) slice was cut and ground from this diamond and studied further by infrared spectroscopy. It possessed a similar concentration of nitrogen to that of the original cube. A selected area of very low birefringence was thinned by ion beam bombardment⁷ to produce a minute perforation, the edges of which were investigated by TEM. This particular diamond thinned perfectly and particularly quickly ($\sim 3 \mu\text{m h}^{-1}$) thereby creating a larger thickness gradient than usual at the edges of the perforation. The large thickness gradient and perfect surface were responsible for the observation of strong 'thickness' fringes by TEM. Small defects showing as black and white dots were seen near the edges of the dark thickness fringes in a bright-field micrograph (Fig. 3). The white dots were seen on the thin side of the dark fringes, the dark dots were seen on the thin side of the light fringes and no dots were seen near the centre of the fringes. In thicker parts of the foil the effective contrast of the dots was reduced because of increased absorption in the crystal. These observations fit the description of the common type of structure factor contrast usually observed when a small inclusion has a lower structure factor than the matrix⁸. It is debatable whether the defects would be detected in the absence of strong thickness fringes. The strain field of the defect seemed spherical although ill-defined under high resolution (0.2 nm point to point) TEM and typically $\sim 5 \text{ nm}$ in size. They are presumably quite homogeneously distributed in the areas which have been observed by TEM. On this assumption, their concentration was $\sim 2 \times 10^{21} \text{ m}^{-3}$ which is a typical 'platelet' concentration in normal IA material. To ensure that the defects were not artefacts the specimen was re-thinned weakly to remove $\sim 10 \text{ nm}$ of the sample surface and further TEM observations were made. This did not alter the appearance of the defects and we feel, therefore, in the light of previous experience of thinning diamond by ion bombardment, that the defects are real. To ensure that the small diamond slice was IB material a further ESR experiment was performed on the slice alone. A characteristic spectrum was observed which indicated that the slice possessed a similar spin concentration to the original cube.

Further detailed investigation by TEM showed that a small number of these minute defects possessed an appearance characteristic of platelets in IA diamond (Fig. 4). The defects were too small and ill-defined for a definite plane to be assigned to them but $\{100\}$ is consistent with the data. It is suggested that these are minute platelets and that the common defect shown in Fig. 3 is the genesis of such platelets. It is unlikely that the minute platelets are

the genesis of these 'bubbles'. If platelets are subjected to high temperatures and pressures, they form larger structures which have some similarities to giant platelets^{7,9}. Other defects seemed to be at an intermediate stage of development.

It is not possible to determine the exact nature of these defects but voids, bubbles of gas or liquid, amorphous material or crystalline material of a low structure factor are all consistent with the results. It is also possible that the defect could be considerably smaller than 5 nm if a strong strain field is present. Some evidence suggests that hydrogen might be present in the bubbles for, of all the diamonds investigated by ion beam spectrochemical analysis, this IB specimen gave the highest hydrogen content¹⁰.

Presumably if the specimen were subject to an appropriate high temperature and pressure environment the bubbles would transform into minute platelets which could then grow up to $\sim 80 \mu\text{m}$ in length^{7,9,11,12}. It should be noted that the common platelets also have a lower effective scattering factor than the diamond matrix¹³.

These defects or bubbles will act as phonon scattering centres and in an interesting fashion. The dominant phonon wavelength in diamond is equal to the bubble size at a temperature $\sim 100 \text{ K}$. Thus for $T \ll 100 \text{ K}$ one would expect a phonon ω^4 -scattering dependence (Rayleigh scattering) to be most important (where ω is the phonon angular frequency) while for $T \gg 100 \text{ K}$ frequency independent scattering would be prevalent. In the intermediate range ($T \sim 100 \text{ K}$) a complex oscillatory scattering mechanism will be most relevant^{14,15}. Therefore, one would not expect the thermal conductivity of specimens C8 and C9 (Fig. 1) to be explained solely by the addition of ω^4 -Rayleigh scattering if they also contained similar defects to those described here. The magnitude of the phonon scattering associated with these particular defects would be sufficiently high to explain the data on C8 and C9. Generally, if the 'bubble' defect size in IB diamond were smaller ($\leq 2 \text{ nm}$) such that Rayleigh-type scattering could adequately describe the phonon defect scattering at all temperatures for which data were available (that is, $T < 300 \text{ K}$), this contribution would be confused with the

Fig. 3 Small defects in a IB diamond showing structure factor contrast. The edge of the specimen is labelled E. Bright-field micrograph. Scale bar $0.1 \mu\text{m}$.





Fig. 4 Small defects in a IB diamond (as used in Fig. 3). Some of these defects appear as minute platelets (P) surrounded by characteristic strain fields. Larger platelets are common in type IA diamond. Scale bar 0.1 μm .

nitrogen cluster scattering mechanism which is identical in its phonon scattering frequency dependence.

We conclude that the thermal conductivity of our sample would be similar to the conductivity of diamonds C8 and C9, and suggest that smaller defects of the same nature might have existed in the other four type IB diamonds for which the thermal conductivity has been measured^{2,3}. It could be that such defects are a general feature of IB diamonds, in which case it would seem that IB diamond is a preliminary state of IA diamond. Synthetic diamond must be the most preliminary state, and a IB diamond will be fabricated if nitrogen is introduced, whereas it has not been possible to synthesise IA diamond¹⁶. If the bubbles contained nitrogen, this would account for the generally higher nitrogen concentration (as detected by infrared) found in IA samples.

P. R. W. HUDSON

P. P. PHAKEY

Physics Department,
Monash University,
Clayton, Victoria 3168, Australia

Received 21 December, 1976; accepted 28 July, 1977.

- ¹ Hudson, P. R. W. *The thermal conductivity of various types of diamond paper presented at Diamond Conf.*, Bristol (1972).
- ² Berman, R., Hudson, P. R. W. & Martinez, M. J. *Phys. C. Solid St. Phys.* **8**, L430 (1975).
- ³ Berman, R. & Martinez, M. *Diam. Res.* **7**, 13 (1976).
- ⁴ Hudson, P. R. W. thesis, Univ. College, Oxford (1972).
- ⁵ Smith, W. V., Sorokin, P. P., Gelles, I. L. & Lasher, G. J. *Phys. Rev.* **115**, 1546 (1959).
- ⁶ Dyer, H. B., Raal, F. H., Du Preez, L. & Loubser, J. H. N. *Phil. Mag.* **11**, 763 (1965).
- ⁷ Hudson, P. R. W. & Phakey, P. P. *Phys. stat. solid* **a36**, 627 (1976).
- ⁸ Ashby, M. F. & Brown, L. M. *Phil. Mag.* **8**, 1649 (1963).
- ⁹ Evans, T. & Rainey, P. *Proc. R. Soc. A344*, 111 (1975).
- ¹⁰ Hudson, P. R. W. & Tsong, I. S. T. *J. mater. Sci.* (in the press).
- ¹¹ Woods, G. S. *Phil. Mag.* **34**, 993-1012 (1976).
- ¹² Mendelsohn, M. J. thesis, Univ. London (1971).
- ¹³ Bursill, L. A., Barry, J. C., Hudson, P. R. W. (to be published).
- ¹⁴ Schwartz, J. W. & Walker, C. T. *Phys. Rev.* **155**, 969 (1967).
- ¹⁵ Hudson, P. R. W. *Phys. stat. Solid* **a37**, 645 (1976).
- ¹⁶ Woods, G. S. *Diam. Res.* **25-27** (1973).

Correlation between dielectric constant and defect structure of non-stoichiometric solids

To understand the properties and behaviour of inorganic non-stoichiometric compounds it is vital to know how the stoichiometric imbalance in the material is accommodated structurally. It has been usual to assume that point defect populations have been solely responsible for such departures from the normal stoichiometric formula. But, recent structural investigations have shown that for a growing number of materials this does not seem to be true and instead changes in the anion-cation ratio are accommodated by planar faults. This report points out that an empirical correlation seems to exist between the magnitude of the low frequency dielectric constant of a compound and the structural mode by which it accommodates its non-stoichiometry. Those materials with a low dielectric constant are found to prefer point defect populations, while those with a high dielectric constant prefer planar faults. Some reasons why this correlation should hold are suggested.

Recent studies have mainly led to a recognition of two broad groupings of inorganic non-stoichiometric compounds. First, there are those materials which accommodate stoichiometric changes by way of point defects, either in the classical sense or, in materials with relatively large departures from stoichiometry, by point defect clusters or by ordered arrays of point defects. Examples of this are: Fe_{1-x}O (wüstite), many compounds with the fluorite or fluorite-related structures and the metallic oxides $\text{TiO}_{1 \pm n}$ and $\text{VO}_{1 \pm n}$ (ref. 1).

The second grouping consists of materials in which any stoichiometric variation seems to be taken up by planar faults or intergrowths. In these materials the point defect contribution to the stoichiometric imbalance seems minimal. Instead the structure of the non-stoichiometric crystal can be regarded geometrically as an intergrowth between two phases which have compatible structures, or else as a parent structure which has collapsed along a particular crystallographic plane so as to eliminate one atom type rather than another from the parent matrix. The intergrowth or fault usually takes the form of a lamella only one or two cation-anion polyhedra thick and hence is related to, but differs in scale, from syntaxy.

As these materials are less well known, some examples will illustrate their behaviour and the range of compounds covered. Perhaps the best documented are the crystallographic shear (CS) phases. These are formed when WO_3 , Nb_2O_5 , TiO_2 and other materials are reduced or doped with (generally) lower valance cations. Characteristically they are slabs of the parent higher oxide containing fault planes, the CS planes, which geometrically are planes along which the structure has collapsed to eliminate oxygen. If these faults are ordered, a homologous series of oxides is formed; typical examples being $\text{W}_n\text{O}_{3n-2}$, $\text{Ti}_n\text{O}_{2n-1}$. More examples of CS phases are given in several reviews (refs 1-3 and refs therein.) A second group consists of oxides often described as intergrowths. In these phases, stoichiometric variation is achieved by the ordered or disordered intergrowth of phases with different compositions. Examples are the perovskite-related oxides formed by intergrowth between NaNbO_3 and complex silicate-related phases $\text{Ba}_3\text{Nb}_2\text{Si}_4\text{O}_{26}$ and $\text{Ba}_3\text{Nb}_2\text{Ti}_4\text{O}_{21}$ (ref. 5). Ordered intergrowths can again be expressed in terms of homologous series formulae. Finally, some of the materials described as polytypes can also be placed into this category. Of particular note are the hexagonal ferrites⁶ where structural complexity is associated with intergrowth between units of the spinel structure. The titanium sulphides with compositions lying near to Ti_2S_3

Table 1 Dielectric constant and defect structure type of some non-stoichiometric compounds

Material	Dielectric constant*	Frequency (Hz)	Non-stoichiometry†
FeO	14.2	2×10^6	P.D.
NiO	11.9	10^9	P.D.
CaO.ZrO ₂	~ 12.0	2×10^6	P.D.
CaF ₂	6.8	10^3	P.D.
UO ₂	24	3×10^5	P.D.
ThO ₂	18.9	3×10^5	P.D.
CeO ₂	7.0	2×10^6	P.D.
V ₂ O ₅	~ 15 (11)	~ 10^6	P.D. (15)
WO ₃	~ 10^3 (12)	~ 10^6	CS
TiO ₂	$\epsilon_c = 167$ (13)	~ 10^6	CS
H-Nb ₂ O ₅	~ 200 (14)	~ 10^6	CS
SrTiO ₃	~ 350	10^3	I (16)
PbS	~ 200	i.r.	I ₊
Bi ₃ Ti ₃ O ₁₂	~ 200	10^3	I
NaNbO ₃	$\epsilon_{33} \sim 670$	—	{ 1 (4)
Ca ₂ Nb ₂ O ₇	~ 45	5×10^7	

*Values are room temperature values, taken from Young and Frederikse (ref. 10), or the references in parentheses.

†P.D., ordered, clustered or random point defects; CS, crystallographic shear; I, intergrowth. Fuller descriptions of defect type will be found in refs 1–3 or the references in parentheses.

‡A. N. Bagshaw, personal communication.

also fall into this group. Stoichiometric variation here can be described as being due to intergrowth between the TiS phase and the TiS₂ phase⁷.

For evaluating materials growth methods, or predicting the chemical or physical behaviour of materials it is valuable to have a reliable guide as to which of these modes of stoichiometric behaviour is likely to predominate in any particular system but at present no such guide has been found.

Calculations of the electrostatic interaction between CS planes in reduced WO₃ (ref. 8) showed that a high value of the low frequency dielectric constant was of importance in reducing this energy term to a small value. The fact that CS planes only seemed to be found in materials which possessed a high dielectric constant was also pointed out by Bursill *et al.*⁹. Taking these two observations as starting points, I have examined the literature in an attempt to determine whether there is a definite correlation between the occurrence of intergrowth as a means of accommodating non-stoichiometry in a material and low frequency dielectric constant. Although the data on both these topics are scanty a very good correlation is found, provided that those materials in which a high value of the low frequency dielectric constant is associated with hydrogen bonding are excluded, as the crystal-chemical relationship between dielectric constant and structure is rather different in these compounds than in, for example, the perovskite oxides.

Table 1 reports some materials with well known modes of accommodating non-stoichiometry which also have recorded dielectric constant values. Unfortunately data are not available for many interesting compounds, but there are enough to show that systems using point defects have low values of dielectric constant, less than approximately 24, while all the materials with known intergrowth or CS behaviour have values >100 .

In order to apply this empirical correlation usefully it is important to remember that most experiments involving non-stoichiometric behaviour are carried out at moderate to high temperatures. The room temperature dielectric constant which is the value most usually reported and which

is given in Table 1 may well be considerably lower than that at high temperatures, and temperature variation can be large, as in the case of WO₃ (ref. 12). Thus for some materials, a change in behaviour with temperature may be expected, MoO₃ may be such an example¹⁷.

In the non-metallic materials we are considering, the low frequency dielectric constant is composed of two components, the electronic and ionic polarisabilities of the atoms in the structure. A high value of the static dielectric constant is often attributable to a high ionic polarisability term, which is largely due to the presence of small off-centre cations in metal-oxygen coordination octahedra which form the building units of many oxides. The ferroelectric perovskites fall into this class. In other materials, such as PbS or SbSI the presence of large easily polarisable anions and lone pairs of electrons on the cations produces large electronic polarisation. Neither of these features in themselves, however, seem to explain why a point defect population should be resisted in these materials, although recent lattice energy calculations for materials containing point defects have shown that polarisation terms have a considerable effect on defect formation energies.

A possible reason for the rejection of high point defect populations may be found by considering the lattice dynamics of crystals. The low frequency dielectric constant of a crystal and hence the ionic and electronic polarisabilities of the component atoms are closely connected with the allowed lattice vibrations of the matrix. The simplest form this relationship takes is expressed by the Lyddane-Sachs-Teller relationship which shows that a high static dielectric constant is associated with a transverse optical phonon, ω_T , of very low frequency in the lattice¹⁸. These soft transverse modes are temperature dependent and tend to exhibit instability as the frequency falls, that is, as the dielectric constant increases. Barker¹⁹ has shown that soft mode instability can be strongly influenced by the local electric field within a dielectric material. The presence of defects in a material, either some form of point defect arrangement or intergrowths or planar faults, will lead to significant changes in the local fields in some regions of the crystal which will have a bearing on the stability of the soft ω_T modes. The interaction will almost certainly be complex and has not been analysed quantitatively, but it seems reasonable to suggest that in materials with a high dielectric constant the existence of potentially unstable soft ω_T modes favours the formation of sheets of defects, intergrowths or crystallographic shear planes, over point defects or point defect clusters.

The lack of a detailed theoretical treatment, together with the paucity of experimental data make these suggestions very speculative. Despite these qualifications the empirical correlation between dielectric constant and defect structure seems to work well and be useful. It provides a guide to the structural behaviour of non-stoichiometric solids where none has existed before. Theoretical studies are now underway which, it is hoped, will provide a more quantitative understanding of the problem. In particular it is important to determine the extent to which there may be a causal relationship between low frequency dielectric constant and the mode of accommodation of non-stoichiometry which, at present, is by no means certain.

R. J. D. TILLEY

School of Materials Science,
University of Bradford,
Bradford,
W. Yorkshire, UK

Received 23 June; accepted 25 July 1977.

- ¹ Anderson, J. S. in *Defects and Transport in Oxides* (eds Seltzer, M. S. & Jaffee, R. I.) 24–54 (Plenum, New York, 1974).
- ² Anderson, J. S. & Tilley, R. J. D. in *Surface and Defect Properties of Solids 3*, (eds Roberts, M. W. & Thomas, J. M.) 1–56 (Chemical Society, London, 1974).
- ³ Hyde, B. G., Bagshaw, A. N., Anderson, S. & O'Keeffe, M. in *A. Rev. mater. Sci.* 4, 43–92 (1974).
- ⁴ Nanot, M., Queyreu, F., Gilles, J. C., Portier, R. & Fayard, M. *Mater. Res. Bull.* 10, 313–316 (1975).
- ⁵ Nguyen, N., Studer, F., Groult, D., Choisset, J. & Raveau, B. *J. Solid St. Chem.* 19, 369–382 (1976).
- ⁶ McConnell, J. D. M., Hutchison, J. L. & Anderson, J. S. *Proc. R. Soc. A* 339, 1–12 (1974).
- ⁷ Tilley, R. J. D. *J. Solid St. Chem.* 7, 213–221 (1973).
- ⁸ Iguchi, E. & Tilley, R. J. D. *J. Solid St. Chem.* (in the press).
- ⁹ Bursill, L. A., Hyde, B. G. & O'Keeffe, M. in *Solid State Chemistry*, N. B. S. spec. Pub. 364, (eds Roth, R. S. & Schneider, S. J.) 197–204 (National Bureau Standards, Washington, 1972).
- ¹⁰ Young, K. F. & Frederiske, H. P. R. *J. phys. Chem. Ref. Data* 2, 313–409 (1973).
- ¹¹ Chernko, I. M. & Ivon, A. I. *Fiz. Tverd. Tela* 16, 2130–2133 (1974); English translation, *Sov. Phys. Solid St.* 16, 1391 (1975).
- ¹² Lefkowitz, I., Dowell, M. B. & Shields, M. A. *J. Solid St. Chem.* 15, 24–39 (1975).
- ¹³ Samara, G. A. & Percy, P. S. *Phys. Rev. B* 7, 1131–1148 (1973).
- ¹⁴ Emmenegger, F. P. & Robinson, M. L. A. *J. phys. Chem. Solids* 29, 1673–1681 (1968).
- ¹⁵ Hyde, B. G. & Tilley, R. J. D. *J. phys. Chem. Solids* 31, 1613–1619 (1970).
- ¹⁶ Tilley, R. J. D. *J. Solid St. Chem.* (in the press).
- ¹⁷ Bursill, L. A. *Proc. R. Soc. A* 311, 267–290 (1969).
- ¹⁸ Peterson, G. E. in *Treatise on Solid State Chemistry* 2 (ed. Hannay, N. B.) 183–236 (Plenum, New York, London, 1975).
- ¹⁹ Barker, A. S. in *Ferroelectrics* (ed. Weller, E. F.) 213–250 (Elsevier, Amsterdam, 1967).

Crystal structure of rustumite

RUSTUMITE was first described by Agrell¹ as $\text{Ca}_4\text{Si}_2\text{O}_7(\text{OH})_2$, space group Cc or C2/c, $a = 7.62(5)$, $b = 18.55(5)$, $c = 15.51(5)\text{\AA}$, $\beta = 104^\circ 20'(10')$, with $Z = 10$. The crystal structure we describe here, in space group C2/c ($R = 11.3\%$ for all data) gives, instead, an ideal formula $\text{Ca}_{10}(\text{Si}_2\text{O}_7)_2\text{SiO}_4\text{Cl}_2(\text{OH})_2$, with $Z = 4$. The important features of the completed structure are the presence of chlorine and of orthosilicate groups in addition to Si_2O_7 groups.

The experimental intensity data, from a naturally occurring single crystal, comprised 2,100 independent reflections for layers 0–9, k, l ($\sin^2\theta < 0.2$) obtained with a Hilger and Watts Y-190 linear diffractometer and Mo-K α radiation, using the cell dimensions given by Agrell¹. Of this data, 282 reflections were classed as 'unobserved', and were excluded from all further calculations.

Solution of the Patterson map was hindered by the large number of atoms in the unit cell (~ 150), permitting the detection of only four (or more)-multiple peaks, and the presence of subtranslations of $\sim a/4$, $\sim b/6$ and $\sim c/6$ among the Patterson

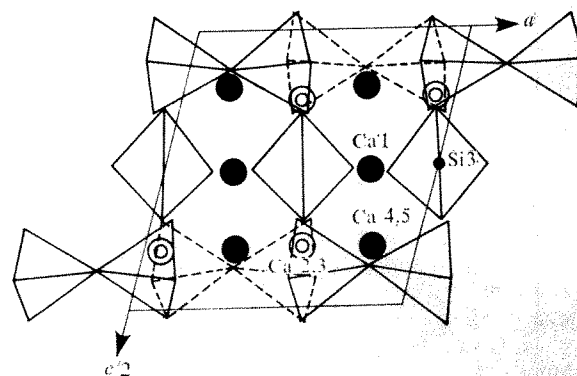


Fig. 1 Rustumite—(010) projection of one half of the unit-cell. The Si_2O_7 and SiO_4 groups are indicated as polyhedra. The Ca atoms overlap in pairs: ●, (Ca1, Ca1'), (Ca4, Ca5); ○, (Ca2, Ca3).

peaks. Nevertheless, an initial structure was established in the space group C1 by double superposition using two multiple peaks as shift vectors² and consideration of the vector subsystem³.

In the course of refinement by the usual Fourier methods, as the remaining atoms of the structure appeared, the presence of first the twofold axis and finally the c -glide became obvious. The final solution in the space group C2/c, refined by block diagonal least squares using all of the observed reflections except 171 which showed especially poor agreement between F_o and F_c , gave the parameters of Table 1. At this stage R was 7.4% for the data included in least squares (11.3% over all observed data).

In the asymmetric unit it is easy to pick out, apart from the five Ca atoms, an $(\text{Si}_2\text{O}_7)^{6-}$ group (involving Si1 and Si2) and an $(\text{SiO}_4)^{4-}$ group involving Si3 on a twofold axis (Fig. 1), leaving only O10 and O11. The atom O10 yields a peak in the electron density maps comparable in size with Si3, gives an occupancy factor (refined as oxygen) of ~ 2.0 in least squares refinement and has no nearest-neighbour Ca atom closer than 2.75 Å, all consistent with this atom being Cl. Bogomolov and Organova (personal communication) have given analytical data for chloride in rustumite.

Whereas all other O atoms, including O10, are four-coordinate, O11 has only three Ca nearest neighbours (Ca—O, 2.33–2.37 Å) all to one side of it, clearly allowing completion of its tetrahedral coordination by a hydrogen atom. Therefore, although there is no clear evidence for the H atom in the electron density or difference maps, O11 can reasonably be regarded as a hydroxyl group to give the formula $\text{Ca}_5(\text{Si}_2\text{O}_7)(\text{SiO}_4)_{0.5}\text{Cl}(\text{OH})$ for the formula of the asymmetric unit (eight per cell) of rustumite. The cell contents are then $\text{Ca}_{40}\text{Si}_{20}\text{O}_{72}\text{Cl}_8(\text{OH})_8$ compared with $\text{Ca}_{40}\text{Si}_{20}\text{O}_{70}(\text{OH})_{20}$ from Agrell's formula.

We thank Professor H. F. W. Taylor for bringing this problem to our attention and the SRC for a grant to V.V.I.

R. A. HOWIE

V. V. ILYUKHIN*

Department of Chemistry,
University of Aberdeen,
Meston Walk,
Aberdeen, UK

Received 1 July; accepted 28 July 1977.

*Permanent address: Institute of Crystallography, Academy of Science, Moscow, USSR.

¹ Agrell, S. O. *Min. Mag.* 34, 1–15 (1965).

² Ilyukhin, V. V. *Direct and Patterson Methods for Solving Crystal Structures* (in Russian, Institute of Applied Physics, Academy of Science (Moldavia), U. S. S. R., Kishinev, 1972).

³ Kuz'min, E. A., Ilyukhin, V. V. & Petrunina, A. A. *The Method of Vector Subsystems* (in Russian, Institute of Geochemistry and Mineral Physics, Academy of Science (Ukraine), U. S. S. R., Kiev, 1976).

Atom	Fractional coordinates			B_{iso} Å ² (10 ²)	Occupancy (10 ²)
	x/a	y/b (10 ⁴)	z/c		
Ca(1)	2,582(4)	3,520(1)	2,498(2)	95(4)	98(1)
Ca(2)	4,399(4)	3,024(1)	6,192(2)	96(4)	97(1)
Ca(3)	5,760(4)	2,068(1)	4,039(2)	95(4)	96(1)
Ca(4)	3,298(4)	232(2)	4,017(2)	102(4)	98(1)
Ca(5)	3,106(4)	5,148(1)	3,964(2)	93(4)	97(1)
Si(1)	4,420(5)	6,319(2)	5,614(2)	76(5)	98(1)
Si(2)	3,692(5)	1,340(2)	5,643(2)	83(5)	99(1)
O(1)	3,349(12)	3,786(5)	4,161(6)	99(16)	93(2)
O(2)	4,782(12)	1,759(5)	6,518(6)	96(15)	98(2)
O(3)	4,318(12)	523(5)	5,559(6)	118(15)	98(2)
O(4)	4,629(12)	5,760(5)	1,594(6)	95(15)	97(2)
O(5)	3,503(13)	1,770(5)	4,725(6)	110(17)	89(2)
O(6)	3,767(13)	6,870(5)	4,777(6)	105(16)	93(2)
O(7)	4,236(13)	3,277(5)	1,516(6)	136(16)	98(2)
O(8)	3,168(13)	4,778(5)	2,481(6)	107(15)	97(2)
O(9)	3,695(13)	4,476(5)	429(6)	119(16)	94(2)
O(10)	2,384(6)	2,961(2)	7,458(3)	54(6)	204(2)
O(11)	560(14)	5,881(5)	3,535(6)	123(17)	91(2)
Si(3)*	1/2	5,276(3)	1/4	82(8)	97(2)

Estimated standard deviations applicable to the least significant digits are given in parentheses.

*Si(3) is in the special position 4e of C2/c, while all other atoms occupy general (8f) positions.

Imaging practical surfaces in a field ion microscope

APPLICATIONS of the field ion microscope (FIM), have been restricted probably because of the need for specially prepared specimens. We present here, for the first time, experimental data on imaging practical surfaces with the FIM. This should provide more applications for this instrument especially in studying multi-protrusions or whiskers.

The techniques used in the FIM including the vacuum system are conventional. In the first stage of the experiments, we tried imaging multi-tips from a single tip holder. Surprisingly, we could identify the individual images of the single tips, and these were geometrically superposed. The images of twin tips of tungsten are shown in Fig. 1a. Figure 1b shows a simulated pattern obtained by superposing two negatives of a single tip image of tungsten. The close correspondence between the two patterns is striking. This imaging was repeated for three and so on up to seven tips. In each case it was possible to get the resolved images although their stability was poor because of the unavoidable differences in specimen tip size, shape and relative displacements, all of which led to a vast difference of field concentrations about each tip.

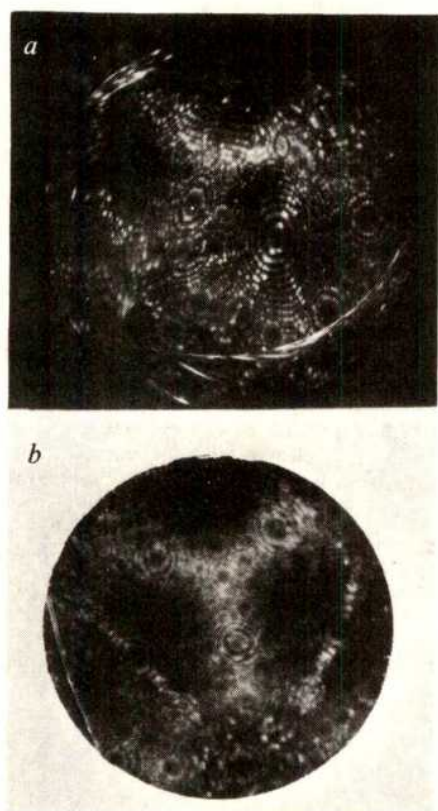


Fig. 1 a, Twin tips of tungsten imaged. b, Superposed images of two tungsten tips. Voltage, 17 kV.

In each case, another striking observation was made after the tips were blunted by rapid field-evaporation. Even at the highest voltage of 28 kV (limited by our high voltage unit), no resolved image could be seen. In all these cases, however, the image pattern exposed bright circles—usually rings with haloes around them—the number corresponding to the number of multi-tips mounted for the projection (Figs 2, 3). Using scanning electron microscope (SEM) patterns for these tips, and assuming an approximate field-factor, the magnification for these tips was estimated and a good experimental fit was observed. But the reason for

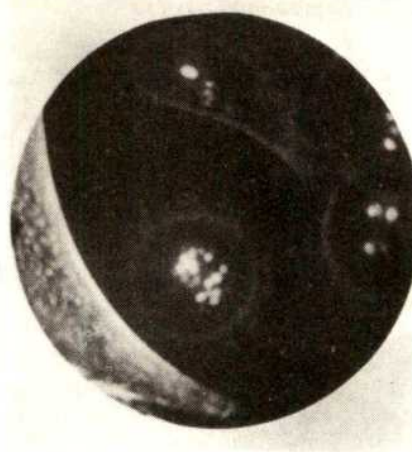


Fig. 2 Image of four tips depicting four rings. Voltage, 25 kV.

these bright ring images is not clear. As tips were blunt, the available voltage was probably insufficient to obtain best imaging voltage (BIV) with helium gas. All imaging was done at background pressure of 10^{-6} torr or less. At the liquid nitrogen temperatures at which our voltages were applied, nitrogen and other residual gases probably reached the tip by surface migration along the shank¹, and then field-

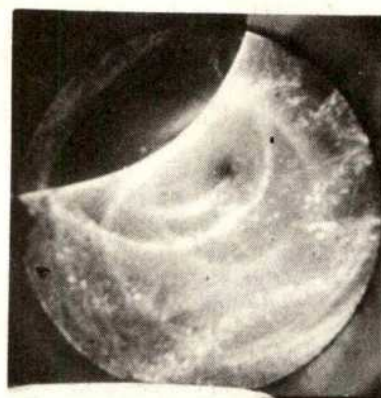


Fig. 3 Image of seven tips depicting seven rings. Voltage, 42 kV.

evaporated. Did the dimension of the ring, then, represent the tip diameter? If the relative displacements of these tips could be assumed, then these images would help trace the electric field contours and field-factor determination for these complex conditions. At times, one could even observe very strange cross lines (Fig. 4) which can only be understood as representing the arc of field distribution which



Fig. 4 Cross lines depicting potential profile. Voltage, 45 kV.

would be too complicated to map for the multiple tips case where equipotential overlappings are expected.

In a later experiment carried out on three tip specimens, an interesting feature was observed. After the first flashings and bluntings of the tips, one microprotrusion, probably belonging to one of the blunt tips became very well resolved at a voltage of 32 kV. When the imaging gas helium was cut out, the resolved tip image disappeared completely leaving the surrounding random adsorption pattern unchanged (Fig. 5*a, b*). This resolved image could also be field-evaporated later atom by atom in a controlled fashion until the image completely vanished. The protrusion size calculated from the evaporated rings was 21 Å.

Our most interesting results were obtained when a good low voltage single tip (Fig. 6) slipped and fractured during rapid field-evaporation. On raising the voltage, distinct circles were seen (Fig. 7), representing different microprotrusions created on the main tip by the fracture. One ring was seen as distinctly separated from an overlapping group (estimated to be four) of rings. At a higher voltage,

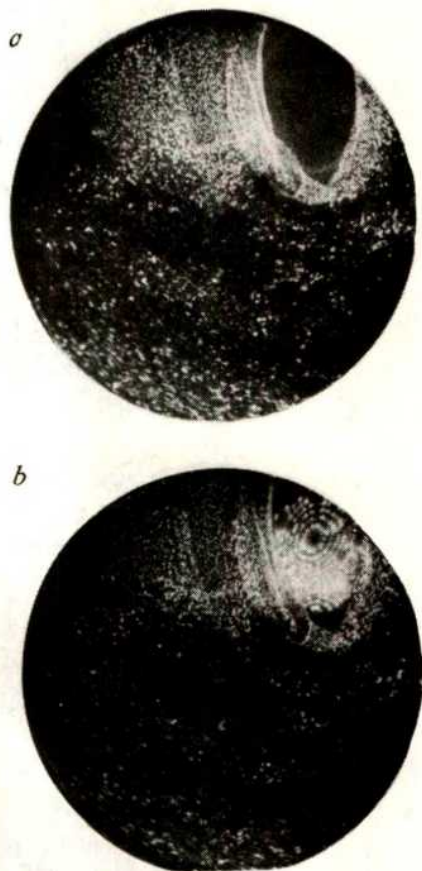


Fig. 5 *a*, Image of deformed tip without imaging gas. *b*, Image of the same tip with helium gas. Voltage, 32 kV.

~41 kV, this group of overlapping rings resolved (Fig. 8) and at least one well defined symmetric tungsten image could be clearly detected. This microprotrusion could be clearly field-evaporated and imaged without any interference. The radius of the original tip was calculated using both Bowkett's² and Müller's³ methods and a value of 220 Å was obtained. By a similar method, the radius of the microprotrusion was calculated to be 35 Å. Using Rose's⁴ formula for magnification enhancement for a conducting protrusion on a field emitter, we calculated the magnification expected for our micro-tip and, finally, its image diameter as observed on the screen. The calculated diameter was about 4 cm and the experimentally observed image was a ring 3.7

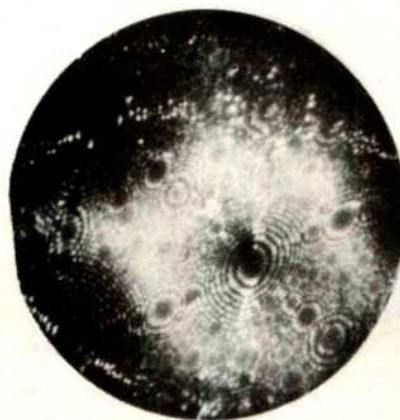


Fig. 6 Single tip of tungsten. BIV, 9.8 kV.

cm in diameter. The SEM pattern taken at the end of this experiment did not indicate the presence of a protrusion. This renders our technique more sensitive for the detection of such extremely small protrusions unresolved by other microscopic techniques available.

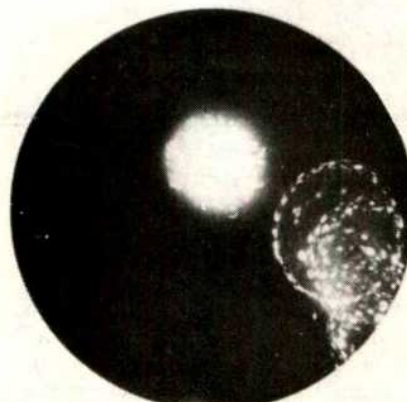


Fig. 7 Tip of tungsten (the same as in Fig. 6) after a flash. Voltage, 18 kV.

To conclude, it is possible to resolve individual microprotrusions of a few tens of ångströms on a practical surface using the field ion microscope.

Further it seems feasible to (1) map equipotentials for complex distribution of conducting protrusions on a surface and calculate field factors which hitherto was not possible;

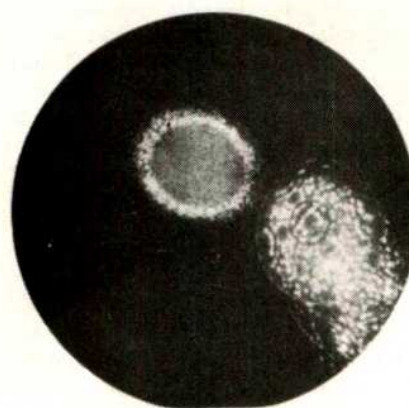


Fig. 8 Resolved microtip. Voltage, 43 kV.

and (2) image simultaneously and without mutual interference, dissimilar elements that can be grown as microprotrusions or whiskers. This will enable us to understand specific sites on the substrate for nucleation, in relation to crystallographic features and so on. The atom probe³ coupled to this mode of operation of FIM will permit us to chemically identify individual atoms from different protrusions.

We are grateful to the late Dr D. Y. Phadke who encouraged this unconventional approach.

P. VIJENDRAN

DEVAKI RAMANATHAN

SUNDER DASS

Technical Physics Division,
Bhabha Atomic Research Centre,
Bombay 400 085, India

Received 10 May; accepted 25 July 1977.

- ¹ Muller, E. W. *Field Emission Studies of Metal Surfaces* 47 (Tech. Rep. AF 49 (638)—504 Solid State Division Air Force Office of Scientific Research, Washington, 1961).
- ² Bowkett, K. M. & Smith, D. A. *Field Ion Microscope* 37–39 (North Holland, Amsterdam, 1970).
- ³ Muller, E. W. & Tsong, T. T. *Field Ion Microscopy—Principles and Applications* 187–189 (Elsevier, New York, 1969).
- ⁴ Rose, D. J. *J. appl. Phys.* 27, 215 (1956).
- ⁵ Muller, E. W. *Lab. Pract.* 22, 408–415 (1973).

Archaeological evidence for Subrecent seismic activity along the Dead Sea–Jordan Rift

EXAMINATION of archaeological sites and records in the Levant reveals features of destruction attributable to ancient earthquakes, which may help in verification of old earthquake catalogues and in assessment of seismic hazards.

Regional seismicity in Israel is characterised by sporadic shocks of up to $M=7$, separated by long quiescent periods. The shocks, believed to originate mainly along the Dead Sea–Jordan Rift, where microtremors occur also during the quiescent periods, are documented by instrumental and historical data^{1–7}. Historical earthquake evidence, however, is based on biblical, ecclesiastical and historical chronicles, which are tainted by superstition, exaggeration and misinterpretation of natural phenomena. Consequently, the estimates of incidence, intensity and distribution of individual shocks presented by different cataloguers differ considerably^{1–6,8,9}.

To obtain material evidence¹⁰, we have examined the record of archaeological excavations in Israel, searching for reports of catastrophic damage at explored sites. Summaries of archaeological findings^{11,12} and responses to questionnaires circulated among Israeli archaeologists revealed numerous cases in which destruction and desertion were attributed to major earthquakes (Table 1). Field examination of the reputed features of seismic damage (for example, joints, cracks, faults and breakage; tilted and distorted walls, buildings and aqueducts; oriented collapse and subsidence; and parallel alignment of fallen columns and masonry) showed them to be less unequivocal than generally assumed by archaeologists, because very similar effects may be caused by poor construction or unfavourable geotechnical conditions, unrelated to any seismic events. At the same time, it seems that these 'earthquake indicators' were used by archaeologists fairly consistently, and that the assignment of such features of architectural damage to seismic shocks at

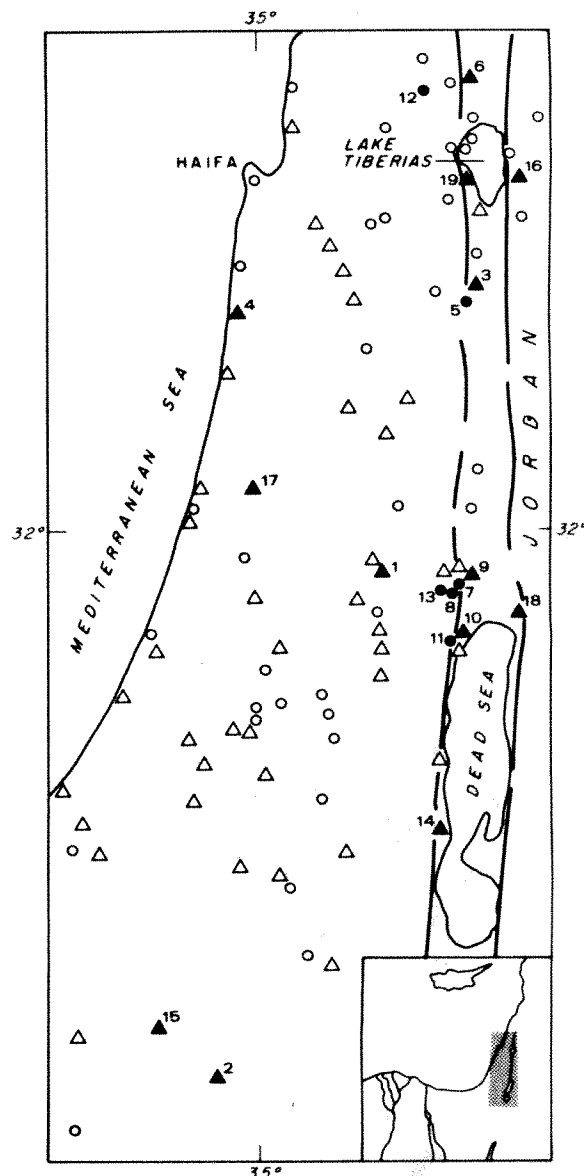


Fig. 1 Distribution of suspected pre-Crusader archaeoseismic damage in Israel. ○, Sites with one or two exposed buildings. △, Sites with numerous exposed buildings and structures. ●, ▲, Sites with reported archaeoseismic damage. Numbers refer to locations presented in Table 1. Heavy lines: major Rift faults.

any individual site, though probably prejudiced by interpretation of historical records, was unbiased by previous geological or geotechnical considerations.

Of all the excavated pre-1000 AD (pre-Crusader) sites, which are fairly evenly distributed over northern and central Israel, although sparser in the more arid south, we plotted in Fig. 1 only those where excavations uncovered building elements of extent and quality sufficiently high for features of architectural damage not to pass unnoticed. On the same map we marked all the sites with supposed seismic damage. The shock-affected sites differ in lithological and topographical setting, and most of them are located along the Dead Sea–Jordan Rift and its margins. This concentration (about 75%) is greater than that indicated in the historical catalogues (15–30% of references mention the Rift zone). The present distribution of these sites does not show whether they are located along the major Rift faults or along subsidiary crescentic faults and blocks or both. It

Table 1. Reports of ancient seismic damage at archaeological sites in Israel

Location number (Fig. 1)	Site	Evidence	Age of damage	Reference
1	Ai	Widespread destruction, collapse of buildings, breakage, tilting	27th century BC	11, 12
2	Avdat (Eboda)	Damaged city walls and buildings, cracks, subsequent repairs	5–6th century AD	11, 12
3	Beit Shean	Disturbances and collapse in the northern cemetery	?	11, 12
4	Caesarea	Disturbed offshore moles and port installations, tilted walls	2nd century AD	11, 12
5	Ein Hanaziv	Oriented collapse, alignment of fallen masonry	7th century AD	13
6	Hazor	Tilted columns, walls and buildings	8th century BC	11, 12
7	Jericho-Tel el Sultan	Collapse and slides	?	11, 12
8	Jericho-Tel Abu Alaik	Tilted and distorted walls, collapse, subsidence and breakage	1st century BC	14
9	Jericho-Khirbet el Mafjar	Destruction and collapse	8th century AD	11, 12
10	Khirbet Qumran	Displacement and breakage, cracks	1st century BC	11, 12
11	Khirbet Magari	Violent destruction, collapse	7–8th century BC	11, 12
12	Khirbet Shama	Collapse, tilted and distorted (?) walls	4–5th century AD	15
13	Kypros	Collapse, imbricated fallen masonry	?	Meshel (unpublished)
14	Massada	Disturbed floors Tilted walls, aligned fallen masonry, cracks, collapse	1st century BC 1st century BC and later shocks	11, 12
15	Shivta	Damaged buildings and collapse	5–6th century AD	11, 12
16	Susita	Tilted buildings and walls, aligned fallen columns	?	11, 12
17	Tel Afeq (Antipatris)	Tilted and distorted walls, breakage, subsided arches	5th century AD	16
18	Tellat Ghassul	Tilting, faulting, cracks, subsidence and collapse	40th century BC	17, 18
19	Tiberias	Desertion of the southern city	11th century AD	Foerster (personal communication)

seems to show, however, that the Rift region is seismogenic and that the seismic hazards here are relatively high.

A part of this study was aided by grants to I. K. from the US Academy of Sciences (Day Fund) and the SUNY Faculty Awards Committee. The advice of M. Ben Dov, D. Bahat, M. Kochavi, Y. Mincker, E. Nezer and F. Vitto is gratefully acknowledged.

IAAKOV KARCZ

Department of Geology,
SUNY Binghamton,
Binghamton, New York 13901
and Geological Survey of Israel

URI KAFRI

Geological Survey of Israel,
Jerusalem, Israel

ZEEV MESHEL

Institute of Archeology,
Tel Aviv University,
Tel Aviv, Israel

Received 16 May; accepted 5 July 1977.

- Willis, B. *Bull. seism. Soc. Am.* **18**, 73–103 (1928).
- Sieberg, A. *Denkschriften Mediz. Naturwiss. Gesellschaft zu Jena* **18**, 2, 161–273 (1932).
- Shalem, N. *Jerusalem* **2**, 1–3, 22–54 (1949) in Hebrew.
- Amiran, D. *Israel Expl. J.* **1**, 223–246 (1951); 48–65 (1952).
- Shalem, N. *Bull. Res. Coun. Israel* **2**, 2, 1–16 (1952).
- Ben Menahem, A., Nur, A. & Vered, M. *Phys. Earth planet. Int.* **12**, 1–50 (1976).
- Wu, F., Karcz, I., Arie, E., Kafri, U. & Peled, U. *Geology* **1**, 159–161 (1973).
- Ambraseys, N. *Nature* **232**, 375–379 (1971).
- Ambraseys, N. in *Historical Geography of the Middle East* (ed. Brice, W.) (Academic, London, 1975).
- Ambraseys, N. *Antiquity* **47**, 229–230 (1973).

¹¹ *Encyclopaedia of Archeological Excavations in Eretz Israel*, Hebrew edn, 2 vols (Massada, Jerusalem, 1970).

¹² *Encyclopaedia of Archeological Excavations in Eretz Israel*, English edn (updated), vols 1 and 2 (from 4) (Massada, Jerusalem, 1975).

¹³ Vitto, F. *Qadmoniot* **8**, 119–123 (1975).

¹⁴ Nezer, E. *Israel Expl. J.* **25**, 89–100 (1975).

¹⁵ Meyers, E. M., Kraebel, A. T. & Strange, J. F. *Israel Expl. J.* **22**, 174–175 (1972).

¹⁶ Kochavi, M. *Israel Expl. J.* **26**, 51–53 (1976).

¹⁷ North, R. *Analecta Biblica* **14**, 1–88 (1960).

¹⁸ Hennessey, I. B. *Levant* **1**, 1–25 (1969).

Bacterial sulphate reduction and calcite precipitation in hypersaline deposition of bituminous shales

BITUMINOUS shales of the Ghareb Formation of Maastrichtian age from Nebi Musa (near Jericho, Jordan Valley) were analysed for their texture, mineralogical and chemical composition. The results from the lowermost bed of the formation are of broad interest, and indicate special diagenetic processes affecting sediments rich in organic material deposited in an anaerobic hypersaline environment. Single crystal calcite replacement of gypsum infilling of foraminifera and a predominance of even over odd carbon numbers among C_{25} – C_{30} *n*-alkanes indicate sulphate reducing bacterial activity during deposition of bituminous shales in a hypersaline environment.

The samples taken consist of hard black calcareous shale with up to 15% organic carbon, containing also kaolinite, detrital phosphate and pyrite. Foraminifera constitute a major component—particularly thin walled, small plank-

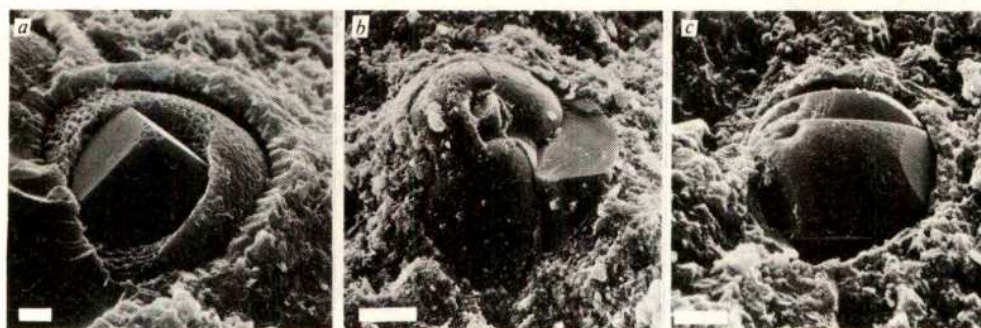


Fig. 1 Scanning electron micrographs of calcite replacement of gypsum infilling of foraminiferal tests. *a*, Chamber filled with gypsum (pitted surface) and a calcite crystal growing in its centre. Scale bar 5 µm. *b*, Chamber partially filled with a euhedral single calcite crystal. Scale bar 5 µm. *c*, Foraminifera completely filled with single calcite crystals. Note the preservation of the original thin walls. Scale bar 50 µm.

tonic ones. The benthonic foraminiferal fauna is composed exclusively of numerous specimens of Buliminidae of one or two species only. Exclusively benthonic Buliminidae assemblages occurring in the phosphates of the underlying Mishash Formation were interpreted as indicating semi-closed, near-shore basins with low surface salinity and oxygen deficiency¹. The addition of abundant planktonic foraminifera to the Buliminidae assemblages in the Ghareb Formation may indicate a change of salinity in the surface waters. The organic matter (mostly kerogen) is dispersed and no structure indicative of its source can be observed even in the scanning electron microscope (SEM).

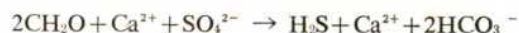
A mineralogical investigation using petrographic microscopy, X-ray diffraction and microprobe analysis revealed that the foraminiferal tests are filled with either of the following minerals: gypsum, single crystals of calcite, and pyrite, which is also dispersed in the matrix. The calcite infilling in the form of a single crystal in a foraminiferal chamber or a whole test was observed optically under the petrographic microscope and morphologically in the SEM. Locally, stages in the replacement of gypsum by single crystal calcite growth can be observed with the SEM (Fig. 1*a*), as well as steps towards complete single crystal calcite infilling of a foraminiferal test (Fig. 1*b*, 1*c*). This infilling cannot be regarded as a late diagenetic sparry calcite mosaic from which it differs in texture. Moreover, sparry calcite development through dissolution and precipitation is not likely to have taken place in the impervious shales.

The samples from the lower Ghareb Formation showing these mineralogical features also exhibit particular organo-geochemical markers, especially with regard to the *n*-alkanes extracted. There is a high concentration of *n*-C₁₉ alkane and a prominent envelope in the C₂₆–C₃₂ carbon number range, in which even carbon number C₂₈ and C₃₀ predominate over their odd carbon number homologues (Fig. 2*b*). This *n*-alkane distribution differs from that of living and fossil higher plants, which are characterised by a prominent envelope in the C₂₃–C₃₃ range, with a marked odd over even preference^{2,3}, as well as from that of algae and photosynthetic bacteria marked by a higher concentration around *n*-C₁₇ (refs 4–6), such as is found in higher strata of this formation (Fig. 2*a*).

Even over odd preference in the C₂₆–C₃₂ range in rock extracted *n*-alkanes was attributed to highly saline environments mainly on the basis of local geological and sedimentological considerations^{7,8}. Studies of the *n*-alkane content of algae and bacteria grown in the laboratory showed that non-photosynthetic anaerobic bacteria have a high concentration of *n*-C₁₉ and longer chain alkanes^{4,5}. An even over odd preference in the C₂₆–C₃₂ range of *n*-alkanes was reported from cultures of *Desulfovibrio hildenboroughi*⁴, a common sulphate reducing bacterial species. It seems, therefore, that the *n*-alkane distribution of the samples investigated is indicative of contributions from both anaerobic, non-photosynthetic, as well as sulphate reducing bacteria.

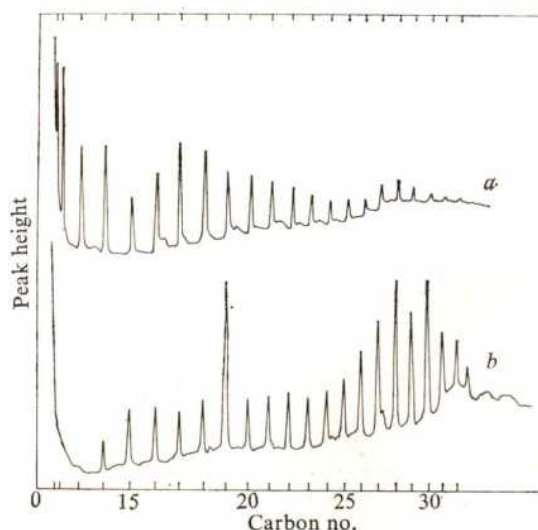
The palaeontological and sedimentological record of the lowermost beds of the Ghareb Formation, the occurrence of gypsum infillings of foraminiferal tests, the single crystal calcite replacement of gypsum, as well as the peculiar *n*-alkane distribution which resembles that of anaerobic and sulphate reducing bacteria, can all be interpreted in terms of environment of deposition and early diagenetic processes as follows.

(1) The bituminous shales were deposited on the bottom of a hypersaline, stratified lagoon, the upper oxygenated, highly productive layer of which was rich in planktonic foraminifera, mainly in the outer part of the lagoon. Organic matter accumulated on the anoxic bottom. (2) Empty foraminiferal tests transported to the inner margin of the lagoon were filled with gypsum either in the upper layer or on the bottom immediately after settling. (3) Anaerobic non-photosynthetic and sulphate reducing bacterial activity resulted in dissolution of gypsum according to the generalised formula⁹



thus increasing the bicarbonate and calcium ion concentration. (4) The waters of the lagoon were already over-saturated with regard to calcium carbonate, therefore, calcite precipitation took place concomitantly where gypsum was dissolved. (5) The presence of pyrite is at least partly due to the production of H₂S during these reactions.

Fig. 2 Gas chromatograms of *n*-alkanes from the Ghareb Formation. *a*, Characteristic *n*-alkane distribution of the bituminous Ghareb Formation; *b*, *n*-alkane distribution of the lowermost bed. Separation on 6 foot $\frac{1}{8}$ inch column packed with 2% Apiezon L on Gas Chrom Z, programming 100–290 °C, 4 °C per min.



The process of single crystal calcite replacement of gypsum infillings of foraminiferal tests, thus envisaged, takes place in a combination of strictly defined conditions: water oversaturated with respect to calcium carbonate and gypsum, in the presence of organic material and sulphate reducing bacteria. It is probable that such replacement is generally indicative of this type of sedimentary environment.

BARUCH SPIRO

Department of Geology,

Zeev Aizenshtat,

The Energy Research Center,

The Hebrew University of Jerusalem, Israel

Received 13 June; accepted 5 July 1977.

- 1 Reiss, Z. *Israel. Geol. Surv. Israel Bull.* 34, 1-23 (1962).
- 2 Albrecht, P. & Ourisson, G. *Angew. Chem.* 10, 209-225 (1971).
- 3 Calvin, M. *Chemical Evolution* (Oxford University Press, Oxford, 1969).
- 4 Han, J. & Calvin, M. *Proc. natn. Acad. Sci. U.S.A.* 64, 436-443 (1969).
- 5 Han, J., McCarthy, E. D., Van Hoven, W., Calvin, M. & Bradley, W. H. *Proc. natn. Acad. Sci. U.S.A.* 59, 29-37 (1968).
- 6 Winters, K., Parker, P. L. & Van Baalen, C. *Science* 158, 467-468 (1969).
- 7 Dembicki, H., Jr, Meinschein, W. G. & Hattin, D. E. *Geochim. cosmochim. Acta* 40, 203-208 (1976).
- 8 Welte, D. H. & Waples, D. *Naturwissenschaften* 60, 516-517 (1973).
- 9 Berner, R. A. *Principles of Chemical Sedimentology* (McGraw-Hill, New York, 1971).

Inducer of pectic acid lyase in *Erwinia carotovora*

PECTIC acid (polygalacturonic acid) lyase (EC 4.2.2.2., PAL) formed in bacteria, catalyses the transesterificative cleavage of pectic acid¹. Because PAL both macerates plant tissues and causes cell leakage and death^{2,3}, it has often been investigated biochemically from the industrial and plant pathological point of view. Physiological studies, however, have been limited, probably because PAL is in part an extracellular enzyme⁴. Thus most studies of its inducibility have compared activities in filtrates of cells cultured on different carbon sources^{5,6}. But assay of an extracellular enzyme does not necessarily reveal changes in its rate of synthesis. In *Erwinia carotovora*, however, the PAL that can be purified from cell-free extracts is probably the same protein as the extracellular enzyme¹. In strain EC-1 of *E. carotovora*, non-induced PAL activity is three to four times greater in cell-free extracts than in the culture filtrate during log phase growth, and the relative rate of increase of intracellular enzyme activity after induction is 130 times greater than the rate of release of PAL into the culture filtrate (unpublished data). I therefore used this strain to study induction by measuring PAL activities in cell-free extracts. I report here that the inducer of PAL in *E. carotovora* may not be pectic acid itself but its breakdown product.

Maximum differential activity has been reported to be in the culture fluid grown on pectic acid⁶. To test this, after the addition of pectic acid into minimal medium of Mikula *et al.*⁷ (without sodium citrate) time course samples were taken, washed, sonicated and assayed by the thiobarbituric acid method⁸. The inducibility of PAL by pectic acid was shown by an increase in PAL specific activity (Fig. 1a). The long lag before the onset of induction, however, suggested that pectic acid was not inducing directly. It is unlikely that the lag simply reflects a low initial rate of uptake of pectic acid because (1) induction was further delayed and a growth lag was observed when pectic acid was added to washed cultures rather than directly to cultures grown in glycerol (Fig. 1a and b) or citrate (data not shown); (2) the lag for the induction by pectic acid was further delayed when 10^{-3} M EDTA was added in addition to pectic acid, and this could be abolished by addition of 10^{-3} M CaCl_2 in addition to EDTA and pectic acid (Fig. 1a). Because Ca^{2+} has been reported to activate many bacterial pectic

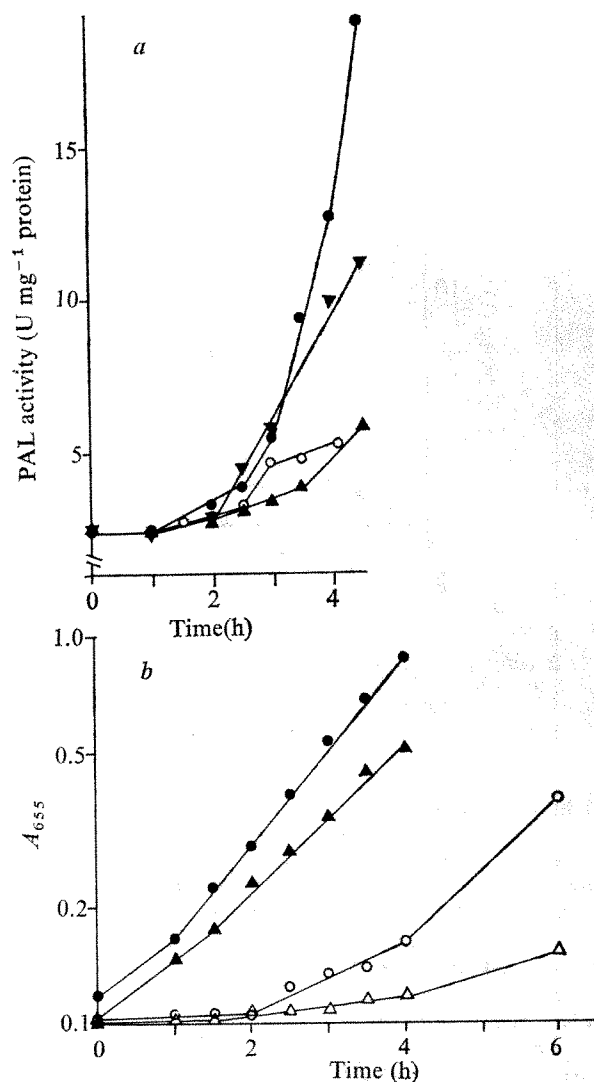


Fig. 1 Kinetics of the induction of PAL by pectic acid. *a*, Induction was initiated by the addition of 0.5% pectic acid, pH 8.3, with 10^{-3} M CaCl_2 or 10^{-3} M EDTA, either directly (closed symbols) or after washing (open symbols), to EC-1 cultures grown in minimal⁷ + 2% glycerol medium to A_{655} of about 0.1. At intervals, 6-ml samples were withdrawn, washed twice and resuspended in 1.5 ml of Tris-HCl buffer (0.05 M, pH 8.0). Suspensions were sonicated and the cell debris was removed by centrifugation for 15 min at 10,000g. PAL activity was measured by a modified thiobarbituric acid method⁸. One unit of PAL is defined as the amount of enzyme which produces 1 μmol of UDG per h. Specific activity is expressed as units of enzyme activity per mg of protein. Protein concentrations were determined by the method of Lowry¹⁴. *b*, During the induction experiment growth was measured as the increase in absorbance at 655 nm after the addition of pectic acid. ●, ○, Pectate; ▲, △, pectate + EDTA; ▼, pectate + EDTA + CaCl_2 .

acid lyases (including the *Erwinia* enzyme¹), these two observations suggest that some product of pectic acid degradation by the non-induced level of PAL in culture filtrate is the true inducing metabolite.

The true inducing metabolite of PAL was sought in time-course induction experiments after the addition of one of the purified metabolites of pectic acid into the minimal medium⁷ as a sole carbon source. The major end product of the cleavage of pectic acid by *E. carotovora* PAL is *O*-(4-deoxy- β -L-threo-hexo-pyranos-4-enyluronate)-(1-4)-D-galacturonic acid (unsaturated digalacturonic acid, UDG). The rapid induction was observed specifically when 2×10^{-3} M purified UDG⁹ was added (Fig. 2). Pectic acid is also degraded by hydrolytic polygalacturonase (EC 2.3.1.15., PG) of *E. carotovora*¹⁰. No induction was observed, however, after the addition of galacturonic acid,

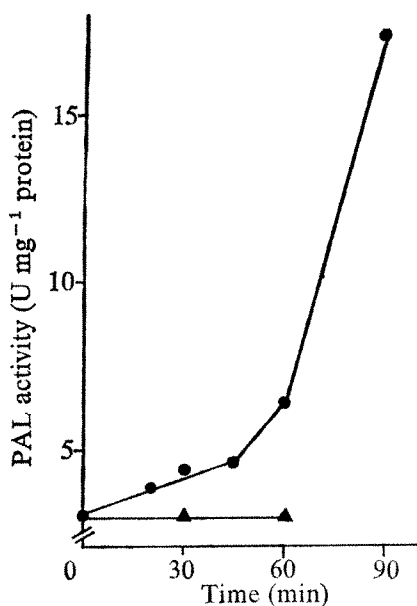


Fig. 2 Kinetics of the induction of PAL by UDG. The procedures were the same as in Fig. 1 except that 2×10^{-3} M UDG or 26×10^{-3} M galacturonic acid instead of pectic acid was added after washing the cultures. ●, UDG; ▲, galacturonate.

which is the major product of the cleavage of pectic acid by *E. carotovora* PG (Fig. 2)¹.

UDG is further metabolised to 4-deoxy-L-threo-5-hexoseulose uronic acid by oligogalacturonic acid lyase (EC 4.2.2.6., OGL)^{11,12}, but as the induction of PAL and OGL is uncoordinate in *Erwinia*¹², UDG rather than its further breakdown product is likely to be the true inducer. The inducer, however, might be a product of unsaturated digalacturonic acid formed by way of a minor pathway, as shown with *Escherichia coli* β -galactosidase, where the transfer products of lactose, 1,6-galactosido-glucose and galactosido-glycerol are inducers¹³.

Data concerning other plant pathogenic bacteria and evidence for catabolite repression of PAL will be published elsewhere. Product-induction may well be a mechanism for regulating other enzymes which have macromolecular substrates. The specific inhibition of PAL induction may also provide an approach to the control of plant diseases with non-toxic agents.

I thank Professors N. Okabe and M. Goto for encouragement, Dr C. Hatanaka for UDG, and Drs R. Bigelis, I. Tsujino, T. Mizuno and S. Nasuno for helpful discussions.

SHINJI TSUYUMU

Faculty of Agriculture,
Shizuoka University,
836 Ohya,
Shizuoka, Japan

Received 18 April; accepted 22 July 1977.

- ¹ Moran, F., Nasuno, S. & Starr, M. P. *Arch. Biochem. Biophys.* **123**, 298–306 (1968).
- ² Mount, M. S., Bateman, D. F., Basham, H. G. *Phytopathology* **60**, 924–931 (1970).
- ³ Hall, J. A. & Wood, R. K. S. *Nature* **227**, 1226 (1970).
- ⁴ Dean, M. & Wood, R. K. S. *Nature* **214**, 408–410 (1967).
- ⁵ Zucker, M. & Hankin, L. J. *Bact.* **104**, 13–18 (1970).
- ⁶ Macmillan, J. D. & Vaughn, R. H. *Biochemistry*, **3**, 564–572 (1964).
- ⁷ Mikula, J. W., Stieglitz, B. I. & Calvo, J. M. J. *Bact.* **109**, 584–593 (1972).
- ⁸ Weissbach, A. & Hurwitz, J. *J. biol. Chem.* **234**, 705–709 (1959).
- ⁹ Okamoto, K., Hatanaka, C. & Ozawa, J. *Agric. biol. Chem.* **28**, 331–336 (1964).
- ¹⁰ Goto, M. & Okabe, N. *Ann. Phytopathol. Soc. Jap.* **27**, 1–9 (1962).
- ¹¹ Preiss, J. & Ashwell, G. *J. biol. Chem.* **238**, 1571–1576 (1963).
- ¹² Hatanaka, C. & Ozawa, J. *Agric. biol. Chem.* **36**, 2307–2313 (1972).
- ¹³ Platt, T., Müller-Hill, B. & Miller, J. H. in *Experiments in Molecular Genetics* (ed. Miller, J. H.) 358 (Cold Spring Harbor Laboratory, New York, 1972).
- ¹⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. J. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).

Steroids stimulate secretion by insect Malpighian tubules

DURING the growth and development of insects there are rapid changes in the concentrations of the moulting hormones (ecdysteroids). In the locust *Locusta migratoria* the rate of excretion of the moulting hormones is one factor which regulates the ecdysteroid titre^{1,2}. The experiments reported here show that the presence of ecdysteroids causes an increase in the rate of urine production by the Malpighian tubules of the tsetse fly *Glossina morsitans*. Previously there has been no evidence to suggest that steroid hormones are involved in the control of excretion in insects³. These results may therefore indicate both a new function for steroids in insects and a new control system for insect excretory systems.

Teneral adult male flies, 24–48 h after emergence, were used in all experiments. Sections (about 1 cm) of their Malpighian tubules were removed and set up *in vitro* in drops of Ringer solution under liquid paraffin⁴. In the absence of a stimulant the tubules secreted very slowly but the addition of extracts of the thoracic ganglion of the fly (which contains the diuretic hormone⁵) or cyclic AMP caused a rapid increase in the rate of secretion. The high rate was maintained as long as the stimulants were present but rapidly reverted to the unstimulated level when they were removed (Fig. 1). The addition of ecdysone (α -ecdysone) or ecdysterone (β -ecdysone) to the bathing drop also caused an increase in the rate of secretion but the time course of this response was very different from that which was elicited by the diuretic hormone or cyclic AMP (Fig. 1). A delay of 20–40 min occurred between the addition of the ecdysteroid and the commencement of fluid secretion by the isolated Malpighian tubule. After the ecdysteroid was removed the rate of secretion returned to its unstimulated level only very slowly. Where tubules were left in bathing drops con-

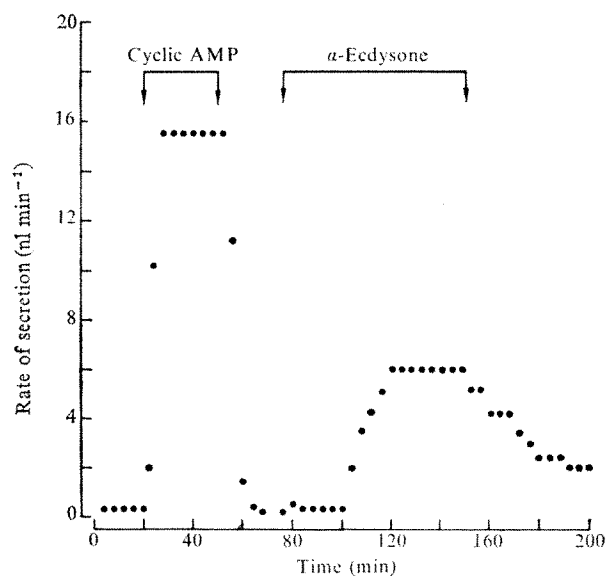


Fig. 1 Rate of secretion of a typical *in vitro* preparation of a *Glossina* Malpighian tubule. A tubule was isolated into a bathing drop of Ringer solution, composition (mM) NaCl, 120; KCl, 10; glucose, 20; malic acid, 3; citric acid, 2; $MgCl_2 \cdot 6H_2O$, 2; $NaH_2PO_4 \cdot 2H_2O$, 1.5; $CaCl_2 \cdot 6H_2O$, 2; pH adjusted to 7 with NaOH and phenol red added to keep constant check on pH. The bathing drop was replaced by Ringer containing 10^{-3} M cyclic AMP for the period shown, after which the tubule was returned to basic Ringer before being transferred to Ringer containing 10^{-6} M ecdysone. Finally, the tubule was returned to basic Ringer. Experiments were performed at 25–28 °C.

taining the ecdysteroids they continued to secrete for many hours. At higher temperatures (28–31 °C), though initially the tubules secreted more rapidly in the presence of the ecdysteroids, the preparations deteriorated after 2–3 h and secretion ceased.

Ecdysone and ecdysterone elicited secretory responses of similar magnitudes. Expressed as a percentage of the maximum response of the tubule stimulated by cyclic AMP, 10^{-6} M ecdysone produced a response of $32.7 \pm 7.0\%$ (mean $\pm 2 \times \text{s.e.}$, $n = 8$) and 10^{-6} M ecdysterone a response of $26.3 \pm 3.8\%$ ($n = 5$). Concentrations above 10^{-6} M were found to cause no further increase in the rate of secretion. But, the rate of secretion decreased with decreasing steroid concentrations below 10^{-6} M, down to a threshold response at 2×10^{-8} M. The response of the tubules at these low concentrations indicates that they would be stimulated to secrete by ecdysteroids at the concentration which is present in the haemolymph of teneral adult male *Glossina*. This was estimated by radioimmunoassay to be 375 pmol g^{-1} . Whole insect extracts of teneral male flies of the same age contained $203 \pm 38 \text{ pmol g}^{-1}$ (mean $\pm 2 \times \text{s.e.}$, $n = 12$; unpublished observations of D.L.W.). Approximately half the total ecdysteroid content of the fly is therefore present in the haemolymph. Whole fly extracts assayed by radioimmunoassay have shown a concentration of 600 pmol g^{-1} in adult male *Calliphora* (J.K. unpublished observations) and the concentration of ecdysterone in adult male *Drosophila* measured by gas-liquid chromatography (350 pmol g^{-1} fresh weight) is in fact higher than at any other stage of its life cycle⁶. It has been known for some time that ecdysteroids are present in adult insects⁷. In female *Locusta*⁸ and *Bombyx*⁹ they are present in the ovaries and oocytes but previously there has been no hormonal function attributed to the high concentrations of ecdysteroids in adult male insects.

The rates of secretion of Malpighian tubules in Ringer's solution alone, in Ringer containing ecdysteroids and in Ringer containing other biologically active steroids are compared in Table 1. In all cases a delay between the introduction of the steroid and the commencement of secretion occurred (range 20–80 min) and the return to the base rate of secretion was slow. The time course of the response by *Glossina* Malpighian tubules to these steroids seems similar both to that of larval *Drosophila* salivary glands, exposed to ecdysterone¹⁰ and to the response of vertebrate transporting epithelia to the mineralocorticoid aldosterone¹¹. It was thought that the delayed response of the *Glossina* tubule preparation might similarly be due to the time required for the sequence of events (receptor interaction, transcription and translation) necessary for cells to respond to a steroid hormone. The delayed recovery might reflect activity of the proteins mediating the action of the steroid hormone continuing after the stimulating steroid was removed. To verify this, Malpighian tubules were stimulated to secrete by ecdysone in the presence of $10 \mu\text{g ml}^{-1}$ actinomycin D which irreversibly inhibits transcription. Their response was not reduced (Table 1). If ecdysone stimulates secretion by *Glossina* Malpighian tubules by controlling protein synthesis it presumably therefore acts post-transcriptionally.

Another way in which steroids might increase the rate of secretion is by altering the permeability of the basal membrane of the tubule cells. The rate of secretion of *Glossina* Malpighian tubules is thought to be determined by the permeability of their basal membrane to sodium¹² and it has been suggested that ecdysteroids are able to alter the permeability of cell and nuclear membranes to sodium and potassium¹³. Cholesterol is also known to alter the permeability of cell membranes¹⁴.

The results of these experiments suggest new lines of investigation into both the control of insect Malpighian

Table 1 Rate of secretion of isolated Malpighian tubules in the presence of steroids

Steroid present	Mean rate of secretion (nl min ⁻¹)	2 × s.e.	n
None	0.3	0.09	12
Ecdysone	4.2	0.98	20
Ecdysterone	3.4	1.06	9
Cortisol	3.1	1.16	7
Cholesterol	3.3	1.88	6
Aldosterone	4.5	1.13	9
Ecdysone + $10 \mu\text{g ml}^{-1}$ actinomycin D	5.1	1.22	10

Steroids were used at a concentration of 10^{-6} M, except for cholesterol which is only slightly soluble in water. A saturated solution of cholesterol in Ringer (containing approximately 0.2 mg per 100 ml H₂O) was centrifuged before it was added to the isolated preparation. The concentration of cholesterol in solution was approximately 5.17×10^{-6} M.

tubules and the function of ecdysteroids in insects. It has subsequently been shown that in *Locusta* also ecdysterone and cholesterol stimulate secretion by *in vitro* preparations of the Malpighian tubules (A. Bernstein and W. Mordue, in preparation). *Glossina* tubules respond to ecdysteroids at concentrations within the physiological range and this response suggests a function for the high concentrations of ecdysteroids present in adult male insects. The action of ecdysteroids (which control the growth and development of the larval stages of the insect) on the process of excretion in the adult insect is especially interesting since the Malpighian tubules are one of the few organs which remain intact throughout metamorphosis. We have little knowledge of the identities or titres of steroids circulating in the haemolymph of adult insects. But, as this investigation suggests that they may have regulatory functions in insects, over and above that of controlling the development of the larval and pupal stages, this may become an interesting new field of study.

We thank Dr J. D. O'Connor and Professor A. E. Kellie for advice about preparation of anti-sera for the radioimmunoassay of ecdysteroids. J. K. thanks the British Council for a grant to visit Britain and J. D. G. and D. L. W. thank the Ministry of Overseas Development for support.

JULIAN D. GEE*
DAVID L. WHITEHEAD

Tsetse Research Laboratory,
University of Bristol, School of Veterinary Science,
Langford, Bristol, UK

JAN KOOLMAN

Physiologisch-Chemisches Institut,
Phillips-Universität Marburg,
D-3550 Marburg, West Germany

Received 25 April; accepted 26 July 1977.

*Present address: Parasitology Department, Central Research, Pfizer Limited, Sandwich, UK.

- Koolman, J., Hoffman, J. A. & Dreyer, M. *Experientia* **31**, 247–249 (1975).
- Hoffman, J. A. & Koolman, J. *J. Insect Physiol.* **20**, 1593–1601 (1974).
- Gee, J. D. in *Transport of Ions and Water in Animals* (eds Gupta, B. L., Moreton, R. B., Oschman, J. L. & Wall, B. J.), (Academic, New York and London, 1977).
- Gee, J. D. *J. exp. Biol.* **64**, 357–368 (1976).
- Gee, J. D. *J. exp. Biol.* **63**, 391–401 (1975).
- Ginsburg, D. thesis, Univ. York (1977).
- Karlson, P. & Stamm, D. *Hoppe-Seyler's Z-physiol. Chem.* **306**, 109–111 (1956).
- Lagueux, M., Hirn, M. & Hoffman, J. A. *J. Insect Physiol.* **23**, 109–119 (1977).
- Legay, J.-M., Calvez, B., Hirn, M. & De Reggi, M. L. *Nature* **262**, 489–490 (1976).
- Ashburner, M. & Richards, G. in *Insect Development* (ed. Lawrence, P. A.) 203–225 (Blackwell, Oxford, 1976).
- Edelman, I. S. in *Transport Mechanisms in Epithelia*, Alfred Benzon Symposium **5**, (eds Ussing, H. H. & Thorn, N.) 185–204 (Munksgaard, Copenhagen, 1973).
- Gee, J. D. *J. exp. Biol.* **65**, 323–332 (1976).
- Kroeger, H. in *Metamorphosis* (eds Etkin, W. & Gilbert, L. I.) 185–219 (North-Holland, Amsterdam, 1968).
- Szabo, G. *Nature* **252**, 47–49 (1974).

Meiotic crossing-over in lily and mouse

CYTOLOGICAL descriptions of meiosis have long been a staple of biology textbooks, but a biochemical account of the process with a comparable claim to universality has been unavailable. We present here evidence for a common pattern of DNA metabolism in male meiotic cells of lilies and rodents during the zygotene-pachytene stages when crossing-over is presumed to occur. The prevalence of a common pattern between species that are as phylogenetically distant as lily and mouse has led us to conclude that the organisation for meiotic crossing-over in at least higher eukaryotes is probably universal in distribution. Four components of the pattern have been identified in separate studies of lilies, mice and rats, most of which have been or are being published elsewhere¹⁻³: the transient appearance of a protein ('R-protein') that facilitates DNA reannealing; the programmed introduction of single strand nicks on completion or near completion of chromosome pairing; the repair of endogenously formed nicks and gaps either immediately following or overlapping the interval of nicking; the preferential localisation of gaps and nicks in specific DNA sequences.

We described previously a protein in nuclei of *Lilium* meiocytes but not of its somatic cells which, like the gene-32 protein, facilitated DNA reannealing⁴. A preliminary report on the presence of a similar protein in testicular nuclei of various mammals was also published⁵. This protein was judged to be significant to chromosome pairing and crossing-over because of its absence during premeiotic chromosome replication, its prominence during zygotene and pachytene, and the reduction of pairing and chiasma formation on disrupting its normal association with nuclear lipoprotein by colchicine treatment⁶. A more detailed study⁷ of the R-protein purified from rat testicular nuclei showed it to have properties distinctly different from those of unwinding proteins in calf thymus which have been thoroughly analysed by Herrick and Alberts⁸. The R-proteins from rat and lily, on the other hand, are very similar to the T4 gene-32 protein with respect to promotion of DNA reassociation and of duplex unwinding in Mg^{2+} -free medium. They are distinctive, however, in that the capacity for reassociation and DNA binding can be destroyed by phosphorylation with a cyclic AMP-dependent protein kinase. Dephosphorylation with alkaline phosphatase restores the binding capacity⁷, but, as we have since observed, not the capacity for reassociation.

We fractionated mouse spermatocytes according to spermatogenic stage and analysed each of the fractions for R-protein activity. Although the different meiotic stages of spermatocytes cannot be separated as well as those of *Lilium* microsporocytes, the respective profiles of R-protein activity during meiosis are very similar (Fig. 1). In both organisms, the R-protein is most prominent during the intervals of zygotene and pachytene when pairing and crossing-over occur. In neither organism is there any evidence for a substantial presence during premeiotic DNA replication when DNA synthesis is at least 300 times greater than during meiotic prophase. Circumstantial evidence thus indicates that in the case of the R-protein, unlike the gene-32 or similar microbial proteins, the recombinational function has been separated from the replicative one. Although the precise role of the R-protein in meiosis remains to be defined and its apparently marginal presence in somatic tissues needs to be explored, the evidence now available points to the conclusion that the R-protein is essential to chromosome pairing and/or crossing-over among higher eukaryotic organisms.

In *Lilium*, single strand nicks and gaps appear in nuclear

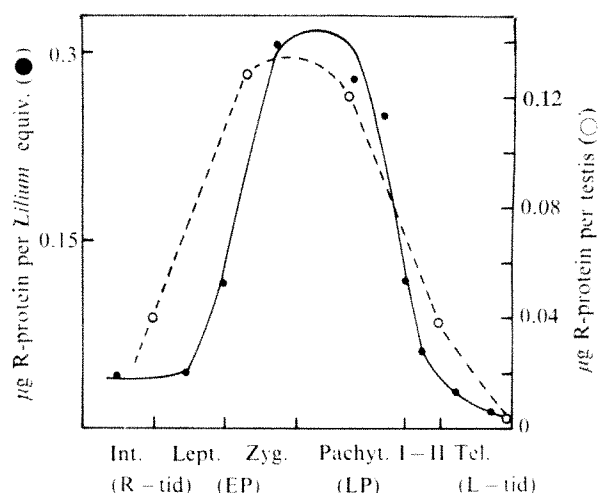


Fig. 1 Changes in R-protein during meiosis in lily microsporocytes (●) and rat spermatocytes (○). The data on lily are from previous studies⁴. To permit a rough comparison in R-protein content between the two organisms, we have recalculated the values on lilies so that the numbers for lily and mouse reflect approximately equal amounts of meiocyte DNA. Spermatocytes were separated by a modified Staput procedure⁹ into fractions designated by the predominant cell type: round spermatid (R-tid), early prophase (EP), late prophase (LP), and late spermatid (L-tid). Although R-tids are postmeiotic, the fraction is the only one containing cells in premeiotic S-phase and very early prophase. Separation of meiotic stages in the lily is unambiguous: Premeiotic interphase including S-phase (int.), leptotene (lept.), zygotene (zyg.), pachytene (pachyt.), Divisions I and II (I-II), telophase (tel.). Nuclei from pooled spermatocytes were suspended in a buffered medium consisting of 20mM Tris-HCl, pH 7.5-25mM KCl-3mM $CaCl_2$ -0.1mM dithiothreitol, sonicated for 45 s at 0°C, and the chromatin-free lipoprotein fraction isolated^{4,11}. The lipoprotein material was resuspended in buffered medium and assayed for duplex and single-stranded DNA binding activity¹¹. A discontinuous sucrose gradient was used to recover the bound DNA. The difference between the amounts of single strand and duplex DNA bound was considered to be R-protein. The activities are expressed in terms of R-protein content, based on assays of a purified protein which bound approximately 0.09 μ g (³H)T7-DNA per μ g R-protein.

DNA at about the time that the pairing of homologous chromosomes is completed⁹. We have interpreted such endogenously regulated nicking as a necessary condition for crossing-over and have adduced evidence for the absence of nicking in situations where crossing-over fails to occur. A pattern of DNA nicking similar to the one in lilies has now been found in mouse spermatocytes³. In both species, zonal sedimentation profiles of single stranded DNA are bimodal at pachytene and unimodal at all other stages of meiosis, thus reflecting the introduction of nicks at the pachytene stage. The unimodal profiles peak at about 105S in the lily and 160S in the mouse, corresponding to molecular weights of 1.8 and 5.1×10^8 (ref. 10). Significantly perhaps, the slower sedimenting component in each of the bimodal profiles peaks at about 62S corresponding to a molecular weight of 48×10^6 . The changes in proportion of 62S DNA at different stages of meiosis in lily and mouse are shown in Fig. 2. Although the fraction of 62S DNA at pachytene is slightly greater in the mouse, the total number of nicks per meiocyte is much larger in lilies, which have a haploid DNA content about 20 times that of mice. Since there are approximately 36 chiasmata per lily microsporocyte and 20 per mouse spermatocyte, the efficiency of converting nicks into crossovers is greater in mouse than in lily. There may indeed be an inverse relationship between genome size and efficiency of such conversion. In lily, there are about 1×10^4 nicks per crossover; in yeast, where a pattern of nicking similar to the one in lily has been

observed, 50% of the nicks seem to be converted into reciprocal crossovers¹¹. Although we have no information on the mechanism relating nicking to the ultimate event of crossing-over, the occurrence of meiotic nicking in yeast lily and mouse, is compelling evidence for the universality of endogenously regulated nicking in eukaryotes as a background condition for crossing-over.

Implicit in the profiles shown in Fig. 2 is the occurrence of sufficient repair synthesis during pachytene to restore the linear integrity of the DNA to its prepachytene level. Direct evidence for repair replication has been obtained in both mouse and lily by pulse labelling of pachytene cells that had previously incorporated bromodeoxyuridine during the premeiotic S-phase^{3,12}. But, meiotic cells, like somatic ones, can repair DNA lesions at almost any stage of development, so that nicking, whether endogenous or exogenous in origin, would be expected to promote repair synthesis. That endogenous pachytene repair synthesis shares many features with exogenously induced repair has been adequately documented. There are, nevertheless, two distinguishing features of the endogenous process. One of these is the elevated capacity for repair in pachytene cells, a property that has been well demonstrated in the mouse¹², and can also be demonstrated in lily. The other, more significant feature, concerns specific sites of nicking and repair, as discussed below.

Commonly, frequency of crossing-over between two loci in a chromosome is considered to be a function of the physical distance between them. Although broad exceptions to this relationship have been documented¹³, it might, nevertheless, be supposed that endogenous meiotic repair synthesis would be randomly distributed in the genome. This, however, is not the case. C_0t profiles of DNA synthesised during pachytene show that the endogenous synthesis occurs preferentially in moderately repeated sequences (Fig. 3). Such preference is a unique characteristic of meiotic repair and not of repair in general; radiation-induced repair tracks the C_0t profile of total DNA regardless of meiotic stage. In lily, 50%–70% of endogenous pachytene repair occurs in sequences that are repeated about 1–2,000 times per genome and constitute at least 0.1% of

Fig. 2 Changes in sedimentation behaviour of single-stranded DNA prepared from microsporocytes and spermatocytes at different stages of meiosis. The profiles are constructed from data obtained in analyses of gently lysed preparations over alkaline glycerol gradients. The proportion of 62S DNA is calculated from the weight distribution of DNA in the region of the gradient containing the 62S component. Designation of meiotic stages is the same as in Fig. 1. Stages beyond pachytene have been grouped under post-pachytene (Post-pachy.). Assignment of sedimentation characteristics to specific spermatocyte stages is easier than in the case of the R-protein because the 62S component is prominent only during the pachytene stage. ●, Lily; ○, mouse.

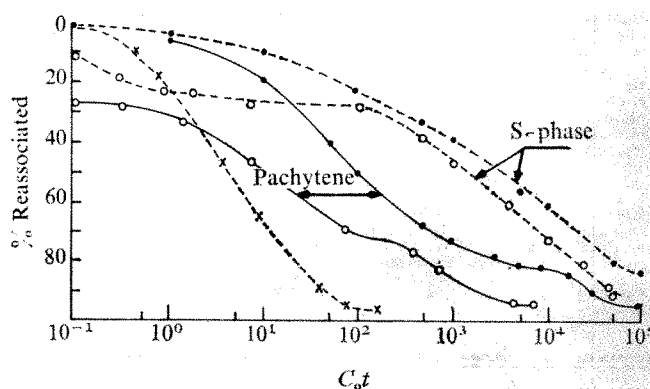
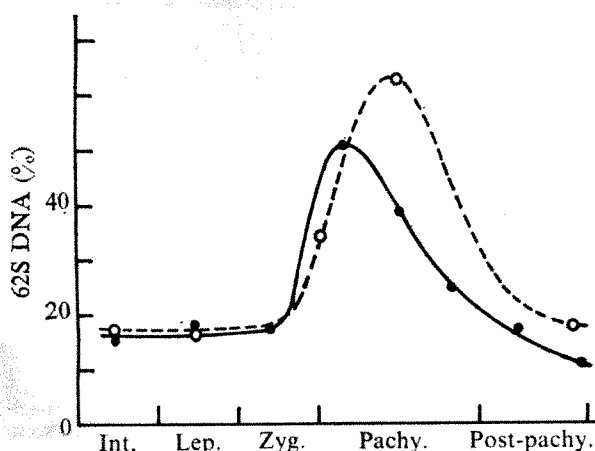


Fig. 3 Reassociation characteristics of mouse and lily DNA synthesised during the pachytene stage. The data displayed are from earlier analyses of *Lilium* microsporocytes¹⁸ and from recent analyses of mouse spermatocytes⁷. In the case of the mouse, the data on pachytene are from mice treated with hydroxyurea to inhibit semiconservative synthesis. In the absence of hydroxyurea, the pachytene profile, though essentially the same, is less pronounced because of contamination by S-phase cells⁶. C_0t characteristics of S-phase labelled DNA are included for comparison. ●, Lily; ○, mouse; ×, *Escherichia coli*.

the total DNA¹⁴. The precise lengths of the repeats have not been determined, but we estimate them to be in the neighbourhood of 100–200 bases¹⁵. In mouse, 50% of the repair occurs in sequences that are repeated about 400 times per genome; the proportion of DNA thus involved is undetermined but it cannot be detected by absorbance measurements, and is probably less than 1–2%. There is, however, one feature of pachytene synthesis in mouse spermatocytes which is not evident in lilies. In the profile shown in Fig. 3, about 25% of the pachytene label reassociates at a C_0t value below 10^{-2} . This rapidly reannealing region is being analysed in greater detail, but it is already clear that with 400–500 nucleotide fragments about 15% of the total pachytene label reassociates as foldback DNA, and that 1/3 of the label present in the foldbacks is in duplex form. This observation is pertinent to speculations about the involvement of hairpin loops in crossing-over^{16,17}. The location of repeated sequences in lily and mouse in relation to single-copy ones needs to be determined, but it is evident from Fig. 3 that pachytene labelling does not occur exclusively in repeat sequences but is also present among the lower repeat sequences.

The evidence, limited though it is, points unambiguously to the conclusion that in organisms as diverse as lily and mouse, metabolic events that are temporally associated with meiotic crossing-over share a common pattern. The preferential and programmed nicking of DNA in regions of moderate repeats, the similarity in spacing between nicked sites, and the temporal correlation between endogenous repair synthesis and cytological stage, are impressive examples of a conserved biochemical program for meiosis. In our opinion, these examples provide for the observed regularity in crossing-over among higher organisms.

This work was supported by grants from the USNIH (HD 03015) and the NSF (PCM 76-09660).

YASUO HOTTA
ANN C. CHANDLEY*
HERBERT STERN

Department of Biology
University of California, San Diego
La Jolla, California 92093

Received 21 April; accepted 26 July 1977.

*Permanent address: MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK.

- ¹ Stern, H. & Hotta, Y. *Phil. Trans. R. Soc. Lond. B.* **277**, 277-293 (1977).
- ² Chandley, A. C., Hotta, Y. & Stern, H. *Chromosoma* **62**, 243-253 (1977).
- ³ Hotta, Y., Chandley, A. C. & Stern, H. *Chromosoma* **62**, 255-268 (1977).
- ⁴ Hotta, Y. & Stern, H. *Dev. Biol.* **26**, 87-99 (1971).
- ⁵ Hotta, Y. & Stern, H. *Nature new Biol.* **234**, 83-86 (1971).
- ⁶ Hotta, Y. & Shepard, J. *Molec. gen. Genet.* **122**, 243-260 (1973).
- ⁷ Mather, J. & Hotta, Y. *Expl Cell Res.* (in the press).
- ⁸ Herrick, G. & Alberts, B. *J. biol. Chem.* **251**, 2124-2132 (1976).
- ⁹ Hotta, Y. & Stern, H. *Chromosoma* **46**, 279-296 (1974).
- ¹⁰ Studier, F. W. *J. molec. Biol.* **11**, 373-390 (1965).
- ¹¹ Jacobson, G. K., Piñon, R., Esposito, R. E. & Esposito, M. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1887-1891 (1975).
- ¹² Kofman-Alfaro, S. & Chandley, A. C. *Expl Cell Res.* **69**, 33-44 (1971).
- ¹³ Jones, G. H. *Heredity* **32**, 375-387 (1974).
- ¹⁴ Smyth, D. R. & Stern, H. *Nature new Biol.* **245**, 94-96 (1973).
- ¹⁵ Hotta, Y. & Stern, H. in *The Eukaryote Chromosome* (eds Peacock, W. J. & Brock, R. D.) 283-300 (Australian National University Press, Canberra, 1975).
- ¹⁶ Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 279-283 (1975).
- ¹⁷ Wagner, Jr, R. E. & Radman, M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3619-3622 (1975).

Evidence for two active X chromosomes in germ cells of female before meiotic entry

THERE is considerable evidence that, as a result of some early embryonic event, the genes of one X chromosome in each somatic cell of the human female are not expressed¹. Studies of somatic tissues and cultured cells (including fibroblast clones) from human embryos, heterozygous for the common electrophoretic variant of glucose-6-phosphate dehydrogenase (G6PD), indicate that a single active X is present in cells from various tissues, at least by 5 weeks from conception². On the other hand, the presence of the heteropolymorphic form of the enzyme in oocytes of the heterozygous adult female³ and a 16-week-old foetus⁴ is compelling evidence that, in meiotic stage germ cells, there are two active X chromosomes; however, the basis for the two active chromosomes in oocytes is not clear. It is conceivable that germ cell progenitors escape inactivation because inactivation occurs when cells destined to become germ cells have already been imprinted. Alternatively, only a single X chromosome may be active in all cells of the early zygote, with the second X activated only in germ cells at some time after their differentiation. The third possibility, that germ cells are subject to inactivation, but that reactivation occurs when meiosis commences, has been suggested by Gartler *et al.* because they did not observe the heteropolymer in ovaries from a 12-week-old human foetus heterozygous for the G6PD variant^{5,6}. The ovary at that developmental stage contains approximately 23% germ cells, with a preponderance of premeiotic and leptotene germ cells⁷. But the implication that two functional X chromosomes are required for the onset of mammalian meiosis is not supported by evidence that XO mice produce normal gametes⁸. Using human foetal material, we have now obtained evidence against the third possibility.

We have studied ovarian extracts from foetuses heterozygous for the G6PD_A variant. Specimens were obtained

(according to an approved protocol and with maternal consent) from dead foetuses in the Fertility Control Unit of the Johns Hopkins Hospital, where every effort is made to assure the accuracy of historical data. Electrophoresis for G6PD was carried out on cellulose acetate gels as before². During this study, we obtained G6PD isozyme patterns from 35 males and 49 females of foetal age 14-21 weeks. The patterns observed in lung tissue from these specimens indicate that the frequencies of the G6PD_A and G6PD_B alleles in the foetus are essentially that observed in somatic cells of a comparable adult population, and that, as expected, males have a single isozyme (Table 1). The age of each specimen was estimated from time of conception, on the basis of last menstrual period and measurements of mean foot length⁹ and crown-rump length of the foetus (Table 2). From specimens obtained after urea-prostaglandin infusion, we ascertained 17 females of 14-21 weeks gestation who were heterozygous for the G6PD variants, and obtained ovarian tissue from 10 of them. From examination of more than 100 specimens obtained from suction procedures, we obtained a single appropriate specimen of ovary from a foetus of 8 weeks'

Table 1 G6PD phenotype of foetal lung specimens

	Male	Female
No. of foetal specimens	35*	49
G6PD _{AB}	0	17
G6PD _A	7	6
G6PD _B	28	26

* 23 further male specimens were ascertained, but not analysed for G6PD.

conceptual age. Foetal specimens were assayed directly for the G6PD phenotype and, in each case, samples of lung were used as the somatic cell control for the ovarian specimen. Ovarian extracts were enriched for germ cells by pressing the foetal ovaries gently under a cover glass^{5,10}.

The results of electrophoretic studies of these 11 specimens are presented in Table 2 and Fig. 1. In each case, lung specimens revealed the heterozygosity, having both A and B isozymes. In all ovarian specimens studied, there were three isozymes with the heteropolymer migrating between the A and B bands. The intensity of the heteropolymer varied considerably. The band ratio of 1A:2 heteropolymer:1B, which might be expected for the three enzymes in a pure population of oocytes, was not seen even in the specimens from the most mature foetuses (20-21 weeks), indicating dilution of the ovarian specimen by non-germ cells. We confirmed previous observations that the heteropolymer was less intense in cells of younger

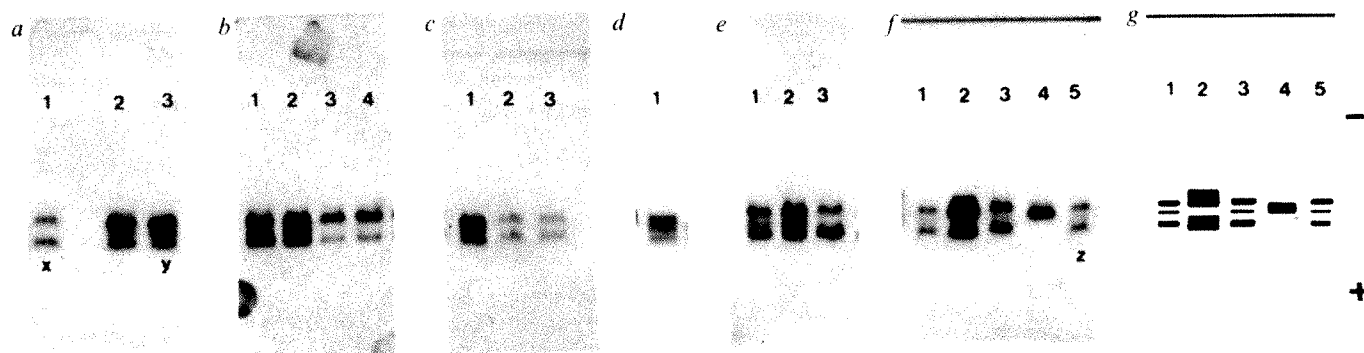


Fig. 1 G6PD isozyme patterns from foetal somatic and germ cell extracts. *a*, M-63, 19-20 weeks: 1, lung; 2, ovary; 3, ovary enriched for germ cells. *b*, M-82, 17 weeks: 1, ovary; 2, ovary enriched for germ cells; 3 and 4, lung. *c*, M-46, 17 weeks: 1, ovary enriched for germ cells; 2 and 3, lung. *d*, M-41, 16-17 weeks: 1, ovary enriched for germ cells; 2, lung; 3, ovary; 4, G6PD_B marker. *e*, M-73, 14-15 weeks: 1, ovary; 2, ovary enriched for germ cells; 3, lung. *f* and *g*, S-19, 8 weeks: 1 and 5, ovary enriched for germ cells. *h*, S-18, 8 weeks: 1 and 5, ovary enriched for germ cells. x, y and z indicate the source of the tracing in Fig. 2.

Table 2 Characteristics of foetal specimens heterozygous for the G6PD variants where ovaries were analysed

Specimen	Foetal age in weeks				G6PD phenotype	
	From last menstrual period	Estimated menstrual*	Estimated gestational†	Probable foetal age	Lung	Ovary
M49	24	22-23	21	21	==	==
M43	18-19	22-23	20	20	==	==
M63	13-17	21-22	19	19-20	==	==
M53	21	20-21	19	19	==	==
M82	22	18	17	17	==	==
M115	20-21	18	17	17	==	==
M46	19	18	17	17	==	==
M41	20	19	16	16-17	==	==
M28	16	—	16	15-16	==	==
M73	14	16	15	14-15	==	==
S19	9-10	9-10	8	8	==	==

* Based on mean foot length.

† Based on crown-rump length.

specimens', most likely because of relatively greater contamination of these specimens by ovarian somatic cells. This is consistent with the lesser number of germ cells known to be in ovaries of younger fetuses^{7,11}. In contrast to previous observations³, we observed a heteropolymer even in the specimen from the 8-week-old foetus (Figs 1f and g and 2), at a time when the germ cell population is essentially premeiotic^{7,11}. Although a few germ cells in the leptotene and zygotene stages of first meiotic metaphase have been described in the 12-13-week ovary, meiotic cells are not found in significant numbers until 16 weeks from conception¹². The ovary of the 8-week specimen was at the lower limit of size necessary for the identification of the pertinent tissue; therefore, it is unlikely that studies of isozyme analysis of ovaries in younger foetal specimens are feasible.

Our observations indicate that two X chromosomes are functioning in female germ cells, even before meiotic entry, and they are supported by the cytological observations of Ohno, who did not observe a precociously condensed X chromosome in mouse oogonia, nor in cells entering meiosis¹². Because there seemed to be one condensed X chromosome in primordial germ cells of the yolk sac, he suggested that germ cells are X-inactivated but reactivated at the time of differentiation of primordial

germ cells into oogonia in the ovary. The presence of two active X chromosomes in premeiotic germ cells, reported here, does not differentiate between escape from inactivation and reactivation (or activation of the second X), but excludes reactivation coincident with the onset of meiosis.

This work was supported by a grant from NIH.

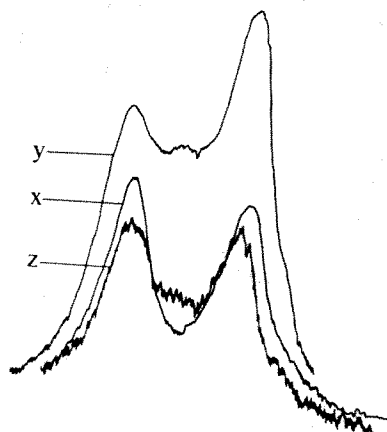
BARBARA R. MIGEON
KAREN JELALIAN

Department of Pediatrics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

Received 11 May; accepted 20 June 1977.

- 1 Lyon, M. F. *Biol. Rev.* 47, 1-35 (1972).
- 2 Migeon, B. R. & Kennedy, J. F. *Am. J. hum. Genet.* 27, 233-239 (1975).
- 3 Gartler, S. M., Liskay, R. M., Campbell, B. K., Sparkes, R. & Gant, N. *Cell Differentiation* 1, 215-218 (1972).
- 4 Gartler, S. M., Liskay, R. M. & Gant, N. *Expl Cell Res.* 82, 464-466 (1973).
- 5 Gartler, S. M., Andina, R. & Gant, N. *Expl Cell Res.* 91, 454-457 (1975).
- 6 Gartler, S. M. & Andina, R. J. *Adv. hum. Genet.* 7, 99-140 (1976).
- 7 Baker, T. G. *Proc. R. Soc., Lond. B158*, 417-433 (1963).
- 8 Cattanach, B. M. *Genet. Res.* 3, 487-490 (1962).
- 9 Streeter, G. L. *Carnegie Inst. Contrib. Embryol.* 11, 145-170 (1921).
- 10 Blandau, R. J., White, B. J. & Rumby, R. E. *Fertil. Steril.* 14, 482-489 (1963).
- 11 Ohno, S., Klinger, H. P. & Atkins, N. B. *Cytogenetics* 1, 42-51 (1962).
- 12 Ohno, S. in *Second int. Conf. Congenital Malformations*, 36-40 (National Foundation, New York, 1963).

Fig. 2 Tracing from Joyce-Loebl recording microdensitometer showing G6PD_{AB} isozyme pattern x without heteropolymer from lung shown in Fig. 1a, slot 1; y with prominent heteropolymer from ovary shown in Fig. 1a, slot 3; and z with heteropolymer from ovary of 8-week specimen shown in Fig. 1f, slot 5.



Transcriptional controls and dosage compensation in *Drosophila melanogaster*

DOSAGE compensation is the equalisation of X-linked gene products in males and females. In *Drosophila* there is substantial evidence to support the contention that both X chromosomes are simultaneously active in somatic cells of females¹, and that the regulatory mechanism operates at the level of transcription^{2,3}. Most discussions of this mechanism have focused on the modulation of regulatory factors controlling the rate of RNA synthesis per template^{4,5}. A change in the level of transcription may also be achieved by altering the quantity of template. We present here various arguments and some experimental evidence discounting controlled variation of total template as the basis for dosage compensation in *Drosophila melanogaster*.

Three means by which the number of copies of X-linked genes may be equalised in male and female nuclei come to mind. First, the X chromosome may replicate earlier during the phase in males than in females. This is thought to be the case in polytenic nuclei of larval salivary glands^{6,7}. During a portion of the cell cycle, old and new copies of X-linked genes would be available

for transcription in male cells; the number of genes per cell would, therefore, be the same in males and females until that time when replication of X-linked genes occurred in the latter. A serious objection to this model is based on the observation that the S phase constitutes at most 30%–40% of the cell cycle (measurements performed on cleaving embryos and cells in culture)^{8–10}. The male cells, therefore, spend a large portion of their existence in G1 or G2 where the dosage of their X-linked genes relative to that of autosomal genes is either 1:2 or 2:4. Furthermore, if the difference in time of X chromosome replication between males and females were significant, a considerable proportion of the cells sampled at any given time in males and in females should have equivalent X DNA content. But, microspectrophotometric measurements of Feulgen-stained chromosomes indicate that the paired X's in the salivary gland nuclei of female larvae have twice the DNA content of the single X in males (each measurement is made in reference to an autosomal, internal control)^{11–12}. It is, therefore, improbable that the early replication of X-linked genes in male cells could lead to equal transcriptional steady states in males and females.

A second possibility for template modulation would be the differential replication of X-linked genes in males. The unequal replication of specific sequences of the polytenic X chromosome in larval salivary glands is well documented. In a fully mature salivary gland chromosome, the centric heterochromatin including the highly repetitive DNA sequences has not undergone any endoreplication; the nucleolus organiser region containing the ribosomal RNA cistrons is present in 150 to 200 laterally redundant copies; the euchromatic portion of the chromosome is represented by more than 1,000 copies^{13,14}. If the coding segments of all structural genes were to undergo an extra round of replication in male cells, their dosage would become equal to that of female cells. Recent experimental evidence makes the differential replication model of dosage compensation unlikely. Electron microscope measurements of nascent RNA transcripts suggest that these molecules are of considerable size, perhaps corresponding to entire chromomeres which, in *Drosophila*, average 30 kilobases (kb) in length¹⁵. The median size of poly(A)-containing nuclear RNA is 6 kb, representing 20% of an average chromomere¹⁶. In terms of the model, this should lead to a substantial amount of extra DNA in males which, as mentioned above, does not seem to be present.

Finally, the equalisation of effective X chromosome

coding sequences in males and females may be achieved through polyteny itself. If only a small fraction of all strands were active in transcription, males may compensate for X-linked gene dosage differences by transcribing twice the number of strands per X chromosome as would be active in females. Implicit to this model is the contention that when gene activity is measured in whole organisms at the level of enzyme activity or of a terminal phenotypic product (eye pigment, for example), the compensatory effect stems from gene activity in polytenic cells. This cell type is found in both larvae and adults and may be ubiquitous enough to mask the lack of compensation in the strictly diploid tissues of the organism.

To test whether dosage compensation is the result of some mechanism inherent to polytenisation, we have compared the expression of X-linked genes in imaginal disks and larval brain to that in larval fat body. The brain is mostly diploid with a very small fraction of cells which may be polytenic^{14,17}; imaginal disk cells are diploid¹⁷; fat body cells are clearly polytenic¹⁸. Table 1 shows activity levels of two X-linked enzymes (glucose-6-phosphate dehydrogenase—G6PD, and 6-phosphogluconate dehydrogenase—6PGD) normalised to the activity levels of three autosomal enzymes (NADP-dependent isocitrate dehydrogenase, α -glycerophosphate dehydrogenase, and aldehyde oxidase). Measurements were performed in larval tissues and adult thoraces and heads. All male individuals carried w^+Y , a Y chromosome bearing a small X fragment which includes the structural gene of 6PGD. Gene dosage for this enzyme is, therefore, the same in males and females, providing control evidence that activity differences of the magnitude expected could be detected by our experimental procedure. The male-female ratios of normalised G6PD activity are equal to or slightly greater than one, while the ratios of 6PGD activity are slightly less than or equal to two, in disks, fat body and adult tissues. These observations suggest the occurrence of dosage compensation in all these tissues.

In summary, three possible models for equalising the number of copies of X-linked genes in *Drosophila* males and females have been considered. Existing data regarding the amount of DNA in X chromosomes and the proportion of the S phase in the cell cycle have been used to discredit early replication of the male X as a possible explanation for dosage compensation. It is unlikely that differential endoreplication of X-linked structural genes is occurring in males. Finally, we have shown that compensation cannot be ascribed to polytenisation *per se*. Therefore, we conclude that mechanisms that vary the relative

Table 1 Normalised X-linked gene activities in males and females

		G6PD/IDH	6PGD/IDH	G6PD/AO	6PGD/AO	G6PD/GPDH	6PGD/GPDH
♂	Disk	0.316 ± 0.03 (5)	0.698 ± 0.039 (5)	0.265 ± 0.054 (5)	0.575 ± 0.094 (5)		
♀	Disk	0.291 ± 0.022 (5)	0.381 ± 0.03 (5)	0.267 ± 0.032 (5)	0.345 ± 0.05 (5)		
♂/♀	Disk	1.08	1.83	0.99	1.67		
♂	Fat body	0.97 ± 0.08 (6)	2.31 ± 0.11 (6)			1.11 ± 0.1 (3)	2.57 ± 0.09 (3)
♀	Fat body	0.79 ± 0.06 (6)	1.11 ± 0.11 (6)			1.09 ± 0.12 (3)	1.57 ± 0.18 (3)
♂/♀	Fat body	1.22	2.08			1.01	1.64
♂	Adult	0.301 ± 0.02 (7)	0.416 ± 0.016 (7)	0.495 ± 0.012 (3)	0.545 ± 0.016 (3)		
♀	Adult	0.324 ± 0.031 (7)	0.254 ± 0.019 (7)	0.410 ± 0.061 (3)	0.296 ± 0.039 (3)		
♂/♀	Adult	0.93	1.64	1.21	1.84		

♂ Signs for male; ♀ signs for female.

Drosophila melanogaster stock, $y w^+/w^+Y$ (y , yellow; w^+ , white-apricot) was raised at 22°C on standard medium. Imaginal disks (with attached brain) and fat body were dissected from late third instar larvae in *Drosophila* Ringer's. Adult flies with abdomens removed were also used to prepare extracts. Imaginal disks from about 80 larvae, fat body from 15 female or 25 male larvae, or the anterior end of eight adults comprised a single sample. Tissues were transferred to a 1.5 ml Brinkman-Eppendorf microtest tube and homogenised in 0.125 ml of distilled water with a Teflon homogeniser. The homogenates were allowed to stand on ice for 20 min, centrifuged at 12,000g, and the supernatant was used as the extract for enzyme assays. Assay conditions for G6PD, 6PGD, α -GPDH and IDH were as described previously¹⁹. AO assay conditions were those of Dickinson²⁰. Normalised activity measurements were obtained by dividing X-linked gene activity by autosomal gene activity for each extract. The mean ratio is given \pm s.e. with the number of extracts in parentheses. The male/female values are ratios of the means.

amounts of template in males versus females are not adequate to account for dosage compensation in *Drosophila*.

We thank Mr James Kitchens for experimental assistance and Dr G. Maroni for critical advice. This work was supported by the US NIH.

RICHARD L. ROEHRDANZ
JOHN C. LUCCHESI

Curriculum in Genetics and
Department of Zoology,
University of North Carolina,
Chapel Hill, North Carolina 27514

Received 24 April; accepted 18 July 1977.

- 1 Kazanian, H. H., Jr, Young, W. J. & Childs, B. *Science* **150**, 1601–1602 (1965).
- 2 Mukherjee, A. S. & Beermann, W. *Nature* **207**, 785–786 (1965).
- 3 Korge, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4550–4554 (1976).
- 4 Maroni, G. & Plaut, W. *Genetics* **74**, 331–342 (1973).
- 5 Lucchesi, J. C. *Ann. Rev. Genet.* **7**, 225–238 (1973).
- 6 Berendes, H. C. *Chromosoma* **20**, 32–43 (1966).
- 7 Lakhotia, S. C. & Mukherjee, A. S. *J. Cell Biol.* **47**, 18–33 (1970).
- 8 Blumenthal, A. B., Kriegstein, H. J. & Hogness, D. S. *Cold Spring Harb. Symp. quant. Biol.* **38**, 205–233 (1974).
- 9 Dolfini, S. A., Courgeon, A. M. & Tiepolo, L. *Experientia* **26**, 1020–1026 (1971).
- 10 Ananiev, E. V., Palakarova, L. G. & Yurov, Y. B. *Chromosoma* **59**, 259–272 (1977).
- 11 Aronson, J. F., Rudkin, G. T. & Schultz, J. J. *Histochem. Cytochem.* **2**, 258–259 (1954).
- 12 Rudkin, G. T. in *The Nucleohistones* (eds Bonner, J. & Tso, P.) 184–192 (Holden-Day, San Francisco, 1964).
- 13 Hennig, W. & Meer, B. *Nature new Biol.* **233**, 70–72 (1971).
- 14 Spear, B. B. & Gall, J. G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1359–1363 (1973).
- 15 Laird, C. D. & Chooi, W. Y. *Chromosoma* **58**, 193–218 (1976).
- 16 Lamb, M. M. & Laird, C. D. *Biochem. Genet.* **14**, 359–371 (1976).
- 17 van de Fliedert, K. *Chromosoma* **50**, 431–434 (1975).
- 18 Ashburner, M. *Adv. Insect Physiol.* **7**, 1–95 (1970).
- 19 Lucchesi, J. C. & Rawls, J. M. *Biochem. Genet.* **9**, 41–51 (1973).
- 20 Dickinson, W. J. *Genetics* **66**, 487–496 (1970).

Endocytosis of red blood cells or haemoglobin by activated macrophages inhibits their tumoricidal effect

MICE with chronic *Bacillus Calmette-Guérin* (BCG) or *Toxoplasma gondii* infection have increased resistance to tumour growth, and peritoneal macrophages from these mice can selectively kill neoplastic cells *in vitro* by non-phagocytic means^{1,2}. Although the actual molecular mechanism of killing is unknown, the tumoricidal macrophage (TM) vacuolar system is probably involved. Phase contrast³ and electron microscopy studies⁴ have suggested the translocation of TM lysosomes into tumour cells with subsequent tumour cell death. Trypan blue, an inhibitor of lysosomal enzymes, or hydrocortisone, a membrane stabiliser and inhibitor of membrane fusion, will suppress the *in vitro* tumour cell killing by TM (ref. 3). We report here that endocytosis of red blood cells (RBC), haemoglobin or haemoglobin degradation products by TM inhibits their tumoricidal effect.

In vitro macrophage-mediated tumour cell killing was assayed as described previously³ with some modifications as described in the legend to Fig. 1. Tumour cell killing was assessed by microscopic examination or by measurement of tritiated thymidine (³HTdR) release from pre-labelled 3T12 cells⁵. The results were comparable using TM from either BCG- or *T. gondii*-infected mice.

Results of a typical experiment are shown in Fig. 1. Control TM destroyed very nearly all the 3T12 cells (Fig. 1a). But if the TM had phagocytosed RBC, the killing was markedly suppressed. In Fig. 1b, with an RBC:TM ratio of 4:1, there is complete inhibition of the killing. The inhibition seems to be proportional to the RBC:TM ratio with less complete suppression of killing apparent in Fig. 1c and d with RBC:TM ratios of 2:1 and 1:1, respectively. Experiments using a ³HTdR release assay for tumour killing gave comparable results. For example, the ³HTdR release expressed as a percentage of maximal release (100% release with no RBC added) was 3.2 ± 2.0%, 9.5 ± 3.4%, and 14.4 ± 2.7% for RBC:TM ratios of 3.00:1, 1.50:1, and 0.75:1 respectively. Figure 2 shows the microscopic features of the inhibitory effects of RBC.

Inhibition was seen when RBC were phagocytosed by the TM. Heat-damaged, glutaraldehyde-treated⁷, or antibody-opsonised⁸ (Cordis Laboratories, rabbit IgG anti-sheep RBC) RBC were all

avidly phagocytosed (>90% of the TM contained ≥2 RBC at an RBC:TM ratio of 4:1). If fresh bovine RBC and serum, or fresh mouse RBC and serum syngeneic to the TM (C₃H-He) were present throughout the assay, no TM phagocytosis of RBC occurred. In these situations, there was no inhibition of tumour killing despite close contact between RBC and TM. Of the species examined (human, bovine, rabbit, mouse, chicken, sheep), none was tested that did not suppress killing as long as the RBC were phagocytosed. Syngeneic RBC inhibited if they were phagocytosed. The TM remained viable after the phagocytosis as judged by Trypan blue exclusion, absence of release of the cytoplasmic enzyme lactic dehydrogenase (LDH) (refs 9, 10) and maintenance of their adherence to plastic. TM that had phagocytosed RBC in pre-incubation (with free RBC then washed away, also had diminished tumoricidal effect. *In vivo* loading of TM by intraperitoneal injection of glutaraldehyde-treated RBC 3 d before the assay also resulted in TM with suppressed tumour killing ability.

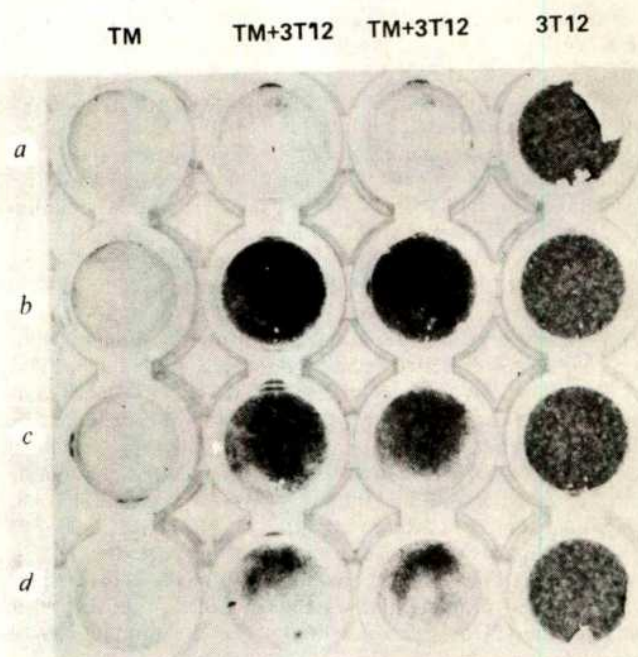


Fig. 1 Photograph of giemsa-stained assay of macrophage-mediated tumour cell killing demonstrating inhibition by red blood cells (RBC). Swiss Webster female mice were infected with either the Paris strain of BCG intraperitoneally (i.p.)³, or the C56 strain of *Toxoplasma gondii* i.p.⁶. At 2–3 weeks after BCG infection or 8–24 weeks after toxoplasma infection, the mice were injected with 2 ml of sterile 10% peptone i.p., and 3 d later the peritoneal cells were collected. Peritoneal cells (4×10^5) in 0.1 ml of Dulbecco's modification of Eagle medium supplemented with streptomycin ($100 \mu\text{g ml}^{-1}$), penicillin (100U ml^{-1}), HEPES buffer (0.02 M), and glucose (1 mg ml^{-1}) (DMM) were placed into the microtitre wells (Falcon plate 3040) and incubated for 60 min at 37°C in humidified 95% air–5% CO_2 . The nonadherent cells were removed then by washing twice with 0.15 M NaCl (saline). Human RBC were obtained from heparin-anticoagulated blood and washed three times with saline. The RBC were fixed for 30 min at room temperature in 1% glutaraldehyde–saline and washed five times with 15–20 volumes of saline each time. After removal of nonadherent peritoneal cells, 3T12 tumour cells (6×10^3 per well) and appropriate RBC additions were applied in DMEM with 10% foetal calf serum. The plates were incubated for 60 h at 37°C in humidified 95% air–5% CO_2 , fixed, and stained with giemsa. Multilayers of 3T12 cells stain dark while TM are not grossly stained. The defects in the tumour cell multilayer apparent in the '3T12' column on rows a and d are artefacts due to detachment and rolling up of the multilayer from the substrate. Row a, (control, no RBC added) reveals the tumoricidal effect of the macrophages seen in the 'TM + 3T12' wells when compared to the control '3T12' well which has no TM. Numbers of RBC in test wells are: b, 1×10^6 ; c, 5×10^5 ; d, 2.5×10^5 . b–d Demonstrate the RBC inhibition of TM-mediated 3T12 killing with grossly visible multilayers of 3T12 in the 'TM + 3T12' wells. Microscopic examination of a '3T12' well stained 3 h after seeding 6×10^3 3T12 (not shown) reveals 33–37 cells per 300 power field. The 'TM + 3T12' wells in (a) have 0–3 3T12 cells per 300 power field. But, in (d), killing is less complete with many fields having more than three tumour cells and several having a multilayer. Virtually all fields in b and c have multilayers of 3T12 cells.

The act of phagocytosis, *per se*, along with all the accompanying metabolic consequences¹¹, seemed not to be responsible for the RBC-induced inhibition of tumour killing. Latex spheres, either 5.7- μm diameter styrene divinyl benzene (SDB) or 1.01- μm diameter polystyrene (Dow Chemical), were avidly endocytosed by the TM. There was, however, no inhibition of tumoricidal activity until the sphere:TM ratio exceeded 1000:1 for the polystyrene spheres or 100:1 for the SDB spheres. At these ratios, the TM were clearly dead as evidenced by Trypan blue uptake and LDH release. For example, at an SDB:TM ratio of 3:1, there was no inhibition of 3T12 killing and 0% extracellular release of TM LDH, whereas there was complete inhibition of 3T12 killing at an SDB:TM ratio of 100:1 with 61.4% release of the total TM LDH.

Red blood cell ghosts that were phagocytosed after glutaraldehyde treatment or antibody opsonisation did not inhibit killing even at a ghost:TM ratio of 100:1. But, endocytosed¹² ghost-free RBC lysate or chromatographically separated haemoglobin (Hb) was inhibitory at Hb levels $\geq 250 \mu\text{g ml}^{-1}$ (either native or heat denatured). Haem-free globin, prepared by acid-acetone extraction of Hb (ref. 13), was quite unstable in culture media at 37 °C and precipitated as 0.5–1.0- μm diameter spherules. These very hydrophobic globin particles were endocytosed by TM and inhibited tumour killing at levels of 500–1000 $\mu\text{g ml}^{-1}$. Methaemalbumin prepared as described by Tenhunen *et al.*¹⁴ was inhibitory at levels of 500–2,000 $\mu\text{g ml}^{-1}$, whereas albumin treated comparably but lacking haem had no effect on tumour killing. To determine whether the iron of the haem was responsible for the inhibition, ferric chloride (FeCl_3), ferrous sulphate (FeSO_4), and iron dextran (Imferon) were examined. Imferon (200–1,000 μg elemental iron per ml) or FeCl_3 or FeSO_4 (1–40 $\mu\text{g ml}^{-1}$), when left in through the killing assay or used in a pretreatment fashion for 3 h in serum-containing media, completely inhibited the killing by TM. Dextran (average molecular weight 40,000) had no suppressive effect. Non-haemoglobin erythrocyte proteins did not inhibit killing. As much as 1,000 $\mu\text{g ml}^{-1}$ endotoxin-free superoxide dismutase (SOD, Truett Laboratories), catalase (Boehringer), or bovine serum albumin (Pentex)¹⁵ did not suppress macrophage-mediated tumour cell killing. Enhancement of endocytosis of SOD, catalase, or albumin by heat denaturation (120 °C for 20 min followed by sonication if necessary to disperse the denatured proteins) likewise had no effect. Native or heat denatured myoglobin did not inhibit. Table 1 summarises the suppressive effects of various additives.

These results suggest that RBC, Hb, globin, and iron inhibit the tumoricidal effect of activated macrophages. Because damaged

Table 1 Effects of various additions on macrophage-mediated tumour cell killing

Addition	Tumoricidal effect
None	4+
RBC (phagocytosed)*	0
RBC (not phagocytosed)*	4+
RBC ghosts† (phagocytosed)*	4+
RBC lysate‡	0
Haemoglobin‡§	0
Myoglobin‡	4+
Methaemalbumin	0
Albumin‡	4+
Globin‡	0
FeSO_4 or FeCl_3	0
Iron dextran (Imferon)‡	0
Catalase‡	4+
Superoxide dismutase‡	4+

Additions were tested throughout the 60 h *in vitro* assay or in a 2 h pretreatment of TM before tumour cell challenge. All were negative for endotoxin as judged by the limulus amoebocyte lysate test¹⁶. The tumoricidal effect was judged from microscopic assay of microtitre plates stained after 60 h culture³. 4+ Tumoricidal effect signifies 0.33T12 cell per 300 power field among the TM; zero tumoricidal effect signifies a multilayer of 3T12 cell per 300 power field among the TM.

*RBC TM ratio of 4:1.

†Prepared by hypotonic lysis¹⁷.

‡1000 $\mu\text{g ml}^{-1}$.

§The haemoglobin was prepared by the method of Haut *et al.*¹⁸, using carboxymethyl cellulose (CM 52, Whatman), eluting the haemoglobin with 0.25 M phosphate buffer, pH 6.8.

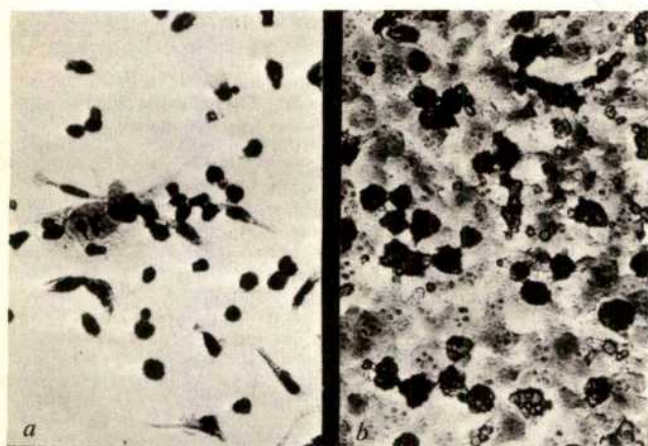
||20 $\mu\text{g ml}^{-1}$.

RBC, Hb, globin, or iron rapidly accumulated in macrophage secondary lysosomes, we interpret our findings as further evidence for involvement of the TM vacuolar system in the non-phagocytic tumour killing process. Vacuolar system and/or plasma membrane damage by iron-induced lipid peroxidation¹⁹, reaction with -SH groups²⁰, or interference with Ca^{2+} transport²¹ may account for the suppressed activated macrophage tumoricidal capability. The lack of inhibition by myoglobin and catalase (which also contain haem moieties with iron) may relate to substrate specificity of haem oxygenase with less or more gradual liberation of 'free' iron from these proteins in the macrophage vacuolar system. Haem oxygenase has little or no degradation effect on myoglobin²². The inhibitory effect of globin is not fully understood, but may relate to its hydrophobic nature. Further elucidation of the mechanisms of the inhibitory effects of RBC, Hb, and Hb degradation products may lead to a more basic knowledge of macrophage-mediated tumour killing. In addition, these observations may aid in understanding the pathogenesis of tumour development in certain clinical situations in man.

There is evidence that iron may contribute to tumour growth in certain experimental and clinical conditions (the occurrence of sarcomas at sites of repeated parenteral iron injections in animals²³ and man²⁴; the increased incidence of hepatomas and other epithelial malignancies in idiopathic haemochromatosis²⁵, acute myeloblastic leukaemia in idiopathic refractory sideroblastic anaemia²⁶, lung carcinoma in mice inhaling iron oxide dust²⁷, and lung carcinoma in haematite miners²⁸). The mechanism by which iron promotes growth of tumours *in vivo* is complex and poorly understood. Some have proposed that iron may act as a primary carcinogen by inducing mutations in somatic cells²⁹, while others have noted a potentiating or cocarcinogenic effect of ferric oxide on chemical carcinogen-induced lung tumours in hamsters³⁰. In addition, some have noted stimulation of tumour cell growth *in vitro* by FeCl_3 (ref 31). Our observed inhibition of the tumoricidal effect of activated macrophages by RBC, haemoglobin, or iron cannot be accounted for by direct effects on tumour cells by the additives, since the inhibition was observed if macrophages had endocytosed the RBC, Hb, or iron before addition of the tumour target cells to the cultures (see Table 1). Therefore, based on the results reported here, we believe that iron could operate through an additional mechanism (inhibition of macrophage tumour killing function) to decrease *in vivo* resistance to tumour development.

Activated macrophages may be the effector component of a

Fig. 2 Photomicrographs from giemsa-stained 'TM+3T12' wells of cytotoxicity assay demonstrating RBC inhibition of macrophage-mediated tumour cell killing. Pictures were made at 60 h from a single experiment as described in Fig. 1 legend. *a*, The TM without added RBC have destroyed the majority of the 3T12 cells. This typical field shows one residual tumour cell among the TM. *b*, The TM with phagocytosed RBC did not kill the 3T12 cells. This typical field shows numerous 3T12 cells with their prominent nucleoli among the RBC-laden TM ($\times 200$).



surveillance system for the detection and destruction of newly emergent clones of neoplastic cells^{3,2}. Pathologic conditions which produce large amounts of RBC, Hb, or Hb degradation products within the macrophage vacuolar system could suppress tumour cell killing and effectively paralyse this surveillance system.

This work was supported by the Medical Research Service of the Veterans Administration, and by USNIH (grants CA14045 and CA15811). We thank C. C. Moore, M. S. Knowlton, C. Henderson, J. E. Brisbay and R. Christensen for technical assistance.

J. BRICE WEINBERG
JOHN B. HIBBS, JR

Veterans Administration Hospital and
Division of Hematology/Oncology,
Division of Infectious Diseases,
Department of Medicine,
University of Utah Medical Center,
Salt Lake City, Utah 84148

Received 23 May; accepted 5 July 1977.

- ¹ Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Nature new Biol.* **235**, 48–50 (1972).
- ² Evans, R. & Alexander, P. in *Immunobiology of the Macrophage* (ed. Nelson, D. S.) 536–573 (Academic, London, 1976).
- ³ Hibbs, J. B., Jr *Science* **184**, 468–471 (1974).
- ⁴ Bucana, C. *et al. Cancer Res.* **36**, 4444–4458 (1976).
- ⁵ Norbury, K. C. & Fidler, I. J. *J. Immun. Meth.* **7**, 109–122 (1975).
- ⁶ Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *J. infect. Dis.* **124**, 587–592 (1971).
- ⁷ Rabinovitch, M. *Proc. Soc. exp. Biol. Med.* **124**, 396–399 (1967).
- ⁸ Bianco, C., Griffin, F. M., Jr & Silverstein, S. C. *J. exp. Med.* **141**, 1278–1290 (1975).
- ⁹ Weissman, G., Dukor, P. & Zurier, R. B. *Nature new Biol.* **231**, 131–135 (1971).
- ¹⁰ Allain, C. C., Henson, C. P., Nadel, M. K. & Knoblesdorff, A. J. *Clin. Chem.* **19**, 223–227 (1973).
- ¹¹ Rossi, F., Romeo, D. & Patriarca, P. *J. reticuloendothel. Soc.* **12**, 127–149 (1972).
- ¹² Ehrenreich, B. A. & Cohn, Z. A. *J. Cell Biol.* **38**, 244–248 (1968).
- ¹³ Rossi-Fanelli, A., Antonini, E. & Caputo, A. *Biochim. biophys. Acta* **30**, 608–615 (1958).
- ¹⁴ Tenhunen, R., Marver, H. S. & Schmid, R. *Proc. natn. Acad. Sci.* **61**, 748–755 (1968).
- ¹⁵ Ehrenreich, B. A. & Cohn, Z. A. *J. exp. Med.* **126**, 941–958 (1967).
- ¹⁶ Elin, R. J., Sandberg, A. L. & Rosenstreich, D. L. *J. Immun.* **117**, 1238–1242 (1976).
- ¹⁷ Dodge, J. T., Mitchell, C. & Hanahan, D. J. *Archs Biochem. Biophys.* **100**, 119–130 (1963).
- ¹⁸ Haut, A., Cartwright, G. E. & Wintrobe, M. M. *J. Lab. clin. Med.* **63**, 278–289 (1964).
- ¹⁹ Aristila, A. U., Smith, M. A. & Trump, B. F. *Science* **175**, 530–533 (1971).
- ²⁰ Jocelyn, P. C. (ed.) *Biochemistry of the SH Group* 94–115 (Academic, New York, 1972).
- ²¹ Romlo, I. & Flatmark, T. *Biochim. biophys. Acta* **325**, 38–46 (1973).
- ²² Tenhunen, R., Marver, H. S. & Schmid, R. *J. Biol. Chem.* **244**, 6388–6394 (1969).
- ²³ Haddow, A. & Horning, E. S. *J. natn. Cancer Inst.* **24**, 109–127 (1960).
- ²⁴ MacKinnon, A. E. & Bancewicz, J. *Br. med. J.* **2**, 277–279 (1973).
- ²⁵ Bomford, A., Walker, R. J. & Williams, R. in *Iron Metabolism and Its Disorders* (ed. Kief, H.), 324–331 (Elsevier, New York, 1975).
- ²⁶ Kushner, J. P., Lee, G. R., Wintrobe, M. M. & Cartwright, G. E. *Medicine* **50**, 139–159 (1971).
- ²⁷ Campbell, J. A. *Br. med. J.* **2**, 275–280 (1970).
- ²⁸ Boyd, J. T., Doll, R., Faulds, J. C. & Leiper, J. *Br. J. ind. Med.* **27**, 97–105 (1970).
- ²⁹ Richmond, H. G. *Br. J. Cancer* **15**, 594–606 (1961).
- ³⁰ Montesano, R., Saffioti, U. & Shubick, P. in *Inhalation Carcinogenesis* (eds Hanna, M. G., Nettiessheim, P. & Gilbert, J.) 353–375 (AEC Symposium Series 18, Oak Ridge, Tennessee, 1970).
- ³¹ Brun, A. & Brunk, U. *Cytobiologie* **12**, 417–428 (1976).
- ³² Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Science* **177**, 998–1000 (1972).

Inhibition of adipose conversion of 3T3 fibroblasts by tumour promoters

TUMOUR-PROMOTING compounds are not themselves carcinogenic, but can induce skin tumours in mice pre-treated with a subcarcinogenic dose of a chemical carcinogen^{1–3}. They have been shown to produce many different biochemical and biological effects on cells^{3,4}, but the mechanism of promotion is not known. Two recent reports describe the effects of the phorbol diester series of tumour promoters on cell differentiation *in vitro*. Cohen *et al.*⁵ observed that 12-O-tetradecanoyl-phorbol-13-acetate (TPA; phorbol myristate acetate), a potent promoter, inhibits myogenesis in chick embryo muscle cultures. We have reported^{6,7} that TPA and other tumour-promoting phorbol diesters inhibit spontaneous and induced differentiation of Friend erythroleukaemia cells in culture. We now describe another cell system, mouse preadipose cells, in which tumour promoters inhibit terminal differentiation, and confirm in these cells our original observation⁶ that there is a good correlation between the ability of a particular phorbol diester to promote tumours in mouse skin and its ability to inhibit terminal differentiation of cells in culture.

A clone of BALB/c 3T3 fibroblasts⁸ was obtained from Dr R. Tennant, Oak Ridge National Laboratory. In our laboratory, cells of this clone (designated clone A31T) behave as preadipose cells and differentiate to mature adipocytes in a manner similar to that already seen in some clones of Swiss 3T3 fibroblasts^{9–12}. When cultures are

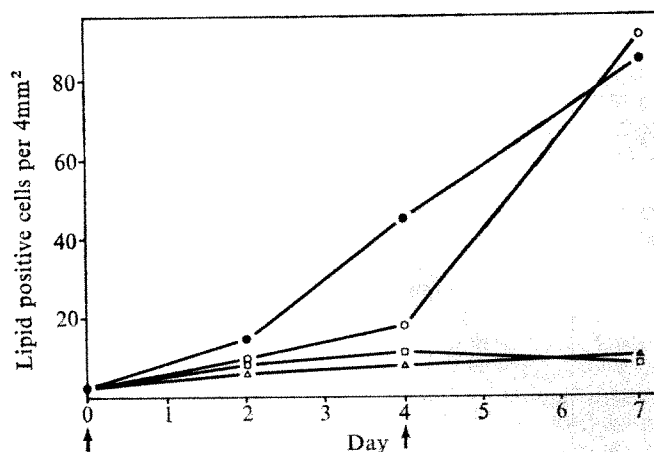


Fig. 1 Effect of TPA on adipose conversion of clone A31T fibroblasts. Cells were seeded into gridded 60-mm plastic Petri dishes at a density of 4×10^4 cells per cm^2 . After 3–4 d, when the monolayers were confluent, and again on day 4, the cultures were refurnished (\uparrow) with fresh medium containing insulin $0.1 \mu\text{g ml}^{-1}$ and TPA. The number of lipid-positive cells per 4 mm^2 was scored by microscopic observation without staining every 2–3 d and confirmed after staining with oil red O at the termination of the experiment. ●, Control; △, TPA $1.6 \times 10^{-7} \text{ M}$; □, TPA $1.6 \times 10^{-8} \text{ M}$.

held as confluent monolayers, the cells begin to accumulate lipids. They soon become mature adipocytes in which the cytoplasm is filled with lipid droplets and lose the ability to divide; after a few weeks, more than half the cells in the monolayer may be mature adipocytes. Both immature and mature adipose cells are easily identified either without staining or after staining with the lipid-specific stain oil red O. Stock cultures of clone A31T were grown in Eagle's basal medium supplemented with 10% foetal calf serum and were subcultured at 1:10 ratios just as the monolayers reached confluence, so that adipose conversion was kept at a minimum.

TPA and 4 α -phorbol-12,13-didecanoate (4 α -PDD) were purchased from Consolidated Midland; phorbol-12,13-didecanoate (PDD), phorbol-12,13-dibenzoate (PDB) and phorbol-12,13-diacetate (PDA) were the gift of Dr R. K. Boutwell, University of Wisconsin. Stock solutions ($1.6 \times 10^{-4} \text{ M}$ in acetone) of the phorbol diesters were stored at -20°C and diluted in medium as required.

The effect of TPA on adipose conversion in clone A31T fibroblasts is shown in Fig. 1. In control cultures, the number of lipid-positive cells increased at the rate of approximately 15 cells per 4 mm^2 per d whereas in cultures treated with $1.6 \times 10^{-7} \text{ M}$ or $1.6 \times 10^{-8} \text{ M}$ TPA, there was no increase in the number of lipid-positive cells during with 7-d period of observation. With a 10-fold lower concentration of TPA, adipose conversion was inhibited for the first 4 d, but by 7 d the number of lipid-positive cells was similar to the number in control cultures.

The inhibitory effect of TPA was reversible after 7 d by refeeding the cultures with control medium. The number of lipid-positive cells increased thereafter at approximately the same rate as in control cultures never exposed to TPA.

The inhibitory effects of TPA on adipose conversion in clone A31T were similar whether or not insulin was in the medium, although insulin did enhance conversion^{11,12}. In one experiment, after 10 d the number of lipid-positive cells in control cultures with insulin $0.1 \mu\text{g ml}^{-1}$ was 82 per 4 mm^2 and without insulin, 58 per 4 mm^2 ; the numbers in TPA ($1.6 \times 10^{-7} \text{ M}$)-treated cultures were 2.1 and 2.8, respectively.

As has been reported with other 3T3 cell lines^{13,14}, TPA stimulated multiplication of clone A31T cells and increased the saturation density of the monolayers. Thus,

at the termination of the experiment shown in Fig. 1, cultures treated with 1.6×10^{-7} M TPA had 3.2×10^6 cells per plate, 0.15% of which were lipid positive, whereas control plates had 1.6×10^6 cells with 2.7% lipid positive.

The effects on adipose conversion of a series of phorbol diesters with a range of tumour-promoting activity in mouse skin^{2,15,16} are shown in Fig. 2. At the same concentration (1.6×10^{-7} M), the three promoters (TPA, PDD and PDB) completely inhibited adipose conversion whereas the three non-promoters (PDA, 4 α -PDD and phorbol) had no effect on conversion. The three active promoters also enhanced the saturation density of the cell monolayers approximately twofold; the three non-promoters had no effect on cell number.

The inhibitory effect of the promoters on adipose conversion was not permanent, even when the cultures were refurbished with fresh solutions of the phorbol diesters every 3–4 d. In five separate experiments, the inhibition of conversion by TPA (1.6×10^{-7} M) persisted for 14–18 d (Table 1), after which time the number of lipid-positive cells slowly increased. PDD and PDB, however, inhibited adipose conversion for only 7 d; after that time there was a rapid and sudden increase in adipose conversion. In the experiment shown in Table 1, for example, the number of lipid-positive cells per 4 mm² in PDD-treated cultures increased from 0 on day 7 to 184 on day 10. In PDB-treated cultures, there was an increase from 1.2 to 167 positive cells. These increases in adipose conversion first became evident on days 8 to 9 when there seemed to be an almost synchronous increase in the number of immature adipocytes; a few days later, these cells were fully mature adipocytes filled with lipid. Following this spontaneous release from the inhibitory effects of PDD and PDB, the percentage of lipid-positive cells gradually approached the percentage in control cultures (Table 1). Thus, on day 14 the absolute number of lipid-positive cells in PDD- and PDB-treated cultures was greater than in control cultures, but since the treated cultures had almost twice as many cells, the percentage of lipid-positive cells in the three groups was similar.

The finding that tumour-promoting phorbol diesters inhibit terminal differentiation in cultures of three different cell types, erythroleukaemia cells^{6,7}, muscle cells⁸ and preadipose cells, suggests that inhibition of terminal differentiation of cells in culture may be a general property of tumour promoters. They inhibit differentiation reversibly at concentrations that are not cytotoxic and that either stimulate or have no effect on cell division.

The phorbol diesters that inhibit adipose conversion of

Fig. 2 Effect of phorbol diesters on adipose conversion of clone A31T fibroblasts (Fig. 1 for procedure). The phorbol diesters were all tested at the same molar concentration, 1.6×10^{-7} M. ●, Control; △, TPA; ▲, PDD; X, 4 α -PDD; □, PDB; ○, PDA; ■, phorbol.

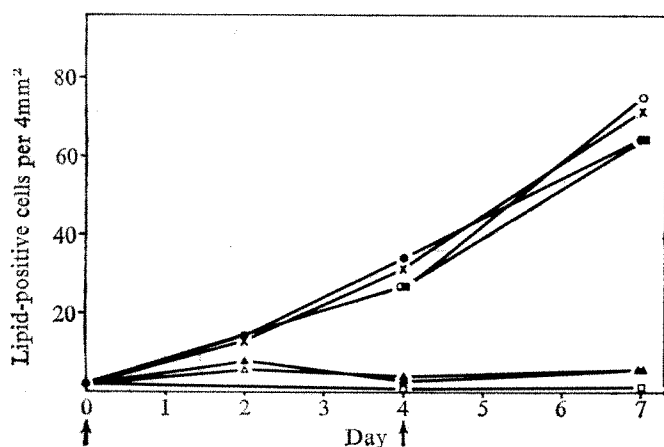


Table 1 Long-term effects of phorbol diesters on adipose conversion

Number of lipid-positive cells per 4 mm ²				
Day	Control	TPA	PDD	PDB
3	3.7 ± 3.0*	0	0	0
7	106 ± 15	0.5 ± 0.9	0	1.2 ± 2.4
10	169 ± 15	12 ± 2.3	184 ± 22	168 ± 22
14	223 ± 30	3.4 ± 1.2	368 ± 65	> 500
17	240 ± 52	6.1 ± 4.3	—	—

% lipid-positive cells				
Day	Control	TPA	PDD	PDB
3	0.1	0	0	0
7	2.7	< 0.1	0	< 0.1
10	4.8	0.1	3.0	2.0
14	6.3	< 0.1	6.0	6.8

Cultures of clone A31T fibroblasts were refurbished on days 0, 4, 7, 11 and 14 with medium containing the phorbol diesters at a final concentration of 1.6×10^{-7} M. On day 0, there were 2.1×10^6 cells per plate. By day 3, the cell monolayers had attained densities that remained essentially unchanged through day 14. The mean number of cells ($\times 10^{-6}$) per plate \pm s.d. from days 3–14 was: control, 2.0 ± 0.3 ; TPA, 3.9 ± 0.1 ; PDD, 3.3 ± 0.4 ; PDB, 4.0 ± 0.5 .

*Mean \pm s.d. of five squares.

clone A31T cells stimulate cell division the first time they are added to confluent cultures. The cells seem to retain their density-dependent growth controls during repeated refurbishing of the cultures with fresh solutions of phorbol diesters; the saturation densities remain essentially constant without cells being shed into the medium. This suggests that inhibition of differentiation in these cells may not be dependent on continuous cell cycling.

The reasons for the spontaneous release of clone A31T cells from the inhibitory effects of the promoters are not known. In contrast to these cells, Friend erythroleukaemia cells do not seem to become refractory to the inhibitory effects of the promoters on spontaneous differentiation; the extent of inhibition has remained the same in erythroleukaemia cells maintained for six months in the continuous presence of TPA⁷.

Tumour promoters induce many diverse effects on cells in culture⁴. It must be determined whether the inhibitory effects of promoters on differentiation are related to any of the other effects or to the mechanism of promotion itself. The ability of promoters to shift the cell population from a commitment to differentiate and become end cells to a commitment to maintain or increase the size of the stem cell pool, does suggest at least one possibility for the mechanism of promotion. The inhibition of terminal differentiation by tumour promoters may give cells in which the potential for malignancy has been initiated by a carcinogen, but is not yet expressed, additional time to convert to tumour cells before becoming end cells. Several investigators^{17,18} have proposed that tumour promoters act *in vivo* by interfering with normal cell differentiation. Our findings support those suggestions.

This work was supported by the NIH. G.R. is a Scholar of the Leukemia Society of America. T.G.O'B is a USPHS postdoctoral fellow.

LEILA DIAMOND
THOMAS G. O'BRIEN
GIOVANNI ROVERA

Wistar Institute of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, Pennsylvania 19104

Received 13 May; accepted 18 July 1977.

- 1 Van Duuren, B. L. *Progr. exp. Tumor Res.* **11**, 31–68 (1969).
- 2 Hecker, E. *Methods Cancer Res.* **6**, 439–484 (1971).
- 3 Boutwell, R. K. *C.R.C. Crit. Rev. Toxic.* **2**, 419–443 (1974).

- 4 Weinstein, I. B., Wigler, N. & Pietropaolo, C. in *Cold Spring Harbor Symposium on the Origins of Human Cancer* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, in the press).
- 5 Cohen, R., Pacifici, M., Rubinstein, N., Biehl, J. & Holtzer, H. *Nature* **266**, 538–540 (1977).
- 6 Rovera, G., O'Brien, T. & Diamond, L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2894–2898 (1977).
- 7 Diamond, L., O'Brien, T. & Rovera, G. in *Symposium on Mechanisms of Tumor Promotion and Cocarcinogenesis* (Raven, New York, in the press).
- 8 Quarles, J. M. & Tennant, R. W. *Cancer Res.* **35**, 2637–2645 (1975).
- 9 Green, H. & Kehinde, O. *Cell* **1**, 113–116 (1974).
- 10 Green, H. & Meuth, M. *Cell* **3**, 127–133 (1974).
- 11 Green, H. & Kehinde, O. *Cell* **5**, 19–27 (1975).
- 12 Russell, T. R. & Ho, R.-J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4516–4520 (1976).
- 13 Sivak, A. & Van Duuren, B. L. *Cancer Res.* **30**, 1203–1205 (1970).
- 14 Diamond, L., O'Brien, S., Donaldson, C. & Shimizu, Y. *Int. J. Cancer* **13**, 721–730 (1974).
- 15 Thielmann, H. W. & Hecker, E. in *Fortschritte der Krebsforschung* (eds Schmidt, C. G. & Wetter, O.) 171–179 (Schattauer, Stuttgart and New York, 1969).
- 16 Baird, W. M. & Boutwell, R. K. *Cancer Res.* **31**, 1074–1079 (1971).
- 17 Raick, A. N. *Cancer Res.* **34**, 2915–2925 (1974).
- 18 Marks, F. *Cancer Res.* **36**, 2636–2643 (1976).

Effect of microtubular antagonists on lymphocyte mitogenesis

SEVERAL different classes of mitogens have been discovered¹. Stimulation of lymphocytes by all classes of mitogens results in cellular division, but it is not known whether all mitogens act on the cell membrane in identical fashion or result in triggering of the same cytoplasmic messenger. We have investigated the inhibitory effect of microtubule disrupting drugs on lymphocytes transformed by two methods. We report here that colchicine and vinblastine inhibit lectin-mediated mitogenesis at low concentrations, while cells stimulated by cell surface oxidation are inhibited only by much higher concentrations of these drugs. It seems that intact microtubules are necessary for lectin-mediated but not for cell surface oxidation-mediated mitogenesis.

Yahara and Edelman have demonstrated that microtubules and microfilaments exist in association with the lymphocyte cell surface². Edelman *et al.* have hypothesised that a colchicine-binding protein system may be a crucial link in the transmission of information from the cell membrane to the nucleus³. In support of this is the finding that drugs which disrupt microtubules block lectin-induced stimulation of lymphocytes at low drug concentrations^{4,5}.

We stimulated lymphocytes with two different classes of mitogens, (1) the plant lectins concanavalin A (con A) and phytohaemagglutinin (PHA) and (2) enzymatic oxidation of the cell surface by means of sequential additions of neuraminidase and galactose oxidase (NG) (ref. 6). Both classes of mitogens are known to selectively stimulate T cells⁷.

The effect of vinblastine on lymphocyte transformation measured by ³H-thymidine uptake is shown in Fig. 1. There is a significant difference between the amount of vinblastine needed to inhibit lectin-mediated stimulation and that amount needed to inhibit NG-treated lymphocytes ($P < 0.02$). Vinblastine had almost no inhibitory effect on NG-treated cells until a concentration of 10^{-5} M was reached. Lectin-treated cells were inhibited by drug concentrations 100 to 1,000-fold less.

The curve describing thymidine uptake as a function of antagonist concentration for NG-treated cells is suggestive of simple saturation kinetics, while the curves for lectin-stimulated cells show evidence of a more complex co-operative interaction. Colchicine gave results similar to those found with vinblastine (Fig. 2). Cytosine arabinoside, a drug known to directly interfere with DNA synthesis¹⁰, inhibits both groups to the same extent while showing similar inhibition kinetics (Fig. 3). This suggests that, not surprisingly, lectin- and NG-mediated stimulation share a common pathway at the level of DNA synthesis.

Table 1 illustrates the $-\log_{10} K_{50\%}$ of colchicine, vinblastine, and cytosine arabinoside for lymphocyte blastogenesis induced by lectins and cell surface oxidation. The significant difference between lectin- and NG-treated cells is evident for both vinblastine and colchicine although vinblastine proved to be a slightly more potent antagonist. Edelman *et al.* have hypothesised that a colchicine-binding

protein system has a role in the mediation of interactions between receptors on cellular membranes and the cytoplasm as well as in mitogenesis³. Microtubular inhibitors also inhibit mitogenesis in neuroblastoma and mouse 3T3 cells as well as in lymphocytes¹¹. Our results support reports^{4,5} that colchicine and vinblastine are effective inhibitors of lymphocyte mitogenesis and do not support the isolated report that vinblastine is not inhibitory¹².

Resch *et al.* have shown that three cellular events which occur in transformed lymphocytes before the initiation of DNA synthesis were not inhibited by colchicine or vinblastine¹³. They concluded that microtubules are not involved in the initiation of lymphocyte activation because the disruption of these cytoplasmic structures should also affect cellular events which occur before DNA synthesis. This conclusion is justified only if those early cellular events

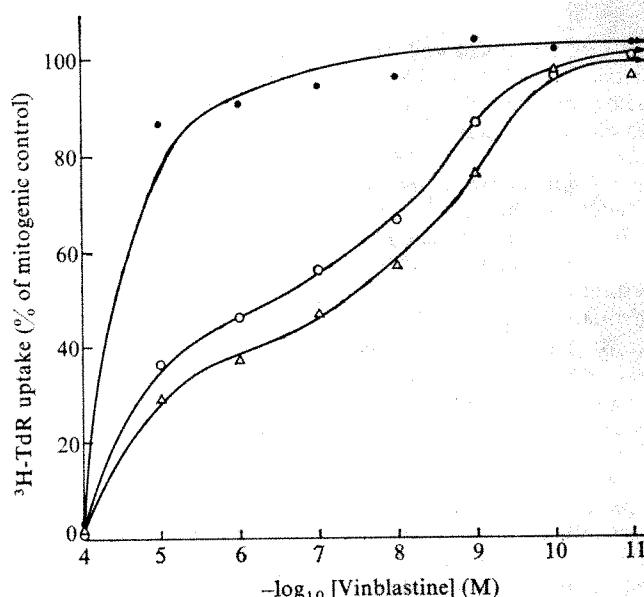


Fig 1. Effect of vinblastine on lymphocyte transformation. Blood was obtained from human donors free of any acute or chronic illnesses and on no medications. Lymphocytes were isolated using the method of Boyum⁸. The cell population was made up of an average of 85% lymphocytes, 12% monocytes and 3% neutrophils. Cell viability following isolation and after treatment with NG or lectin was always over 95% as measured by Trypan blue exclusion and fluorescein diacetate uptake⁹. Cells were cultured in McCoy's 5A Medium (Microbiological Associates) supplemented with 10% autologous serum at a cell density of 1×10^6 cells per ml, in Nunc serum tubes (Vanguard International). The cultures were put in a humidified atmosphere of 5% CO₂, 95% air at 37 °C. At 48 h, 1 μ Ci ³H-thymidine (TdR), specific activity 6.7 mCi mmol⁻¹ (New England Nuclear) was added to each culture. At 72 h, the trichloroacetic acid-insoluble product was separated onto 0.45- μ m Millipore filters. The dried filters were put into a scintillation cocktail of 0.5 g PPO and 5 g POPOP per l toluene and counted overnight in a Packard Tri Carb Scintillation Counter. Cell surface oxidised lymphocytes were treated with 50 units ml⁻¹ neuraminidase for 30 min at 37 °C at a cell density of 20×10^6 cells per ml. Neuraminidase (EC 3.2.1.18 from *Vibrio cholerae* strain Z-4) was obtained from GIBCO as a solution containing 500 units ml⁻¹ where 1 unit releases 1 μ g of N-acetyl neuraminic acid from a glycoprotein substrate in 15 min at pH 5.5 at 37 °C. This was followed by treatment with 0.25 units ml⁻¹ galactose oxidase (Worthington) for 30 min at 23 °C at a cell density of 20×10^6 per ml. A unit is the quantity of enzyme that yields an absorbance of 1.0 at 420 nm by the peroxidase-chromagen assay. PHAMR 69 (Wellcome), con A (Calbiochem), colchicine (Sigma), and vinblastine (Lilly), were added to the cells just before they were put into culture. Each point represents duplicate cultures of 10 different donors. Data is expressed as the mean % of mitogenic control. Absolute c.p.m. for mitogenic controls were 68,500–122,300 for con A, 72,400–131,200 for PHA, and 58,400–118,600 for NG. Mean mitogenic controls were 102,500 for con A, 108,600 for PHA, and 87,400 for NG. ●, NG; ○, PHA; △, con A.

Table 1 Values for $-\log_{10}K_i^{50}$; K_i^{50} is the drug concentration inhibiting mitogenesis by 50%

Mitogen	Vinblastine		N	Colchicine		N	Ara-C		N
Con A	7.45 ± 0.63*		10	6.95 ± 0.66		10	6.60 ± 0.57		5
PHA	6.55 ± 0.42		10	6.50 ± 0.56		10	6.70 ± 0.38		5
NG	4.45 ± 0.46		10	4.45 ± 0.48		10	6.80 ± 0.58		5
	<i>t</i> †	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>			
Con A versus NG	3.02	<0.02	2.95	<0.02	0.221	>0.2			
PHA versus NG	2.64	<0.05	2.57	<0.05	0.115	>0.2			
Con A versus PHA	0.73	>0.2	0.52	>0.2	0.147	>0.2			

N, number of experiments performed.

*Values are means ± s.e.m.

† *t* values determined by Student's *t*-test.

are directly linked and necessary for lymphocyte activation to take place. No evidence exists to suggest that blocking membrane turnover, early RNA synthesis, or production of lymphotoxin leads to inhibition of lymphocyte activation. Furthermore, it has been shown that colchicine inhibits phosphatidylinositol turnover, another early cellular event, in con A-stimulated lymphocytes¹⁴.

It has been suggested that inhibition of mitogenesis by colchicine is due to blockage of thymidine transport. Wilson¹⁵ found the inhibition constant for blockage of thymidine transport by colchicine to be 6.1×10^{-5} M. Interestingly, in our experiments NG-mediated mitogenesis was not 50% inhibited by colchicine until levels of 5×10^{-5} M were reached. Thus for NG-mediated mitogenesis our results are consistent with, but do not prove, the hypothesis that the site of action of colchicine may be at the level of thymidine transport. Furthermore, saturation kinetics, consistent with competitive inhibition of transport were observed. In contrast, con A-mediated blastogenesis was 50% inhibited by 1×10^{-7} M colchicine and 4×10^{-8} M vinblastine, a concentration far too low to act at the level of thymidine transport. This is close to the purified tubulin affinity constant for adult human brain of 3.4×10^{-7} M (ref. 15).

It has been assumed until now that lectins and cell sur-

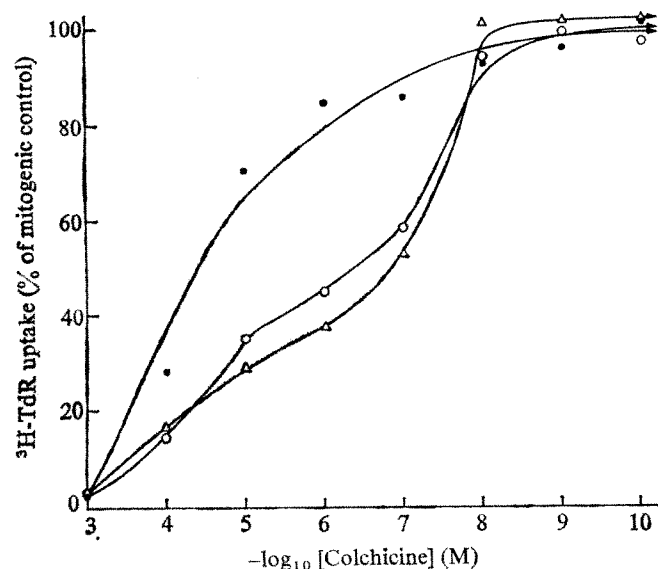


Fig. 2 Effect of colchicine on lymphocyte transformation. Experimental conditions were the same as for Fig. 1. Each point represents duplicate cultures of 10 different donors. Data expressed as in Fig. 1. ●, NG; ○, PHA; △, con A.

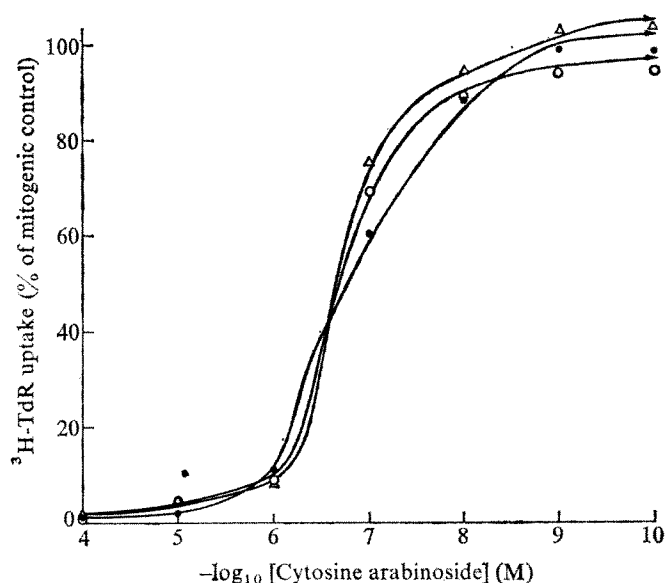


Fig. 3 Effect of cytosine arabinoside on lymphocyte transformation. Experimental conditions as for Fig. 1. Each point represents duplicate cultures of five different donors. Data expressed as in Fig. 1. ●, NG; ○, PHA; △, con A.

face oxidation result in a perturbation of the cellular membrane (perhaps cross-linking^{16,17}) that results in secondary triggering of the same cytoplasmic messenger. It may be, however, that a biological process as complex as mitogenesis is under multifactorial control. There is evidence that the initial membrane event for the two groups is different. Free aldehyde groups generated by cell surface oxidation do not seem to be generated by lectins since mitogenesis due to the former but not the latter stimulus is inhibited by reducing agents⁸. Our results show that although microtubules are necessary for lectin-mediated lymphocyte transformation, they are not a crucial intermediary in transfer of information from cell surface to the genome for cell surface oxidised cells.

STEVEN A. RASMUSSEN
ROBERT P. DAVIS

Division of Biology and Medicine,
Brown University and
Division of Renal and Metabolic Diseases,
The Miriam Hospital,
Providence, Rhode Island 02906

Received 4 April; accepted 5 July 1977.

1 O'Brien, R. L. & Parker, J. W. *Cell* 7, 13–20 (1976).

2 Yahara, I. & Edelman, G. M. *Expl Cell. Res.* 91, 125–142 (1975).

3 Edelman, G. M., Yahara, I. & Wang, J. L. *Proc. natn. Acad. Sci. U.S.A.* 70, 1442–1446 (1973).

- 4 Medrano, E., Piras, R. & Mordoh, J. *Expl Cell Res.* **86**, 295–300 (1974).
 5 Wang, J. L., Gunther, G. R. & Edelman, G. M. *J. Cell Biol.* **66**, 128–144 (1975).
 6 Novogrodsky, A. & Katchalski, E. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1824–1827 (1973).
 7 Novogrodsky, A. *Eur. J. Immun.* **48**, 646–648 (1974).
 8 Boyum, A. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77–88 (1968).
 9 Rotman, B. & Papermaster, B. W. *Proc. natn. Acad. Sci. U.S.A.* **55**, 134–141 (1965).
 10 Chu, M. Y. *Biochem. Pharmac.* **20**, 2057–2063 (1971).
 11 Baker, M. E. *Nature* **262**, 785–786 (1976).
 12 Betel, I. & Martijnse, J. *Nature* **261**, 318–319 (1976).
 13 Resch, K., Bouillon, D., Gerns, D. & Averdunk, R. *Nature* **265**, 349–351 (1977).
 14 Schellenberg, R. R. & Gillespie, E. *Nature* **265**, 741–742 (1977).
 15 Wilson, L. *Ann. N. Y. Acad. Sci.* **253**, 213–230 (1975).
 16 Anderson, J., Edelman, G. M., Moller, G. & Sjöberg, O. *Eur. J. Immun.* **2**, 233–235 (1972).
 17 Greaves, M. & Janossy, G. *Transplant. Rev.* **11**, 87–130 (1972).

Immunoglobulin binding to herpes virus-induced Fc receptors inhibits virus growth

WATKINS¹ reported that sheep red blood cells coated with anti-sheep-red blood cell antibody adsorbed to monolayers of herpes simplex virus (HSV) infected cells. Yasuda and Milgrom² suggested that the haemadsorption was mediated through an Fc-binding site which developed on the surface of the HSV infected cell, and later work from Watkin's laboratory established this interpretation³. Although the receptor for the Fc portion of IgG on the surface of HSV infected cells has been known for some time, its biological role remains unclear^{4–7}. We show here that when the HSV-induced Fc receptor is engaged with IgG or Fc fragments, viral replication is significantly inhibited.

Pools of HSV-2 (strain MS) were grown and titrated in Vero cell monolayers⁸. The Fc receptor studies were done with Vero monolayers and with a human retinoblastoma cell line (Y-79) which grows in suspension⁹. The cells were grown in RPMI 1640 medium (NIH) with 5% foetal calf serum (GIBCO). Fc receptors were demonstrated with sheep red blood cells coated with rabbit 7S antibody to sheep red cell stroma (7SEA). Sheep red cells coated with 19S antibodies were used as control reagents. Haemadsorption assays were done with minor modifications of previously described methods^{8,9}.

Both Vero and Y-79 cultures infected with HSV-2 contained 20–40% of the cells with Fc receptor 20 h after infection. No haemadsorption was seen in infected cultures with 19S-EA reagent, and uninfected cells were negative with both the 7S-EA and 19S-EA. In the Y-79 cultures infected at a multiplicity of 100, haemadsorption was first detected after 6 h, and by 8 h after infection the percentage of rosetting cells was equal to that detected 20 h after infection. Haemadsorption was inhibited when the infected cells were pre-incubated in balanced salt solution (BSS) containing 1 mg ml⁻¹ of IgG of different species (goat, rabbit, mouse). Haemadsorption inhibition was also achieved with Fc fragments but not with (Fab')₂ fragments of rabbit IgG. These results suggest that IgG and Fc fragments occupy the Fc binding sites at the surface of HSV infected cells. Furthermore radiolabelled IgG is known to bind to HSV infected cells³.

We next tested the effects of IgG on virus growth in these cells. The normal concentration of IgG in human serum is 12 mg ml⁻¹, so to avoid unphysiological levels we used concentrations of 1–10 mg ml⁻¹. Serum-free RPMI 1640 medium was prepared with DEAE-purified rabbit IgG in concentrations of 1, 5 and 10 mg ml⁻¹. The IgG used in all experiments was free of HSV neutralising activity in a standard viral plaque neutralisation test. Bovine serum albumin (Sigma) in 1, 5 and 10 mg ml⁻¹ concentrations was used as control medium. Cells were infected at a multiplicity of 100 for 1 h at 35 °C, washed twice in RPMI 1640, and transferred to IgG- or albumin-containing medium. They were incubated for 24–48 h at 35 °C and the total

virus in the cultures after three cycles of freezing and thawing was assayed on Vero monolayers.

As shown in Table 1, there was a 99% reduction in virus production in the cultures treated with 10 mg ml⁻¹ IgG. There was no inhibition of virus growth in 1 mg ml⁻¹ IgG. These results have been consistently reproducible both in Vero and Y-79 cells.

Although the concentrations of IgG we used were in the physiological range, we still thought it would be of importance to determine whether they were cytotoxic in our cell systems. We tested viability of non-infected Vero and Y-79 cells maintained in our 10 mg ml⁻¹ IgG medium for 24–48 h by Trypan blue exclusion and found no cytotoxicity.

We have also tested the effects of purified Fc fragments of rabbit IgG on virus growth. Rabbit IgG was digested with papain and the Fc fragments were purified by the method of Prahl¹⁰. When infected cultures were maintained in medium with 5 mg ml⁻¹ Fc fragments, virus production was 99% inhibited (Table 1). Similar inhibition was seen with Fc fragments at 2.5 mg ml⁻¹ and no effect was demonstrable with 1 mg ml⁻¹.

The infected monolayers of Vero cells maintained on IgG showed a marked decrease in viral cytopathic effect when examined by light microscopy. Electron microscopic examination of Vero and Y-79 cultures 24 and 48 h after infection in 10 mg ml⁻¹ IgG medium showed a marked decrease in viral capsids in the nuclei when compared to control cultures (Fig. 1). Most of the capsids in the IgG treated cells were empty, and accumulations of empty capsids in the cytoplasm were prominent. The same morphological findings were observed when the infected cultures were incubated in medium containing Fc fragments.

In all of the infected cultures examined by electron microscopy, the morphological effects on virus by IgG and Fc fragments were seen in more than 90% of the cells. Although only 20–40% of the cells in infected cultures produced rosettes with the 7S-EA, it is known that haemadsorption with 7S-EA is not an efficient method for demonstrating Fc receptors in other systems¹¹, and our electron microscopical findings suggest that the immunoglobulin exerts an inhibitory effect on most if not all infected cells in the cultures.

We also tested the effect of incubation in IgG containing media on infectious centre production. Y-79 cells were infected with a multiplicity of 100, washed three times in BSS and 10⁴, 10³ and 10² cells plated on Vero cell monolayers. After plating, the monolayers were fed with IgG-containing medium supplemented with 2% of HSV anti-serum in order to obtain plaques. This experiment showed

Table 1 Effect of rabbit IgG and Fc fragments on HSV growth in retinoblastoma and Vero cells

Retinoblastoma	Total virus produced (PFU ml ⁻¹)
IgG (10 mg ml ⁻¹)	2.3 × 10 ⁴
Fc Fragment (5 mg ml ⁻¹)	1.2 × 10 ⁴
Control	1.3 × 10 ⁶
Vero	
IgG (10 mg ml ⁻¹)	1.2 × 10 ²
Fc (5 mg ml ⁻¹)	1 × 10 ³
Control	2.5 × 10 ⁴

Cultures were infected at a multiplicity of 100 and viral growth was measured 24 h (Vero) and 48 h (retinoblastoma) after infection. Control cultures contained equivalent protein concentration of albumin. 10⁴ cells were infected with 10⁶ plaque forming units (PFU) and incubated in IgG or Fc fragment-containing media. At the end of the experiment the number of cells was not changed, indicating no loss of cells or growth during the experiment. The cell viability was 90% by Trypan blue exclusion in both controls and IgG treated cultures.

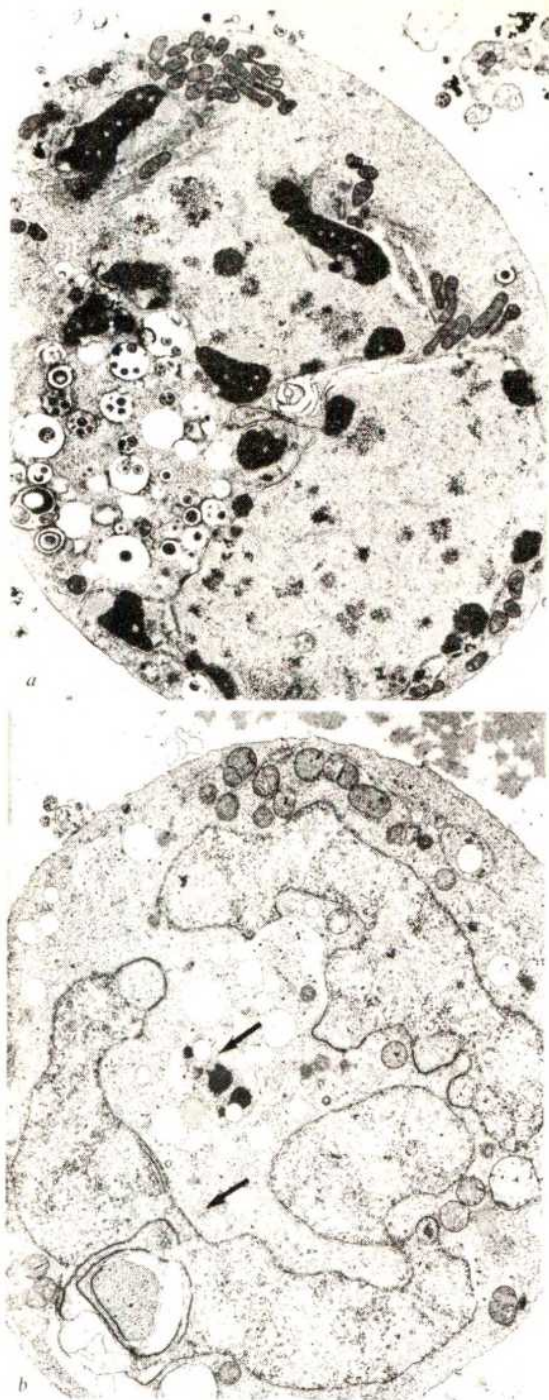


Fig. 1 Electronmicrographs of Vero cells infected with HSV-2. *a*, Cells incubated for 24 h in media containing albumin. Production of complete virions is seen. *b*, Cells incubated with 10 mg ml⁻¹ of rabbit IgG. Empty viral capsids are present in the nucleus and cytoplasm. ($\times 17,000$).

no infectious centres in IgG-containing medium with 10^4 , 10^3 or 10^2 cells while controls in medium free of IgG showed that 12% of the infected cells plated as infectious centres. The low percentage of infectious centres in the controls may be related to the use of a fluid rather than semi-solid overlay.

Our results establish that IgG and its Fc fragment inhibit the growth of HSV and strongly suggest that the effect is due to engagement of the virus-induced Fc receptor. Although the mechanism is not yet known, it is possible that this inhibition obtained with physiological concentrations of IgG may be of relevance in control of primary

infection *in vivo* and perhaps in the establishment or maintenance of latency.

We thank Dr M. Frank for preparation of sheep red blood cell reagents.

J. COSTA
A. S. RABSON
C. YEE
T. S. TRALKA

Laboratory of Pathology, National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 2 June; accepted 22 July 1977.

- ¹ Watkins, J. F. *Nature* **202**, 1364 (1964).
- ² Yasuda, J. & Milgrom, F. *Int. Arch. Allergy* **33**, 151 (1968).
- ³ Westmoreland, D. & Watkins, J. J. *gen. Virol.* **24**, 167 (1974).
- ⁴ Costa, J. C. & Rabson, A. S. *Lancet* **i**, 77 (1975).
- ⁵ Lehner, T., Wilton, J. M. A. & Shillito, E. J. *Lancet* **ii**, 60 (1975).
- ⁶ Costa, J., Yee, C., Troost, T. & Rabson, A. *Nature* **252**, 745 (1974).
- ⁷ Reid, T. W. *et al.* *J. natn. Cancer Inst.* **53**, 347 (1974).
- ⁸ Reynolds, H. Y., Atkinson, J. P., Newball, H. H. & Frank, M. M. *J. Immun.* **114**, 1813 (1975).
- ⁹ Jaffe, E. S., Shevach, E. M., Frank, M. M., Berard, C. W. & Green, J. *New Engl. J. Med.* **290**, 813 (1974).
- ¹⁰ Prael, J. W. *Biochem. J.* **104**, 647 (1967).
- ¹¹ Huber, C. H., Sundstrom, C., Nilsson, K. & Wigzell, H. *Clin. exp. Immun.* **25**, 367 (1976).

Excretion of feline leukaemia virus by naturally infected pet cats

THE transmission of feline leukaemia virus (FeLV) horizontally among cats has been well established¹⁻³ but the source of infectious virus responsible for this transmission has not been determined. C-type particles resembling FeLV have been seen by electron microscopy in many tissues and body fluids from cats having leukaemia and lymphoma⁴⁻⁷. In reference to potential environmental excretion, particles have been seen in the upper and lower respiratory tract, saliva, urine, gut wall, and peripheral blood. These studies, however, have been limited to electron microscopic visualisation and have not studied levels of infectious FeLV in tissues or fluids which could release virus into the environment. Speculation about the source of infectious virus has included urine, saliva, fleas, and blood (from either scratches, bites, or blood sucking arthropods)^{1,8}.

FeLV is so far the only oncogenic RNA virus that is known to be widely disseminated in man's environment, and several epidemiological studies have attempted to determine if an increased risk of human malignancies occurs for individuals exposed to leukaemic cats⁹⁻¹². Furthermore, the US National Cancer Institute has categorised the FeLV and the feline sarcoma virus as agents of 'moderate risk', presumably because they can grow in human cells and/or demonstrate oncogenicity in experimental conditions in certain non-feline species¹³. It thus seems remarkable that little or no information is available on the sources and amounts of infectious FeLV in excretory materials, especially from those taken from naturally infected pet cats.

To understand better the transmission of this disease and to assess the exposure potential of this virus to human contacts of infected cats we have studied the excretion of FeLV in virus-positive cats.

The animals used in all experiments were naturally infected FeLV-positive healthy pet cats who were identified from two sources: a previously characterised cluster house (four cats)¹⁴, or cats referred by community veterinarians (eight cats). The cats were judged positive based on the indirect immunofluorescence test for virus internal antigens in peripheral blood cells². Six of the animals were domestic short hair, three were Abyssinian, two Burmese, and one Siamese. Eight were males and four were females. All were asymptomatic and seemed healthy.

Plasma was collected in heparinised tubes, placed on ice,

separated, and frozen at -90°C until testing. Saliva was collected using small calcium alginate swabs (Calgiswabs, Inolex, Glenwood, Illinois), diluted in 1.5 ml medium and frozen at -90°C until testing. These swabs absorb a constant volume of saliva and allow quantification of infectious virus (unpublished observations). Urine was collected in sterile containers after manual expression from the bladder. Faecal specimens, in approximately 1-g amounts, were collected using calcium alginate swabs and placed in phosphate-buffered saline and frozen before testing at -90°C . After thawing, the suspension was centrifuged briefly at 1,000 r.p.m. and the supernatant was passed through a $0.22\text{-}\mu\text{m}$ filter (Millipore, Bedford, Massachusetts) before placing on cell cultures. Fleas were individually vacuumed off from cats, pooled in groups of ten, and frozen at -90°C until testing. Before being placed in cell cultures, the fleas were diluted in 1 ml cell culture medium, homogenised with a Teflon pestle, and centrifuged briefly at 1,000 r.p.m.

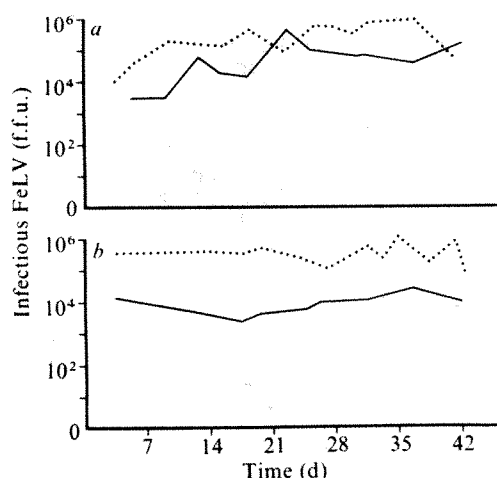


Fig. 1 Simultaneous FeLV titres in plasma (continuous line) and saliva (dotted line) in two cats (a and b) followed for 2 months.

Virus assay was carried out in triplicate using the method of Fischinger¹⁵. Before assay, all specimens were thawed, and tenfold dilutions were made on ice using McCoy's 5a with 15% foetal calf serum containing penicillin 100 IU ml^{-1} , streptomycin $100\text{ }\mu\text{g ml}^{-1}$, and amphotericin $0.25\text{ }\mu\text{g ml}^{-1}$.

Simultaneous saliva and plasma samples collected from nine cats were assayed for virus (Table 1). Saliva was positive in all cats examined, containing 5.4×10^3 – 3.9×10^5 focus forming units (f.f.u.) per ml whereas plasma generally contained about fivefold less. Three cats out of the twelve continued to excrete a cytopathic agent in their saliva for the entire length of the study and we were unable to assay their saliva for virus.

Urine was tested from three cats and was negative for infectious virus at the lowest dilution (undiluted or 1:10) (Table 2). Foecal specimens from three cats were tested, and no infectious virus was demonstrated at the

Table 1 Simultaneous saliva and plasma levels of infectious FeLV

Cat	Number of paired specimens*	Saliva f.f.u.	Plasma f.f.u.
1	11	3.9×10^5 †	1.7×10^5 †
2	12	2.4×10^5 †	6.8×10^4 †
3	2	1.6×10^4 †	6.9×10^3 †
4	1	4.4×10^3	3.2×10^4
5	1	3.0×10^5	2.8×10^4
6	1	5.7×10^4	1.6×10^3
7	1	5.4×10^3	1.0×10^3
8	1	1.8×10^4	6.4×10^3
9	1	3.6×10^5	3.2×10^4

*Each specimen was analysed in triplicate.

†Mean.

lowest dilution (0.5 g ml^{-1}) (Table 2). Ten pools of ten fleas each were examined for infectious virus. None was demonstrated at the highest concentration (5 fleas ml^{-1}) (Table 2).

Two cats were followed for 2 months with once or twice weekly FeLV assay for plasma and saliva (Fig. 1). These cats regularly excreted high titres of FeLV in their saliva (10^4 – 10^6 f.f.u. ml^{-1}) and their plasma (10^3 – 10^5 f.f.u. ml^{-1}). Although there was moderate variation of both saliva and plasma, the titres of virus in both remained high and never dropped below 10^3 f.f.u. ml^{-1} . Plasma FeLV alone was assayed in four additional cats for 3 months. The results were similar to those shown in Fig. 1.

Our results indicate that FeLV-infected healthy cats excrete levels of infectious FeLV in saliva that are as high or higher than levels in blood plasma. Although various other tissues and body fluids have revealed virus particles by electron microscopy or serology, our negative results on these suggest that they may be less significant as sources of transmissible virus. In a previous serological study that was non-quantitative, we found detectable levels of FeLV in salivary gland tissue from five out of five leukaemic cats and in urine from three out of five leukaemic cats². Whether cats that are leukaemic excrete infectious FeLV in the same manner as healthy infected cats is unknown. Our study, however, has emphasised non-leukaemic FeLV-infected cats for two reasons: (1) the prevalence of such cats in most random pet populations is considerably higher than the number with leukaemia; and (2) non-leukaemic FeLV-excretor cats have been regularly overlooked or ignored by epidemiologists who examined either cat-to-cat or potential cat-to-man transmission. Such conventional epidemiological studies, especially those attempting to link feline leukaemia to human malignancy, have usually failed to detect horizontal transmission of FeLV or any increased risk of malignancy in human contacts^{12,16}. These investigations, however, chose cases and controls by pathological diagnosis or pet ownership status, and not by virus positivity. We now know that 10–50% of leukaemic cats ('cases') are non-viraemic^{1,2,17}, and that clinically healthy cats or those ill with diseases other than leukaemia or lymphoma ('controls') are often infected with FeLV. Also, serological studies have indicated that many FeLV-infected healthy cats contain detectable plasma virus for several years^{18,19}. In addition, as many as half of the pet cats in free-roaming

Table 2 Infectious FeLV titres in specimens from healthy excretor cats

Specimen	Cats tested	Total	Samples tested (f.f.u.)*	Mean titre (f.f.u.)	Range (f.f.u.)
Plasma	12	70	70	4.4×10^4	9.4×10^2 – 8.4×10^5
Saliva	9†	30	30	2.2×10^5	5.4×10^3 – 2.0×10^6
Urine	3	5	0	—	—
Faeces	3	3	0	—	—
Fleas	4	10‡	0	—	—

*Each tested in triplicate.

†Three cats consistently and four others periodically excreted an agent toxic to our cell cultures.

‡Total of 100 fleas.

populations have evidence of transient FeLV infection, and only small proportions of those that remain chronically infected ultimately develop leukaemia or lymphoma^{1,20}. Therefore, epidemiological studies to date designed to ascertain human risk of FeLV exposure have not precisely done so since virus exposure was probably present in both the case and control groups.

In conclusion, it seems too early to rule out a link between any human disease and exposure to FeLV. Many FeLV-infected cats exist in the community and the laboratory methods to identify them are readily available. Subsequent studies designed to examine potential human risk from FeLV should compare the incidence of human disease in contacts of FeLV-positive cats to matched controls in FeLV-negative cats.

This work was supported by the American Cancer Society and the US National Cancer Institute. D.F. is a Career Development Award recipient from the Center for Disease Control, Atlanta, Georgia, and M.E. and W.D.H. Jr are Scholars of the Leukemia Society of America. We thank Drs S. Cotter and E. Essex for clinical assistance, and D. Gayzagian for technical assistance.

D. P. FRANCIS
M. ESSEX

Department of Microbiology,
Harvard School of Public Health,
Boston, Massachusetts 02115

W. D. HARDY, JR

Memorial Sloan-Kettering Cancer Center,
New York, New York 10021

Received 29 May; accepted 11 July 1977.

- ¹ Essex, M. *Adv. Cancer Res.* **21**, 175 (1975).
- ² Hardy, W. D., Jr *et al. Nature* **244**, 266 (1973).
- ³ Jarrett, W. *et al. J. natn. Cancer Inst.* **51**, 833 (1973).
- ⁴ Dougherty, E., Post, J. E. & Rickard, C. G. *Can. Vet. J.* **10**, 291 (1969).
- ⁵ Gardner, M. B., Rongey, R. W., Johnson, E. Y., DeJournett, R. & Huebner, R. J. *J. natn. Cancer Inst.* **47**, 561 (1971).
- ⁶ Hardy, W. D., Jr *et al. Science* **166**, 1019 (1969).
- ⁷ Laird, H. M., Jarrett, O., Crighthon, G. W. & Jarrett, W. F. H. *J. natn. Cancer Inst.* **41**, 867 (1968).
- ⁸ Hardy, W. D., Jr *et al. in Comparative Leukemia Research* (eds Ito, Y. & Dutcher, R. M.) **67** (University of Tokyo Press, Tokyo, 1975).
- ⁹ Schneider, R. *Int. J. Cancer* **10**, 338 (1972).
- ¹⁰ Hanes, B. *et al. J. natn. Cancer Inst.* **45**, 1155 (1970).
- ¹¹ Bross, I. D. J. & Gibson, R. J. *Med. exp. Clin. I.* **180** (1970).
- ¹² Essex, M. & Francis, D. P. *J. Am. Animal Hosp. Ass.* **12**, 386 (1976).
- ¹³ *Biologic Safety Manual for Research Involving Oncogenic Viruses*, National Cancer Institute, 3 October, 1974.
- ¹⁴ Essex, M. *et al. J. natn. Cancer Inst.* **54**, 637 (1975).
- ¹⁵ Fischinger, P., Blevins, C., Nomuro, S., *J. Virol.* **14**, 177 (1974).
- ¹⁶ Schneider, R. *Int. J. Cancer* **10**, 345 (1972).
- ¹⁷ Jarrett, W. F. H. *Adv. vet. Sci. comp. Med.* **19**, 165 (1975).
- ¹⁸ Essex, M., Hardy, W. D., Jr, Cotter, S. M., Jakowski, R. M. & Sliski, A. *Inf. Immunity* **11**, 470 (1975).
- ¹⁹ Cotter, S. M., Essex, M. & Hardy, W. D. Jr *Cancer Res.* **34**, 1061 (1974).
- ²⁰ Jarrett, W. F. H. *Bibl. Haemat.* **43**, 209 (1975).

Blood group-like activity released by human mammary carcinoma cells in culture

TUMOUR cells synthesise and release antigenic membrane associated components which circulate either in a free state or complexed with host immunoglobulins¹⁻⁵. A membrane derived N-like glycoprotein, which inhibited *Vicia graminea* lectin haemagglutination, has been demonstrated in serum and ascites fluid of mice bearing the Ha subline of TA₃ murine mammary adenocarcinoma⁶. Springer *et al.*⁷ have shown that M and N blood group reactive substances were present in both benign and malignant lesions of the human breast. But, T-like *Arachis hypogaea* reactive substances were only found in malignant breast tissue and not in benign tumours or healthy breast tissue⁷. Isolation and long term cultivation of a human mammary tumour cell line, BOT-2 was recently reported⁸. Immunological tests indicated that women with diagnosed mammary cancer had circulating antibodies that reacted with these cells⁹ and induced cell surface shedding¹⁰. Here we report the use of blood-typing antisera and lectins to characterise the proteins synthesised and spontaneously

released from BOT-2 cells growing in defined medium. To our knowledge this is the first demonstration of M, N, T, Tn erythrocyte-like glycoproteins being spontaneously shed from human epithelial tumour cells in culture.

BOT-2 cells, after 2 d growth on Eagle's minimum essential medium with 10% foetal calf serum, were grown to confluency (2 weeks) in albumin containing, serum free medium (Hi-WO₃/BA₂₀₀₀; International Scientific). After the 2-week interval, each third day the medium was collected from the culture flasks, centrifuged at 200g for 10 min to remove debris and lyophilised. The dry solids were resuspended in distilled water, dialysed against phosphate buffered saline pH 7.2 (PBS) and chromatographed on Sephadex G-10 equilibrated with PBS. The G-10 excluded proteins were then concentrated to 5 mg ml⁻¹ by pressure dialysis on an Amicon XM-50 membrane. The concentrated protein was tested for reactivity against human blood-typing lectins and antibodies. First the BOT-2 protein was screened for reactivity against various lectins by double diffusion in gel. Then, more sensitive agglutination inhibition assays were performed to show the competition of BOT-2 proteins and M or N red cells for specific anti-M or N antibody binding. Finally because of the potential significance of spontaneously shed glycoproteins with T-like activity to tumour growth and survival, a more sensitive test using shed proteins to inhibit T-erythrocyte agglutination was used.

Double diffusion reactions of BOT-2 proteins and selected blood group typing lectins are shown in Table 1. It may be seen that BOT-2 proteins formed precipitin bands with a variety of lectins having specificities related to blood group antigens M, N, T and Tn. To show cross-reactivity of BOT-2 proteins and the erythrocyte glyco-

Table 1 Blood group active lectins which form precipitin bands with shed human breast tumour cell proteins

Blood group		Lectins	
Activity	Common name	Systematic name	Refs
anti-M	Candytuft	<i>Iberis amara</i>	11, 12
anti-N	—	<i>Vicia graminea</i>	7, 11
anti-T	Peanut	<i>Arachis hypogaea</i>	11, 13
anti-Tn	Blue beard	<i>Salvia horminum</i>	14
anti-Tn	Horse gram	<i>Dolichos biflorus</i>	7

Seeds were ground dry, mixed with four times their weight of 0.15 M NaCl for 3 h at 20 °C, centrifuged 10 min at 3,000g and the supernatant solution collected. *A. hypogaea* lectin was further purified by affinity chromatography on β -galactosyl derivatives coupled to Sepharose 4B. The lectins (5–20 mg ml⁻¹) were reacted with a 10X concentrate (5 mg ml⁻¹) of dialysed BOT-2 cell growth medium by diffusion tests in 1% Agarose in PBS¹⁵.

proteins by a system involving different reaction mechanisms, a specific antiserum-red cell agglutination inhibition was performed. Agglutination of human M and N erythrocytes by both human and rabbit anti-M and anti-N antisera was inhibited by BOT-2 proteins (Table 2). When a more sensitive assay of T-like activity in BOT-2 proteins was used, the agglutination of human T red blood cells (neuraminidase-treated erythrocytes) by anti-T lectin (*A. hypogaea*) could be completely inhibited by BOT-2 proteins (Table 3). Inhibition required 3,000 times as much BOT-2 proteins as anti-T lectin on a weight basis, however.

In all assays two types of controls were performed. In one, unused medium was concentrated to equal the concentration of medium from BOT-2 cells. In the other, growth medium from a human melanoma cell line was prepared in the same manner as the BOT-2 proteins. When the concentrated unused or melanoma growth medium were tested by double diffusion, antibody agglutination inhibition or lectin agglutination inhibition, no M, N, T or Tn activity was found.

Shed glycoproteins from human breast carcinoma cells

Table 2 Effect of shed human breast tumour cell proteins on the agglutination of M or N erythrocytes by human and rabbit M or N antiserum

Erythrocytes and antiserum	Human antiserum BOT-2 cell proteins (μ g)	Inhibition of agglutination	Erythrocytes and antiserum	Inhibition of agglutination
M	65	Yes	M, 1:2	No
M	32	No	M, 1:4	No
M	16	No	M, 1:8	Yes
			M, 1:16	Yes
N	65	Yes	N, undiluted	No
N	32	No	N, 1:2	Yes
N	16	No	N, 1:4	Yes

Using human antiserum, 50 μ l of serum were incubated with 50 μ l of the indicated amount of BOT-2 cell proteins. Using rabbit antihuman M or N antiserum, 25 μ l of serum at the indicated dilutions were incubated with 25 μ l (32 μ g) of BOT-2 cell proteins. After 15 min incubation at 20 °C, 25 μ l of a 3% suspension of washed human M or N erythrocytes were added to the reactions. After incubation for 90 min, the agglutination reactions were read by visual inspection. The haemagglutination titres of the human M and N antisera were both 1:8. The haemagglutination titres of the rabbit M and N antisera were both 1:64.

in culture react with the same lectins as do the membrane antigens isolated from whole human breast tumour tissue⁷ and the glycoproteins in serum and ascites fluid from mice bearing the TA₃-Ha mammary carcinoma⁶. The finding that BOT-2 glycoproteins reacted with anti-M lectin (*I. amara*) and anti-N lectin (*V. graminea*) suggests that the carbohydrate portions of BOT-2 proteins are similar to that of M and N blood group substances. The reaction of BOT-2 proteins with anti-T (*A. hypogaea*) and anti-Tn (*S. horminum*, *D. biflorus*) may be due to incomplete glycosylation of some of the carbohydrate chains by BOT-2 cells, since T and Tn activity may be induced from N substance by selective enzymatic digestion¹⁶⁻¹⁹.

Other workers have found M and N activity in both benign and malignant breast tumours, but T and Tn activities have been reported to occur only in malignant human breast tumour tissue. A significant number but not all patients with active mammary carcinomas had lowered anti-T serum activity, but after surgical excision of the tumour many of these patients had increased serum anti-T activity²⁰.

Our findings show that a human ductal mammary carcinoma cell line growing in chemically defined media spontaneously releases glycoproteins which have M, N, T and Tn activity. These observations are in accord with the idea that early survival of these cells may depend on presentation of a cell surface similar to M and/or N erythrocyte membrane glycoproteins. As the tumour mass increases it ceases to form fully glycosylated membrane glycoproteins. The resulting incompletely glycosylated M-N precursors (that is, T and Tn) may block or overwhelm the hosts immune capabilities with the result that rapidly growing and aggressive tumour cells have decreased restraint upon survival and growth.

We thank R. J. McNeil, P. J. Riggs, and P. L. Munson for technical assistance; Dr Jim Lawson, Tulsa Regional Red Cross Blood Center, for useful conversations and

the gift of some seeds; Richard Murdock (Ortho Pharmaceuticals) for human M and N antisera; Bill Deal and Twanda Bruegggen for typed blood; and the American Cancer Society (BC-230) and the Maizie Wilkonson award for support.

J. H. ANGLIN, JR*

Departments of Biochemistry and Molecular Biology and Research Dermatology,

MICHAEL P. LERNER

Department of Microbiology and Immunology,

ROBERT E. NORDQUIST

Departments of Anatomical Sciences and Research Dermatology

University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Received 13 June; accepted 22 July 1977.

*To whom reprint requests should be addressed.

- Hollinshead, A. C. *et al. Cancer* **34**, 1235-1243 (1974).
- Currie, G. A. & Basham, C. Br. *J. Cancer* **26**, 427-438 (1972).
- Thomson, D. M. P. & Alexander, P. Br. *J. Cancer* **27**, 35-47 (1973).
- Alexander, P. *Cancer Res.* **35**, 2077-2082 (1974).
- Kim, U., Bauml, A., Carruthers, C. & Biel, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1012-1015 (1975).
- Cooper, A. G., Codrington, J. F. & Brown, M. C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1224-1228 (1974).
- Springer, G. F., Desai, P. R. & Banatwala, I. J. *natn. Cancer Inst.* **54**, 335-339 (1975).
- Nordquist, R. E., Ishmael, D. R., Lovig, C. A., Hyder, D. M. & Hoge, A. F. *Cancer Res.* **35**, 3100-3105 (1975).
- Nordquist, R. E., Schafer, F. B., Manning, N. E., Ishmael, D. R. & Hoge, A. F. *J. Lab. clin. Med.* **89**, 257-261 (1977).
- Nordquist, R. E., Anglin, J. H. & Lerner, M. P. *Science* **197**, 366-367 (1977).
- Dahr, W., Uhlenbruck, G. & Bird, G. W. G. *Vox Sang.* **28**, 133-148 (1975).
- Uhlenbruck, G. & Dahr, W. *Vox Sang.* **21**, 338-351 (1971).
- Lotan, R., Skutelsky, E., Danon, D. & Sharon, N. *J. biol. Chem.* **250**, 8518-8523 (1975).
- Bird, G. W. G. & Wingham, J. *Vox Sang.* **26**, 163-166 (1974).
- Ouchterlony, O. *Acta Path. Micro. Scand.* **32**, 231-240 (1953).
- Uhlenbruck, G., Pardoe, G. I. & Bird, G. W. Z. *Immunitätsforsch.* **138**, 423-433 (1969).
- Springer, G. F., Tegtmeyer, H. & Huppikar, S. V. *Vox Sang.* **22**, 325-343 (1972).
- Springer, G. F. & Desai, P. R. *Biochem. biophys. Res. Commun.* **61**, 470-475 (1974).
- Springer, G. F. & Desai, P. R. *Carbohydr. Res.* **40**, 183-192 (1975).
- Springer, G. F., Desai, P. R. & Scanlon, E. F. *Cancer* **37**, 169-176 (1976).

A-specific autoantigenic ovarian glycolipids inducing production of 'natural' anti-A antibody

ACCORDING to current concepts, those antibody populations which appear spontaneously in the sera of many species against components of the widely-distributed ABH (blood-group) antigen system are due to stimulation by corresponding exogenous (environmental) antigenic material¹⁻³. We present here evidence which strongly suggests an alternative origin in the mouse.

We recently reported that the development of 'natural' antibodies against structural elements of the common (blood-group) antigen A is markedly sex-dependent in C57BL/10 inbred mice. In these experiments all the sera of

Table 3 Effect of shed human breast tumour cell proteins on the agglutination of human T erythrocytes by purified anti-T lectin

BOT-2 cell proteins (μ g)	Agglutination of T erythrocytes
300	No
150	No
75	Yes
38	Yes

Human T erythrocytes were prepared by neuraminidase treatment of human O erythrocytes⁷. Purified anti-T lectin (*A. hypogaea*) 50 ng was incubated for 15 min at 20 °C with the indicated amount of shed proteins from BOT-2 cells; 0.2 ml of a 3% suspension of T erythrocytes was then added and the mixture incubated for 90 min. The agglutination reaction was determined by visual inspection. The haemagglutination titre of the peanut lectin was 1:10,000.

the females of healthy, unvaccinated mice displayed high levels of antibodies which selectively agglutinate the erythrocytes of the human blood group A, but only about 50% of the sera of the males exerted detectable activities. This pronounced production of 'natural' anti-A antibody in females having a normal oestrous cycle and fertility correlates with the synthesis of ovarian glycolipids specifically combining with the syngeneic 'natural' anti-A antibody, which they strongly inhibit. Such auto-reactivity was not detected in the glycolipid fractions from any of the other male and female murine tissues at statistically significant levels¹. Moreover, anti-A specific complement-dependent lytic serum activities which discriminate between different (blood-group) A-antigen expressions or qualities and are also exclusively inhibited by the syngeneic ovarian glycolipids could be demonstrated in a further study², and similar observations have been made in limited (unpublished) studies with DBA/2, DDS and NMRI mice.

The genetic background of these findings is as yet unknown, and the site of appearance of the A-active glycolipid structures in the ovarian tissue and the type of cell or cells engaged in their production could up to now also not be determined. But the experiments presented here (Fig. 1) show that these glycolipid structures are probably not expressed at birth but appear during post-natal ovarian maturation and that only low levels of the 'natural' anti-A antibody are produced in the absence of the ovary. At 10 d of age the murine ovarian tissue displays extremely weak A-specific auto-reactivity which then increases rapidly during the maturation process. Marked auto-reactivity can already be measured at the age of 20 d, that is, long before the first ovulation, but complete expression of this reactivity is not reached before the age of 30–40 d and thus correlates with the onset of puberty in the mouse. In fact, no further increase could be measured thereafter. This is illustrated in Fig. 1a, which also shows that the development of the A-specific auto-reactive structures markedly precedes the appearance of the anti-A antibody (Fig. 1b). The antibody develops poorly in mice ovariectomised at the age of 20 d, whereas the development of the antibody levels in sham-operated mice does not significantly differ from that in non-operated mice. Hence, the 'natural' anti-A antibody in the C57BL/10 female mouse is an autoantibody, that is, the syngeneic ovarian glycolipids not only combine with this antibody as a result of, for example, coincidental cross-reactivity, but are also specifically engaged in its induction.

The crucial carbohydrate determinant of these glycolipids involves *N*-acetyl-D-galactosamine in terminal linkage, as the glycolipid material prepared by the method previously described¹ strongly inhibits the agglutinins specifically combining with this amino sugar. Four haemagglutinating units of the anti-A specific lectin from *Dilochos biflorus* (Behringwerke) and the anti-A haemagglutinin from *Helix pomatia* (from Dr E. Cohnen) in volumes of 20 μ l were incubated with equal volumes from twofold serial dilutions of the ovarian glycolipids in 130 mM NaCl–25 mM Na-phosphate buffer, pH 7.2 (PBS), for 60 min at 24 °C. Twenty μ l of a 1% suspension of washed human blood group A₁ erythrocytes in PBS were then added and the incubation was continued for another 60 min. The amounts of the glycolipids producing complete haemagglutination inhibition were determined by microscope. The four haemagglutinating units of the *D. biflorus* lectin and the *H. pomatia* haemagglutinin were completely inhibited by 5 and 2.5 μ g of the glycolipid material respectively.

Whatever their other structural properties, which are currently being investigated, such glycolipids are probably not restricted to ovarian tissue. Moreover, the auto-reactive structures discovered in the ovary may also be produced in other murine tissues but in quantities too low to be

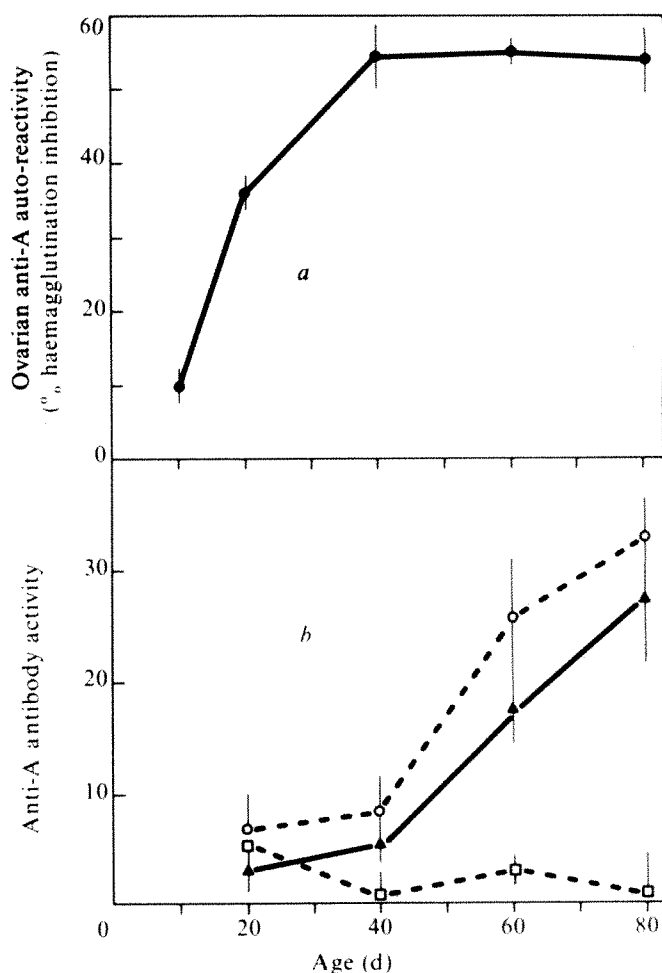


Fig. 1 *a*, Age-dependent appearance of ovarian A-specific auto-reactivity. 10-, 20-, 40-, 60- and 80-d-old healthy, unvaccinated C57BL/10 female mice from our colony (from Jackson Laboratory, Bar Harbor, Maine), each age group consisting of 3 × 20 animals, were killed by ether inhalation in three separate experiments. Whole venous blood was drawn from the caval veins of the 80-d-old mice, pooled, and the serum to be used in the inhibition experiments 'inactivated' for 15 min at 56 °C. The ovaries were removed and pooled according to the respective groups. The ovarian polar glycolipids were prepared as previously described¹ and dissolved in 130 mM NaCl–25 mM Na-phosphate buffer, pH 7.2, at 100 μ g ml⁻¹. The anti-A-inhibitory activities of the glycolipid material from the ovaries of the different age groups were measured and are given as % inhibition of the haemagglutinating power of the pooled syngeneic serum from the 80-d-old C57BL/10 females for human blood group A₁ erythrocytes according to the previously described quantification procedure¹. Values represent the arithmetic mean and standard deviation obtained from the three different inhibition experiments. *b*, Age-dependent appearance of 'natural' anti-A antibody activities in the sera of unvaccinated C57BL/10 female mice. \blacktriangle , Untreated (non-operated) mice; \square , mice ovariectomised at the age of 20 d; \circ , sham-operated mice. Animals to be ovariectomised or sham-operated were anaesthetised by intraperitoneal injection of 0.1 ml (40 mg per kg body weight) of pentobarbital sodium (Nembutal, C. H. Boehringer). Ovaries were removed by standard techniques. In sham-operations performed in parallel, the peritoneal cavity was opened and closed, but the ovaries were left intact. Three different groups, each consisting of 3 × 20 ovariectomised and 3 × 20 sham-operated animals, were killed on the day of the operation and at the age of 40, 60 and 80 d in three separate experiments. Corresponding groups of non-operated animals were killed in parallel. The sera were prepared from blood drawn from the caval veins, pooled according to the respective groups and 'inactivated' for 15 min at 56 °C. The anti-A haemagglutinin activities were determined using human blood group A₁ erythrocytes¹. Each value represents the arithmetic mean and standard deviation obtained from the three different experiments. Values on ordinate derived from¹

$$\sum_{i=1}^k [\text{score}_i \times (-\log_2 (\text{dilution}) + 1)]$$

demonstrable by the methods used in our previous studies^{4,5}. The low anti-A antibody levels in the sera of the males described in those studies and in the sera of ovariectomised females (see Fig. 1b) could reflect extra-ovarian autoantigenic structures, although the possibility of extraneous stimulation due to environmental A-antigenic components cannot be excluded.

Nevertheless, the data presented here strongly suggest that the production of the 'natural' anti-A antibody in the female mouse originates predominantly from autoimmunisation due to an ovarian autoantigen characterised by the A-specific auto-reactivity discovered. It could be argued that this autoimmunisation is induced by material released from the autoantigenic ovarian structures, but that these structures are probably not available to the antibody *in situ*. In fact, many potentially immunoreactive glycolipids in mammalian cell membranes are effectively shielded by proteinaceous cover and do not react with the antibodies directed against them. This applies particularly to a variety of globosides which display N-acetyl-D-galactosamine residues demonstrating predominantly Forssman-type specificity and becoming available to their corresponding antibodies only after protease treatment of the cell surface^{6,7}. Although the murine ovarian A-like autoantigen does not seem to involve this particular 'classic' heterophile specificity, its components might also well be 'masked' *in situ* and protected from immunological attack in normal conditions. Thus the functional significance of the findings reported is still not known. Moreover, the intriguing question of whether these findings may be extrapolated to other species and whether the majority of human 'natural' anti-A (iso)antibody populations may also basically reflect growth processes requires further investigation.

We thank Miss Maria Charlier and Miss Anneliese Rosenbaum for technical assistance and Mrs Beverly B. Niemann for editorial assistance.

P. AREND
J. NIJSSEN

Department of Chemotherapy,
Department of Biometrics,
Research Laboratories,
Chemie Grünenthal,
Postfach 129,
Zweifaller Strasse 112,
D-5190 Stolberg/Rhld, FRG

Received 3 May; accepted 19 July 1977.

- 1 Springer, G. F., Horton, R. E. & Forbes, M. J. *exp. Med.* 110, 221-244 (1959).
- 2 Springer, G. F., Williamson, P. & Brandis, W. C. *J. exp. Med.* 113, 1077-1093 (1961).
- 3 Springer, G. F. & Horton, R. E. *J. clin. Invest.* 48, 1280-1291 (1969).
- 4 Arend, P. & Nijssen, J. *J. Immunogenet.* 3, 373-382 (1976).
- 5 Arend, P. & Nijssen, J. *Z. Immun. Forsch.* 153, 74-84 (1977).
- 6 Uhlenbruck, G., Reifenberg, U. & Heggen, M. *Z. Immun. Forsch.* 139, 486-499 (1970).
- 7 Burger, M. M. *Nature new Biol.* 231, 125-126 (1971).

Reconstruction of photoreceptor membrane potentials from simultaneous intracellular and extracellular recordings

INSERTING a microelectrode into a cell to record electrical events provides a rapid and direct way of investigating many fundamental membrane-based processes^{1,2}. The intracellular signals so obtained do not, however, represent a true picture of the underlying permeability changes if significantly large extracellular potentials are also present. As this is indeed the case in a wide range of tissues, including the cephalopod retina, we have recorded simultaneously the intracellular (V_i) and extracellular (V_e) signals from the photoreceptors of *Sepiolo atlantica* in order to reconstruct the true transmembrane potential (V_m). We show here that the discrepancies between

V_i and V_m depend both on the magnitude and duration of the light stimulus.

Extracellular potentials of the order of 30 mV have been recorded from arthropod^{3,4} and cephalopod retinas (R. B. Clark and G.D., unpublished). These are set up by currents passing through regions of high resistance in the restricted space between adjacent photoreceptor cells (Fig. 1), and several equivalent circuit models that take account of this have been put forward⁵⁻⁶. One implication of these models, which has often been overlooked, is that, when the potential at the back of the eye is used as a reference point, the intracellular recording (V_i) will not give a true picture of the actual photoreceptor membrane potential change (V_m), but will in fact measure V_m in series with V_e (Fig. 1). As most investigators of both invertebrate and vertebrate retinas use this recording arrangement (see refs 1 and 2 for reviews), we felt it worthwhile to find out whether there are significant differences between observed V_i and computed V_m potentials (Fig. 1).

We have found that the most stable intracellular recordings are obtained from the cephalopod when the electrode enters a cell almost immediately after touching the distal surface of the retina. Before entering a cell, we first ensured that the extracellular signals from both recording

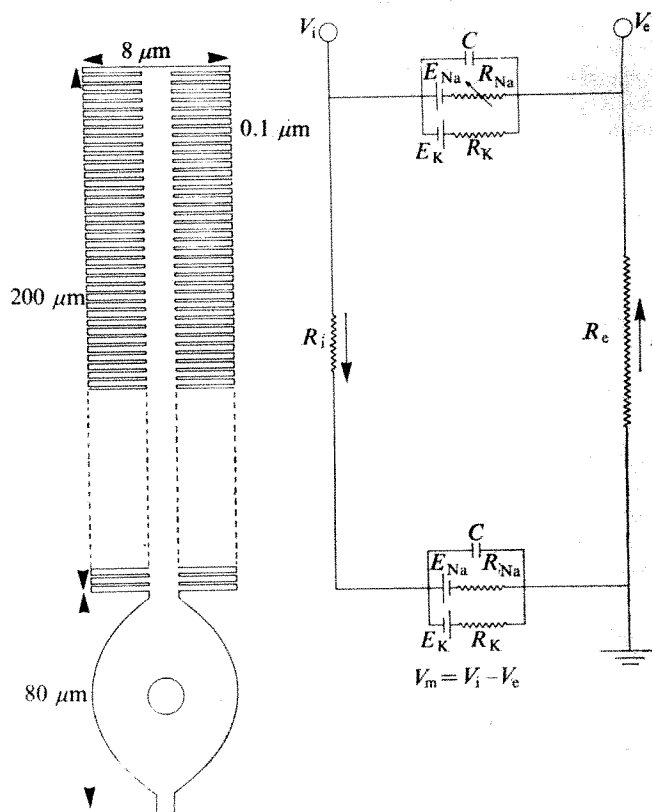


Fig. 1 Diagrammatic representation of a cephalopod photoreceptor cell based on the electron micrographs of refs 18 and 19. There is a minimal extracellular space between cells, whereas in the vertebrate retina the rods and cones are smaller and more widely spaced²⁰. The equivalent circuit is essentially the same cephalopod model as in ref. 6 and resembles an arthropod model⁴. The sodium resistance (R_{Na}) in the distal segment is assumed to decrease in the light. Photocurrents (I) flowing across a large extracellular resistance (R_e) imply a correspondingly large V_e . R_K is usually assumed constant and R_i small, but the validity of neither of these assumptions affects our basic argument which is that the intracellular signal (V_i) does not give a true measure of the primary transmembrane voltage change (V_m). In the recording arrangement used here with the reference electrode (in this case a silver/silver chloride wire loop) at the back of the retina, V_m is given by the difference between V_i and V_e . As V_i and V_e have opposite signs, this means in effect adding the amplitudes of the signals in order to reconstruct V_m .

electrodes were identical. The fact that we could do this with electrodes that were spaced at least 1 mm apart showed the homogeneity of the illumination conditions and, more importantly, the homogeneity of radial current flow across the retina.

Simultaneous extracellular and intracellular records were obtained in response to both short and long flashes from the non-perfused retina maintained at 10 °C (see ref. 7 for experimental details). The short flashes from a stroboscope were 10 μ s in duration and so were well within the latency period of the response at all intensities (Fig. 2). At low intensities ($\log I = -3.2$ and below), the time courses of the two signals are similar whereas the amplitude of the intracellular is the larger. Thus, over this intensity range, the intracellular signals are representative of the true transmembrane potential changes. At higher intensities, the two responses have different time courses and are of comparable magnitude. They are complementary in that they sum algebraically to give a flat region of maximal amplitude. This becomes more apparent as the intensity increases.

The recovery to the baseline is complete within 500 ms at low intensities, whereas there is a significant delay in recovery at the higher intensities. This after-depolarisation, which has been observed in a number of invertebrate preparations^{8,9}, increases with increasing intensity. In vertebrate rods and cones there is an after-hyperpolarisation which increases with intensity¹⁰⁻¹². At the highest intensity used in the present study ($\log I = 0$) there is a very pronounced transient phase in the intra-

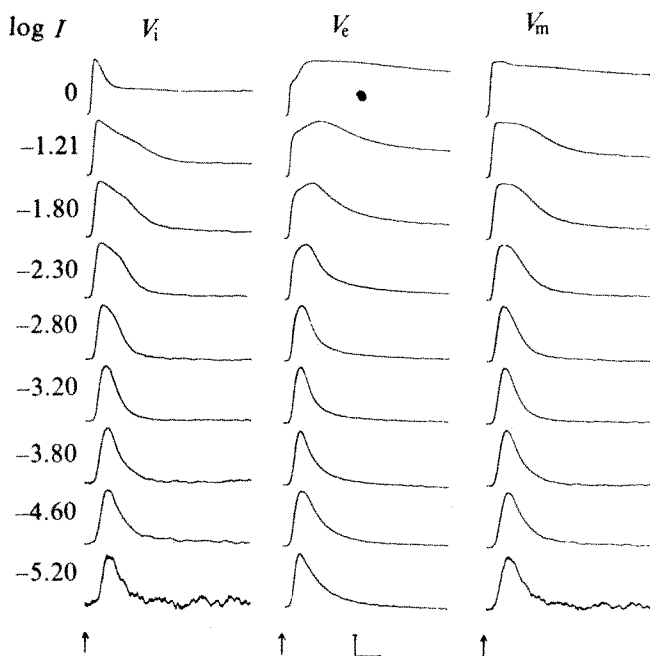


Fig. 2 Response to short flashes. Simultaneous recordings of intracellular and extracellular photoreceptor potentials of the cephalopod retina. The beginning of each trace (arrowed) corresponds to the arrival of a 10- μ s xenon tube flash of unfiltered incident energy equal to 10^{-2} J m $^{-2}$ of white light ($\log I = 0$). The relative intensity of each flash is given on the left of the figure. The resting potential was -45 mV in all of the intracellular recordings (depolarising upwards). The dark potential in the extracellular recordings was zero and an upward deflection in this case corresponds to a negative-going potential at the microelectrode. The signals were digitised, filtered above 1 kHz and plotted. The horizontal scale bar represents 150 ms and the vertical bar represents mV, which for the various intensities is given (in the sequence V_i , V_e , V_m) as follows: $\log I = -5.2$: 0.4, 0.3, 0.7; $\log I = -4.6$: 1.5, 1.0, 2.4; $\log I = -3.8$: 3.6, 2.8, 5.9; $\log I = -3.2$: 6.8, 5.7, 12.1; $\log I = -2.8$: 8.2, 7.6, 15.6; $\log I = -2.3$: 8.8, 9.1, 17.4; $\log I = -1.8$: 9.2, 9.6, 17.7; $\log I = -1.21$: 9.5, 9.7, 17.4; $\log I = 0$: 9.4, 11.1, 16.6.

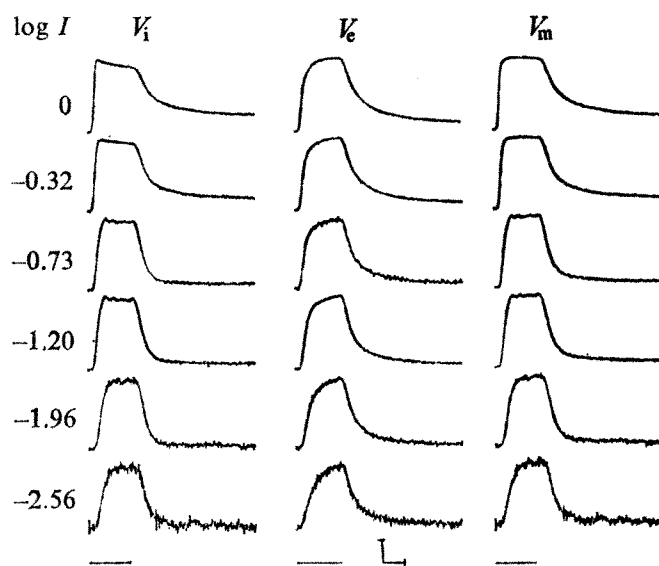


Fig. 3 Response to long flashes. Responses to 500-ms flashes from a light-emitting diode (Plessey GPL5). The light from the diode had an unfiltered energy equal to 0.02 W m $^{-2}$ ($\log I = 0$). The start of each trace corresponds to the stimulus onset which had virtually zero increase-time. The stimulus duration is marked by the solid line below each column. The intracellular dark-adapted potential was -42 mV in each case and the extracellular dark potential was zero. The horizontal scale bar represents 250 ms and the vertical scale bar (mV) for each of the light intensities is given (in the sequence V_i , V_e , V_m) as follows: $\log I = -2.56$: 0.6, 0.2, 0.8; $\log I = -1.96$: 1.3, 0.4, 1.7; $\log I = -1.2$: 3.4, 1.3, 4.7; $\log I = -0.73$: 5.5, 2.3, 7.4; $\log I = -0.32$: 6.7, 3.1, 9.4; $\log I = 0$: 6.8, 3.9, 10.1.

cellular recording which corresponds very closely in time course to the fast response in the extracellular recordings (R. B. Clark and G.D., unpublished). An initial transient has been observed in *Limulus* photoreceptors when short intense flashes were used^{13,14}, and it has been suggested that the transient phase arises from a different mechanism from the remainder of the response¹⁴. It is interesting that the initial transient of V_i in the cephalopod almost completely disappears in the reconstructed membrane response (Fig. 2).

A light-emitting diode was used to deliver 500-ms flashes and in this case the waveforms of the intracellular and extracellular responses appear quite different (Fig. 3). The V_i records are much more rectangular in appearance, and the amplitudes of the responses of V_i were consistently greater than the corresponding V_e records. On closer examination, however, certain similarities between the two types of records emerge. The latencies of both the intracellular and extracellular signals at any one intensity are equal and decrease with increasing intensity, and the initial rate of increase of both signals increases with increasing intensity. At high intensities the intracellular response reaches a maximum value and then decays towards the baseline while the stimulus is still on. This 'sag phase' has also been observed in vertebrate rods and cones¹⁰⁻¹². In the cephalopod, it disappears in the reconstructed membrane responses (Fig. 3). The intracellular records again show a maintained after-depolarisation, which is relatively greater in magnitude than the offset in the V_e records. At high light intensities, as in the short flash responses (Fig. 2), the intracellular and extracellular records sum algebraically to give a flat response over a region in time where both V_i and V_e are changing.

Large extracellular responses are not unique to the invertebrate retina as vertebrate retinal neurones, principally Müller cells¹⁵, can in certain circumstances contribute several millivolts to the overall electroretinogram.

Some workers in this field do take precautions to prevent contamination of primary intracellular photoreceptor recordings by extracellular potentials¹². Extracellular potentials of comparable magnitude are also found, for example, in vertebrate cardiac muscle¹⁶ and olfactory tissue¹⁷. If the membrane events in these tissues are to be analysed in detail, for example in terms of current carriers and permeability changes, then it is important to know the basic time course of the potential change across the receptor membrane. The data presented here strongly indicate that both the intracellular and extracellular signals have to be recorded simultaneously in order to obtain this information. It is also true that in such tissues where the extracellular signal is a function of position (ref. 7 and R. B. Clark and G.D., unpublished), there is no single, true transmembrane potential, but in fact a continuum of transmembrane potentials that require simultaneous recording of the signals from an intracellular electrode and several extracellular electrodes placed at different depths in the tissue. Reconstructions of the primary photoreceptor membrane events based on a simple equivalent model such as that described in Fig. 1 and also a more complex model that takes account of the depth variation of the extracellular signal, will be the subject of a future communication.

We thank Professor E. Rojas and Dr P. C. Croghan for discussions, and Professor P. Fatt of University College for useful criticisms of the manuscript. P.B.P. acknowledges assistance in the form of a scholarship from the University of East Anglia.

P. B. PYNSENT
G. DUNCAN

Biophysics Section,
School of Biological Sciences,
University of East Anglia,
Norwich, UK

Received 12 May; accepted 21 July 1977.

- 1 Tomita, T. *Q. Rev. Biophys.* **3**, 179–221 (1970).
- 2 Rodieck, R. W. *The Vertebrate Retina: Principles of Structure and Function* (Freeman, San Francisco, 1973).
- 3 Burtt, E. T. & Catton, W. T. *J. Insect Physiol.* **10**, 865–886 (1964).
- 4 Shaw, S. R. *Nature* **255**, 480–483 (1975).
- 5 Hagins, W. A. *Cold Spring Harb. Symp. quant. Biol.* **30**, 403–415 (1965).
- 6 Duncan, G. & Croghan, P. C. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 229–233 (Springer, Berlin, 1973).
- 7 Clark, R. B. *Expl Eye Res.* **20**, 499–504 (1975).
- 8 Hochstein, S., Minke, B. & Hillman, P. *J. gen. Physiol.* **62**, 105–128 (1973).
- 9 Minke, B., Wu, C.-F. & Pak, W. L. *Nature* **258**, 84–87 (1975).
- 10 Baylor, D. A., Hodgkin, A. L. & Lamb, T. D. *J. Physiol., Lond.* **242**, 685–727 (1974).
- 11 Grabowski, S. R. & Pak, W. L. *J. Physiol., Lond.* **247**, 363–391 (1975).
- 12 Coles, J. A. & Yamane, S. *J. Physiol., Lond.* **247**, 189–207 (1975).
- 13 Wulff, V. J. & Mueller, W. J. *Vision Res.* **13**, 661–671 (1973).
- 14 Wulff, V. J. & Mendez, C. *Vision Res.* **13**, 2327–2333 (1973).
- 15 Miller, R. F. & Dowling, J. E. *J. Neurophysiol.* **33**, 323–341 (1970).
- 16 Jack, J. J. B., Noble, D. & Tsien, R. W. *Electric Current Flow in Excitable Cells* (Clarendon, Oxford, 1975).
- 17 Ottoson, D. in *Handbook of Sensory Physiology* **4**, 95–131 (Springer, Berlin, 1971).
- 18 Zonana, H. V. *Bull. Johns Hopkins Hosp.* **109**, 185–205 (1961).
- 19 Cohen, A. I. *J. comp. Neurol.* **147**, 351–378 (1973).
- 20 Penn, R. D. & Hagins, W. A. *Biophys. J.* **12**, 1073–1094 (1972).

Immunological identification of rat neurophysin precursors

NEURONES in the supraoptic and paraventricular nuclei of the hypothalamus synthesise two peptide hormones, vasopressin and oxytocin. These hormones and their neurophysin 'carrier' proteins are stored in (and released from) nerve endings in the posterior lobe of the pituitary^{1–3}. On the basis of extensive studies of the synthesis of vasopressin *in vivo* and *in vitro*, Sachs *et al.*^{4–8} have suggested that vasopressin and its associated neurophysin are synthesised as parts of a common precursor by a ribosomal mechanism. We have also presented evidence that the neurophysins are synthesised as parts of larger precursor molecules^{9,10}. These 20,000 molecular weight precursors seem first to give rise to 17,000 molecular weight intermediates which, in turn, yield the 12,000 molecular weight neurophysins. The following data show that the putative neurophysin pre-

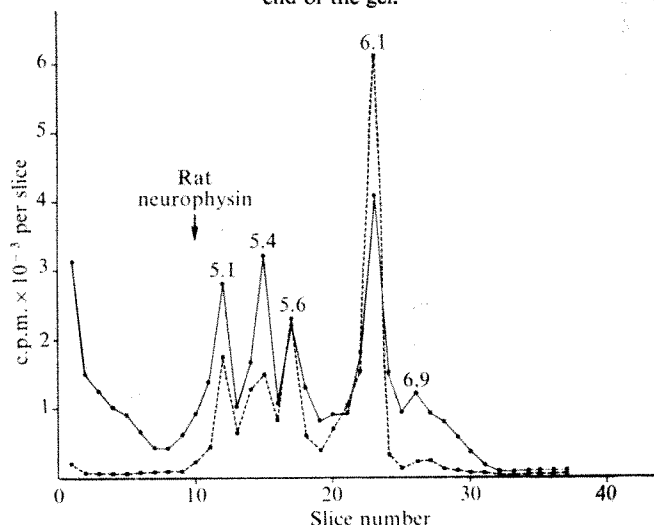
cursors and intermediates bind specifically to anti-rat neurophysin antiserum.

Female Osborne-Mendel rats (250 g) were used. For 5–7 d before use, the rats were given 2% saline to drink to stimulate neurophysin and vasopressin biosynthesis¹⁰. They were then anaesthetised with ether and their heads were fixed in a stereotaxic device. The tips of two 30-gauge needles were positioned just adjacent to the two supraoptic nuclei^{9,10} and 20 μ Ci ³⁵S-cysteine (New England Nuclear; specific activity 40–50 Ci mmol⁻¹) in 1 μ l normal saline containing 1 μ g dithiothreitol were injected through each needle over a 5-min period. The rats were decapitated after an additional 55 min and their brains were quickly removed, mounted on specimen holders, and frozen on dry ice. The brains were placed in a cryostat (–9 °C) and 300 μ m thick frontal sections through the anterior hypothalamus were cut with a microtome. The supraoptic nuclei were dissected from the frozen sections with hollow, stainless steel needles¹¹. The tissue was immediately homogenised in 0.1 M HCl. Tissue pellets from ten animals were pooled into one sample, the final volume of which was 100 μ l. This sample was centrifuged at 10,000g for 5 min, and 10 μ l of the supernatant were subjected to isoelectric focusing in polyacrylamide gels (Fig. 1, solid line)^{10,12}. Four major peaks of labelled protein were present in the supernatant. These had isoelectric points (pI) of 5.1, 5.4, 5.6, and 6.1. A fifth peak (pI 6.9) was also consistently found, but it had less radioactivity than the others. We have previously reported that the pI 5.4 and 6.1 'precursor' proteins—the first ones to be labelled in the pulse-chase paradigm—have molecular weights of about 20,000 and that the pI 5.1 and 5.6 'intermediates' have molecular weights of about 17,000.

The remaining 80 μ l of the supernatant were neutralised with an equal volume of 0.1 M NaOH. An additional 200 μ l of 'buffer A' (0.01 M potassium phosphate, 1.5 M sodium chloride, 1% sodium azide, pH 7.4) were added plus 100 μ l of normal rabbit serum and 0.5 mg of bovine serum albumin. After 4 d at 4 °C the solution was passed over a calibrated Sephadex G-100 column (0.6 \times 17.5 cm) which had been equilibrated with buffer A. Some radioactive material which must either have been of large molecular weight or bound to large serum components appeared in the void volume of the column (Fig. 2a). Analysis of the radioactive substances in the void volume by isoelectric focusing indicated that relatively little of it was 'precursor' or 'intermediate'.

A major peak of labelled material eluted between 2.6 and

Fig. 1 Isoelectric focusing of ³⁵S-labelled proteins in an acid extract of the supraoptic nucleus 1 h after injection of ³⁵S-cysteine adjacent to this nucleus (solid line), and of labelled proteins in the above extract bound by anti-rat neurophysin (broken line). Six per cent polyacrylamide gels were used for isoelectric focusing; the sample buffer contained 8 M urea, 1% Triton X-100, and 2% ampholytes (pH 3–10); and the sample was loaded at the anodal end of the gel.



4.0 ml. Another very large peak came off the column after the bed volume (5 ml), and probably contained cysteine and peptides. A sample containing the material which eluted from the column between 2.9 and 3.5 ml (the '20,000 molecular weight peak') was divided into three 200- μ l aliquots. A specific and well characterised rabbit anti-rat neurophysin (20 μ l)¹³ was added to one of these plus 250 μ l of buffer A; 20 μ l of anti-rat neurophysin plus 20 μ g of rat neurophysin (prepared as described previously¹³) in 250 μ l of buffer A were added to the second; and 20 μ l of normal rabbit serum were added to the

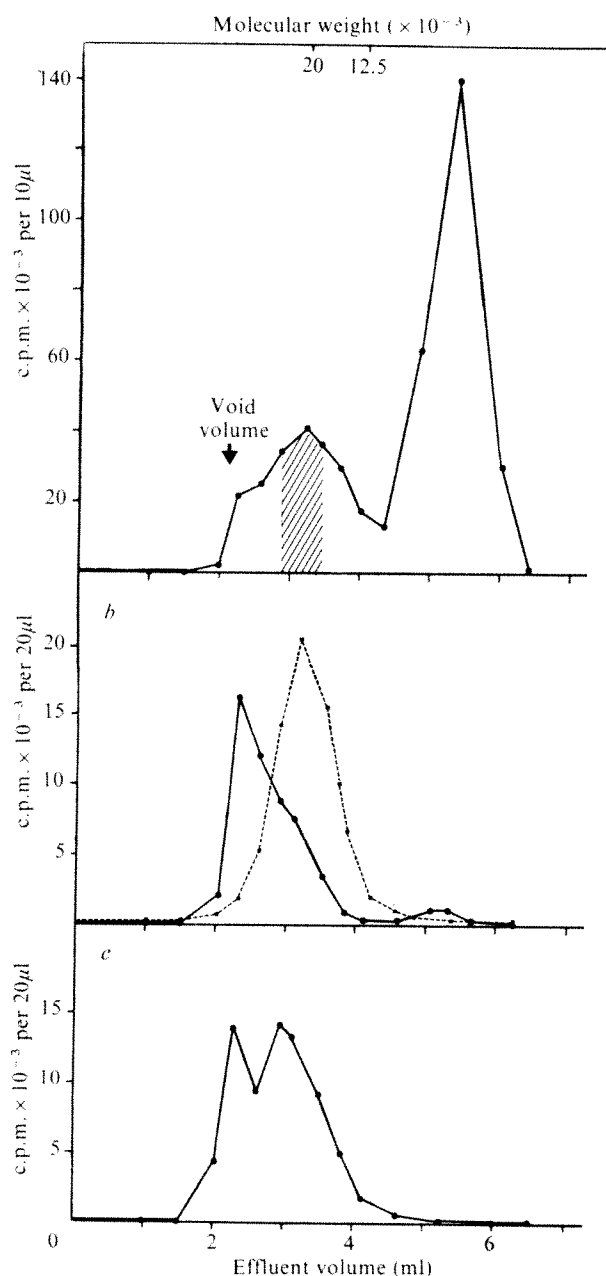


Fig. 2 Sephadex G-100 chromatography of ³⁵S-labelled material extracted from the supraoptic nucleus 1 h after a pulse of ³⁵S-cysteine. *a*, Separation of material which binds non-specifically to large molecular weight components of normal rabbit serum. Large, labelled molecules and smaller molecules bound to large species are found in the void volume. 'Precursors' and 'intermediates' are present in an included volume peak, the middle of which (hatched area) was used for subsequent studies. *b*, Most of the material in the included ('20,000 molecular weight') peak is excluded from the column after addition of rabbit anti-rat neurophysin (solid line). Addition of normal rabbit serum (broken line) caused no change in the elution pattern of the '20,000 molecular weight peak'. *c*, In the presence of rat neurophysin, some labelled protein in the '20,000 molecular weight peak' is excluded from the column (bound to antibody) and part is not.

third. Four days later the three samples were passed in turn over the G-100 column. Few counts appeared in the void volume of the column when normal rabbit serum was added to the '20,000 molecular weight peak'. The labelled material eluted where it had earlier (Fig. 2*b*), and less than 5% of it could be precipitated by adding goat anti-rabbit IgG. On the other hand, about 75% of the labelled material was found in the void volume of the column when anti-rat neurophysin was added to a sample of the '20,000 molecular weight peak' (Fig. 2*b*). The labelled species in the void volume could be precipitated in this case by goat anti-rabbit IgG, showing that they were attached to antibody. The antibody-bound materials that eluted between 2.0 and 2.3 ml were precipitated with trichloroacetic acid and subjected to isoelectric focusing. Four peaks were found with isoelectric points corresponding to those of the 20,000 molecular weight precursors ($pI = 5.4-6.1$) and the 17,000 molecular weight intermediates ($pI = 5.1-5.6$) (Fig. 1, broken line). The pI 6.1 protein seemed to be bound preferentially; the reason for this is unclear. A small amount of pI 6.9 protein may have been bound by the antibody; none of the other components of the original homogenate appeared to be bound.

The counts in the sample of '20,000 molecular weight peak', to which both anti-rat neurophysin and rat neurophysin were added, were distributed between a void volume peak and a 20,000 molecular weight included volume peak (Fig. 2*c*). The counts in the former peak were precipitated by anti-IgG; the counts in the latter were not; the neurophysin seems to have displaced labelled proteins from the antibody. The displacement may, however, have been incomplete because of (1) the presence of an excess of antibody relative to the amount of neurophysin added or (2) the relatively high affinity of the antibody for the precursors as opposed to neurophysin.

We have shown that, in addition to binding neurophysins¹³, anti-rat neurophysin antiserum binds four larger cysteine-rich proteins found in the supraoptic nucleus. These proteins, which do not react with immunoglobulins in normal rabbit serum to a significant extent, can be displaced from anti-neurophysin by neurophysin itself. These data lend credence to our suggestion that larger protein species synthesised in the supraoptic nucleus give rise to the neurophysins^{9,10}.

We have also studied the synthesis of neurophysin in a strain of rats with diabetes insipidus, that is, the Brattleboro strain¹⁴. These animals do not make vasopressin or its associated neurophysin. They lack the pI 4.8 neurophysin as well as the pI 6.1 precursor and the pI 5.6 intermediate. Thus, the vasopressin-associated neurophysin seems to be made from the pI 6.1 precursor by way of the pI 5.6 intermediate, and the oxytocin-associated neurophysin seems to be made from the pI 5.4 precursor by way of the pI 5.1 intermediate. Whether the precursors or intermediates are glycoproteins as are those of ACTH¹⁵ remains to be determined. Whether the precursors contain oxytocin and vasopressin as well as the neurophysins can now be tested directly.

A.G.R. is supported by the NIG (grant no. AM 16166) and is the recipient of an NIH Career Development Award (grant no. AM 00093). Reprint requests to M.J.R.

MICHAEL J. BROWNSTEIN

Section on Pharmacology,
Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland 20014

ALAN G. ROBINSON

Department of Medicine,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15261

HAROLD GAINER

Behavioral Biology Branch,
National Institute of Child Health and Human Development,
Bethesda, Maryland 20014

Received 25 April; accepted 18 July 1977.

- ¹ Bargman, W. in *Neurophysiophysiological Hormones and Similar Polypeptides* (ed. Berde, B.) 1-39 (Springer, Berlin, 1968).
- ² Heller, H. in *Handbook of Physiology*, section 7: Endocrinology, 4 (ed. Knobil, E. & Sawyer, W. H.) 103-117 (American Physiological Society, Washington, DC, 1974).
- ³ Sachs, H. J. *Neurochem.* 5, 297-303 (1960).
- ⁴ Sachs, H. & Takabatake, Y. *Endocrinology* 75, 943-948 (1964).
- ⁵ Sachs, H., Fawcett, P., Takabatake, Y. & Portanova, R. *Recent Progr. Horm. Res.* 25, 447-491 (1969).
- ⁶ Sachs, H., Pearson, D. & Nureddin, A. *Ann. N.Y. Acad. Sci.* 248, 36-45 (1975).
- ⁷ Takabatake, Y. & Sachs, H. *Endocrinology* 75, 934-942 (1964).
- ⁸ Gainer, H., Sarne, Y. & Brownstein, M. J. *Science* 195, 1354-1356 (1977).
- ⁹ Gainer, H., Sarne, Y. & Brownstein, M. J. *J. Cell Biol.* 73, 366-381 (1977).
- ¹⁰ Palkovits, M. *Brain Res.* 59, 449-450 (1973).
- ¹¹ O'Farrell, P. H. *J. Biol. Chem.* 250, 4007-4021 (1975).
- ¹² Seif, S. M., Huellmantel, A. B., Platia, M. P., Haluszczak, C., & Robinson, A. G. *Endocrinology* 100, 1317-1326 (1977).
- ¹³ Brownstein, M. J. & Gainer, H. *Proc. natn. Acad. Sci. U.S.A.* (in press).
- ¹⁴ Mains, R. E. & Eipper, B. A. *J. Biol. Chem.* 251, 4115-4120 (1976).

Several of the adenosine deaminase isozymes are glycoproteins

THE basis for the complex multiple molecular forms (isozymes) of the enzyme adenosine deaminase (ADA) has been widely studied. The ADA found in human erythrocytes (so-called 'red cell' ADA) exhibits genetically determined polymorphism detectable by electrophoresis¹, has reactive thiol groups² and has a low molecular size³. 'Red cell' ADA is present in various tissues other than the red cell³, but additional adenosine deaminases, the so-called tissue ADA isozymes *a*, *b*, *c*, *d* and *e* named in order of decreasing electrophoretic mobility, which exhibit rather different properties, occur in varying amounts in nonerythroid tissues. With the discovery that the deficiency of 'red cell' ADA in certain patients with combined immune deficiency disease is accompanied by the deficiency of all the other isozymes⁴⁻⁶, it became clear that all the forms were probably coded by a single ADA locus. The 'red cell' ADA can be converted into the characteristic 'tissue' isozymes by tissue extracts both from normal individuals^{7,8} and from patients with combined immune deficiency disease. A converting factor has been isolated and partially purified and is thought to be a protein⁹. This protein is presumably associated with one or several 'red cell' ADA molecules to form the 'tissue' isozymes, accounting for the alteration in properties (increase in molecular size, loss of detectable genetically determined electrophoretic variation and loss of thiol reactivity³). We present here evidence, from lectin affinity chromatography and experiments with neuraminidase, that most of the multiple forms of 'tissue' ADA are glycoproteins which differ in their accessible sugar residues. This suggests that the converting factor may be a single glycoprotein with heterogeneous carbohydrate content.

Treatment of extracts of various human tissues with the enzyme

neuraminidase (sialidase) seems to result in the conversion of the most anodal tissue ADA isozymes (*a*, *b* and *c*) into a zone of activity (*d*₂) which is slightly less anodal than the major *d* isozyme (now referred to as *d*₁). The effects of neuraminidase are most easily demonstrated using extracts of liver (Fig. 1) which contain virtually no 'red cell' ADA. The final pattern resembles that seen in kidney extracts. These changes are presumably due to the removal of negatively charged sialic residues from the tissue ADA *a*, *b* and *c* isozymes. The *d*₁ and *d*₂ isozymes were not affected by treatment with neuraminidase and this is most easily demonstrated using extracts of kidney (Fig. 1). The 'red cell' ADA and the *e* isozyme were also not affected by the neuraminidase treatment.

The ADA isozymes in liver, kidney, intestine and various other tissues were investigated by affinity chromatography on concanavalin A-Sepharose and wheat germ lectin-Sepharose. Concanavalin A is known to bind to glycoproteins containing terminal α -D-mannopyranosyl and α -D-glucopyranosyl residues and internal 2-O-D-mannopyranosyl groups^{10,11}. Wheat germ lectin binds *N*-acetylglucosamine residues specifically and sialic acid residues nonspecifically¹².

'Red cell' ADA and the tissue *e* isozyme failed to bind to either lectin, but the other tissue isozymes showed affinity for one or both lectins. Isozyme *a* was adsorbed by both lectins. The relatively weakly active isozymes *b* and *c* seemed to be adsorbed to concanavalin A but their affinity towards wheat germ lectin was not thoroughly investigated. The *d* isozyme showed heterogeneity in its adsorption characteristics. Re-chromatography experiments showed that this was not due to overloading. In the kidney the more cathodal component (*d*₂) was adsorbed by both lectins whereas the more anodal component (*d*₁) was only partly adsorbed. In the liver and intestine where the major component of the *d* isozyme has the mobility of *d*₁, contrasting results were obtained with wheat germ lectin and concanavalin A. The *d*₁ isozyme was mostly adsorbed by concanavalin A but not by wheat germ lectin (Fig. 2).

Lectin affinity chromatography was also conducted using neuraminidase treated liver extracts since the removal of sialic acid residues might alter the affinity characteristics of the isozymes. The affinity of the isozymes towards concanavalin A was unaffected by neuraminidase treatment, but the results of the experiments using wheat germ lectin were hard to interpret and two-stage experiments were therefore conducted. Untreated liver extracts were first of all fractionated by chromatography on wheat germ lectin-Sepharose. Both the fractions were then treated with neuraminidase. The nonadsorbed *d*₁ isozyme was not affected by this treatment. The adsorbed *a* isozyme was converted into the two forms of the *d* isozyme, *d*₁ and *d*₂ (Fig. 2). This material was then re-chromatographed on wheat germ lectin. The *d* isozyme was largely not adsorbed whereas the *d*₂ isozyme was adsorbed. Thus, it seems that the *a* isozyme exists in at least two forms with overlapping electrophoretic mobilities: one *a*₂ which may contain

Fig. 1 Effect of neuraminidase treatment on the ADA isozyme pattern of red cell, liver and kidney extracts. *A*, Photograph of a starch gel and *B*, a diagrammatic representation of the isozymes. Open rectangles represent the 'tissue' ADA isozymes. Shaded rectangles represent the 'red cell' ADA isozymes. On the gel shown the *d*₁ and *d*₂ isozymes were not clearly resolved, but are illustrated as two isozymes in the diagram. Neuraminidase treatment was performed by incubating 50 μ l of aqueous tissue extract with 20 μ l of neuraminidase solution (from *Clostridium perfringens*, Sigma type VI; 1 unit per ml, in 10 mM phosphate citrate buffer pH 5.0) for 2 h. [Similar results were also obtained using *Vibrio cholerae* neuraminidase.] Electrophoresis and detection were done as described by Edwards *et al.*³.

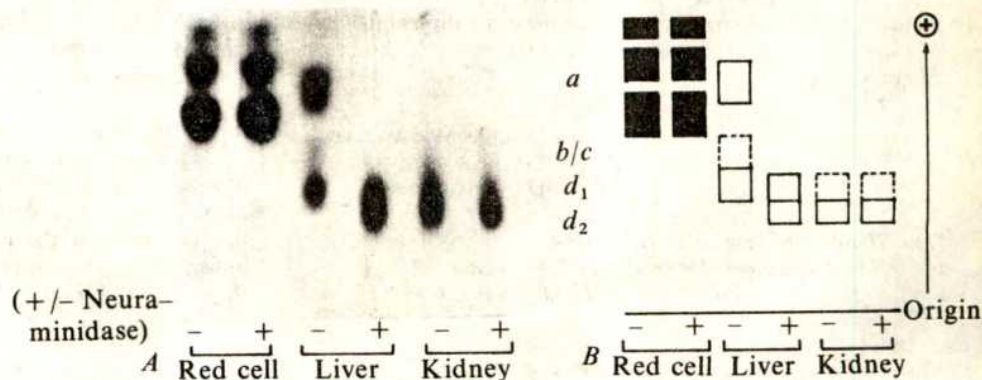
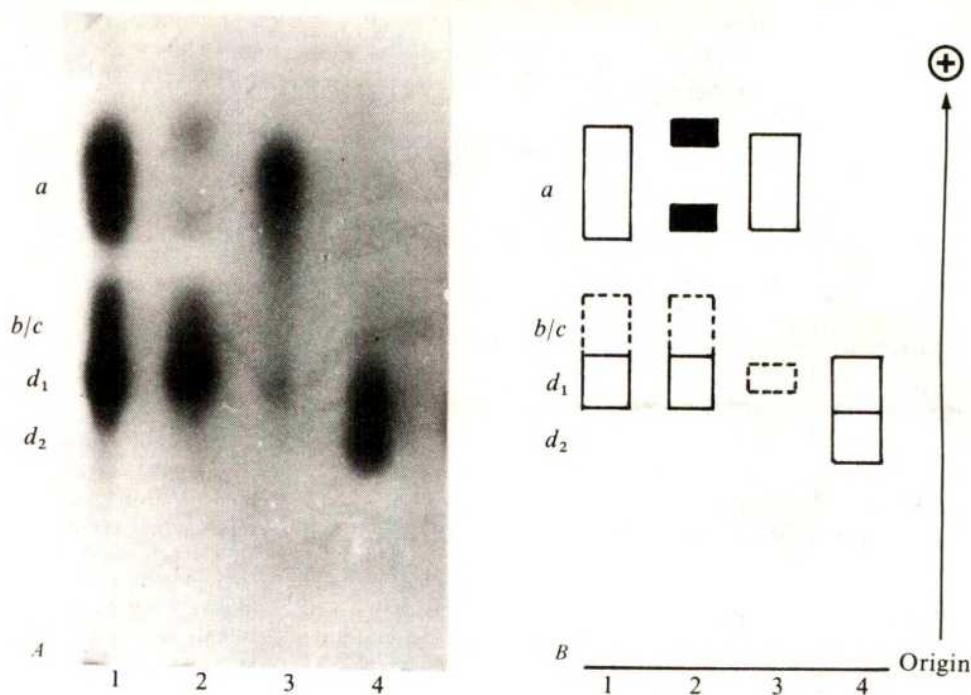


Fig. 2 Affinity chromatography of liver extracts using wheat germ lectin-Sepharose. **A**, Photograph and **B**, diagrammatic representation. 1, Unfractionated control; 2, material that was not adsorbed; 3, material that was adsorbed and subsequently eluted with 10^{-4} M N -acetylglucosamine; 4, sample 3 treated with neuraminidase. The chromatography was conducted using Wheat germ lectin-Sepharose 6MB (Pharmacia) or wheat germ lectin-Sepharose synthesised using Sepharose 4B CNBr-activated (Pharmacia) and wheat germ lectin (Sigma), at 25 °C using a column bed volume of 3 ml and a sample size of up to 0.5 ml crude aqueous tissue extract (1:1 weight to volume). Equilibration and elution of unadsorbed proteins was conducted using a 0.05 M phosphate buffer, pH 7.0, containing 0.2 M NaCl. Elution of adsorbed material was performed using this same buffer containing 10^{-4} M N -acetylglucosamine. These two fractions were concentrated and dialysed before electrophoresis. (The methodology used for concanavalin A-Sepharose affinity chromatography was similar, but in this case the buffers were 0.1 M acetate pH 6.0 containing 1 M NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and the same buffer containing 20^{-4} M methyl α -mannopyranoside for elution.)



appropriately linked N -acetylglucosamine residues and is converted into the d_2 isozyme, and the other, a_1 , without the appropriate N -acetylglucosamine, which is converted into d_1 . Both forms apparently contain sialic acid which is accessible to neuraminidase.

Further experiments using two-stage chromatographic separations involving both wheat germ lectin and concanavalin A indicate that there are several forms of the d isozyme in human tissues: d_{1a} with affinity for concanavalin A, but not wheat germ lectin; d_{1b} with affinity for neither lectin and d_{1c} and d_2 with affinity for both lectins. These isozymes occur in varying amounts in different tissues. It therefore seems likely that the so-called a , b , c and d isozymes each represent a multiplicity of forms with overlapping electrophoretic mobilities which differ only in their carbohydrate side chains.

The tissue ADA e isozyme does not fit readily into this scheme—our evidence suggests that it may not be a glycoprotein. It is also unusual in its restricted tissue distribution, occurring only in large amounts in the intestine, and its much larger apparent molecular size (435,000) (ref. 3) than the other tissue isozymes (230–280,000) (refs 3,5). It is possible that another protein converting factor is involved in the generation of the e isozyme from red cell ADA or the e isozyme may be coded by a separate structural locus. An alternative and perhaps simpler explanation is that the (glyco) protein which leads to the conversion of red cell ADA into the a , b , c and d isozymes is also responsible for the generation of the tissue ADA e isozyme, but the composition of the sugar chains may be different.

DALLAS M. SWALLOW
LORRAINE EVANS
D. A. HOPKINSON

MRC Human Biochemical Genetics Unit,
The Galton Laboratory, University College London,
Wolfson House, 4 Stephenson Way, London NW1, UK

Received 13 June; accepted 27 July 1977.

- ¹ Spencer, N., Hopkinson, D. A. & Harris, H. *Ann. Hum. Genet.* **32**, 9 (1968).
- ² Hopkinson, D. A. & Harris, H. *Ann. Hum. Genet.* **33**, 81 (1969).
- ³ Edwards, Y. H., Hopkinson, D. A. & Harris, H. *Ann. Hum. Genet.* **35**, 207 (1971).
- ⁴ Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, P. & Meuwissen, H. J. *Lancet* **ii**, 1067 (1972).
- ⁵ Hirschhorn, R., Levytsky, V., Pollara, B. & Meuwissen, H. J. *Nature new Biol.* **246**, 200 (1973).

- ⁶ Chen, S. H., Scott, C. R. & Giblett, E. R. *Am. J. Hum. Genet.* **26**, 103 (1974).
- ⁷ Hirschhorn, R. *J. clin. Invest.* **55**, 661 (1975).
- ⁸ Akedo, H., Nishihara, H., Shinkai, K., Komatsu, K. & Ishikawa, S. *Biochim. biophys. Acta* **276**, 257 (1972).
- ⁹ Nishihara, H., Ishikawa, S., Shinkai, K. & Akedo, H. *Biochim. biophys. Acta* **302**, 429 (1973).
- ¹⁰ Lloyd, K. O., Kabat, E. A. & Beychok, S. *J. Immun.* **102**, 1354 (1969).
- ¹¹ Krusius, T., Finne, J. & Rauvala, H. *FEBS Lett.* **71**, 117 (1976).
- ¹² Greenaway, P. J. & LeVine, D. *Nature new Biol.* **241**, 191 (1973).

Translation of human immunoglobulin heavy chain mRNA *in vitro*

MOUSE immunoglobulin (Ig) mRNAs have been the subject of intensive study. Purification of Ig light chain mRNAs from myeloma or plasmacytoma cells has been monitored by translation *in vitro*, and studies have been directed towards sequence determination and gene titration. Moreover, partial purification of Ig heavy chain mRNA has also been achieved^{1–3}. No comparable work on the Ig mRNA of any other species has yet been published. We report here the preparation, fractionation and translation *in vitro* of mRNA from cells of a cloned normal human B lymphocytic cell line, RPMI 1788 (ref. 6), grown in tissue culture. Human Ig μ chains were identified in the translation product by specific immunoprecipitation and electrophoretic mobility in sodium dodecyl sulphate (SDS)-urea polyacrylamide gels.

Preliminary experiments were carried out on a range of human lymphocytic cell lines supplied by Searle Research Laboratories, High Wycombe, and RPMI 1788 was chosen for this work because it produced the highest level of Ig (5%–10% of total protein synthesis) in tissue culture. All of this Ig synthesis in RPMI 1788 is of the IgM type, that is, μ heavy chains and λ light chains. Analysis of the Ig production *in vivo* is shown in Fig. 1. The feasibility of using this cell line for the preparation of Ig mRNA was further indicated by the observation that the rate of protein synthesis and the level of IgM production are maintained when cells are grown in medium containing swine serum rather than the more costly foetal calf serum, and also when the cells are grown to a high density (up to 8×10^5 cells per ml) in suspension culture.

Total RNA was prepared from about 5 g wet weight

RPMI 1788 cells, freshly collected, essentially following the method of Bedard and Huang¹. The total yield of 6 mg RNA was fractionated in sucrose gradients into a high molecular weight (HMW) fraction (5.5 mg) and a low molecular weight (LMW) fraction (0.5 mg) as shown in Fig. 2. The RNA in each of these pools was passed through

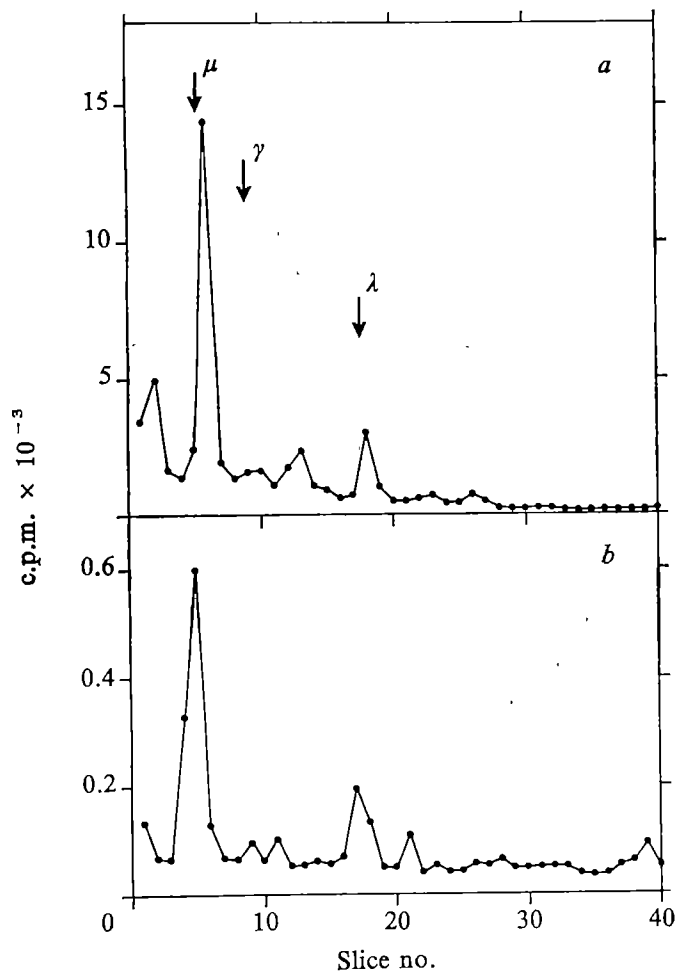


Fig. 1 Polyacrylamide gel electrophoresis identifying Ig chains synthesised by RPMI *in vivo*. RPMI 1788 cells (10^7) suspended in 2 ml RPMI 1640 medium containing 250 μ Ci 3 H-leucine were incubated at 37 °C for 6 h, collected and lysed in 1 ml lysis buffer containing 0.5% Nonidet P40, according to the method of Prekumar *et al.*^{7,8}. Antibody precipitations were performed on 0.5 ml aliquots of the cell lysate and on 1 ml aliquots of the labelled medium as follows: 6 μ l rabbit antiserum to human μ -chain or to bovine serum albumin (Miles) were added and the samples incubated on ice 45 min before the addition of 15 μ l goat anti-rabbit IgG antiserum (Miles). Samples were incubated a further 45 min on ice and the immunoprecipitates were then collected, washed three times with phosphate buffered saline and once with ethanol-ether (1:1) containing 1% β -mercapto-ethanol, dried in a vacuum, and then dissolved and reduced by the addition of 50 μ l 6 M urea, 10 mM sodium phosphate, pH 7.5, 2% sodium dodecylsulphate (SDS), 1% β -mercapto-ethanol, 10% sucrose and incubation at 90 °C for 2 min. Each sample was electrophoresed in 10% polyacrylamide disc gels (6 mm \times 6 cm) containing 6 M urea, 0.1 M sodium phosphate, pH 7.5, and 0.4% SDS, for 16 h at 2.5 mA per gel with a reservoir buffer of 0.1 M sodium phosphate, pH 7.5, 0.1% SDS and 5 mM thioglycolic acid. Gels were frozen and sliced at 1 mm intervals and each slice was incubated in 0.3 ml Soluene 350 (Packard) and the radioactivity monitored in toluene scintillant. Panels show anti- μ -chain immunoprecipitates of the cell lysate (a), and of the medium (b). Unlabelled human μ , γ , and λ chains were run in a parallel gel and their positions at the end of electrophoresis are indicated. A similar gel of the anti-bovine serum albumin immunoprecipitate (not shown) revealed no μ or λ chains.

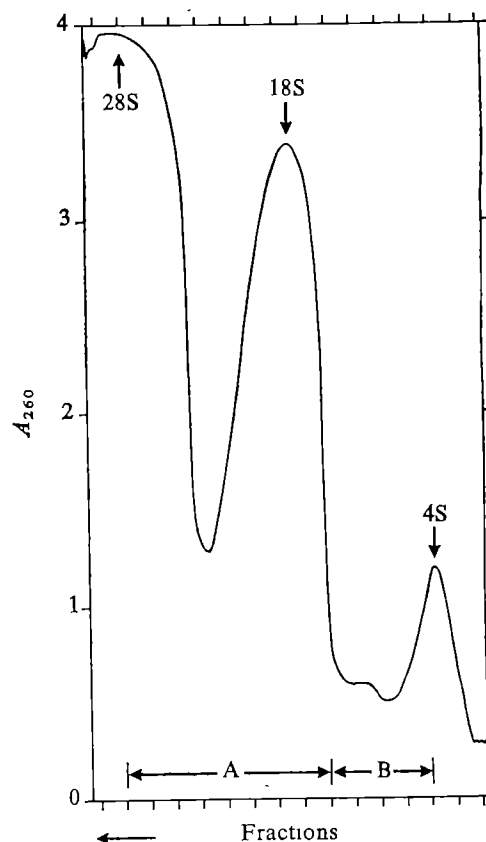
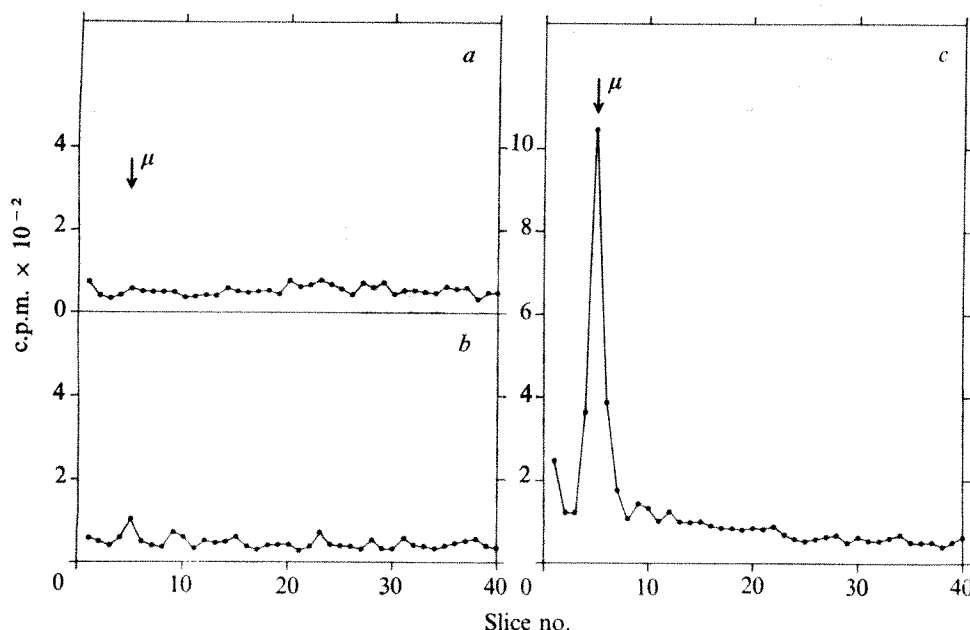


Fig. 2 Sucrose gradient fractionation of RPMI 1788 microsomal RNA. Microsomal RNA was prepared and sedimented through sucrose gradients using the method of Bedard and Huang². All manipulations were at 4 °C unless otherwise indicated. The cells were homogenised by six strokes in a tight fitting glass-Teflon homogeniser in 5 volumes of 4 mM Tris (pH 7.4), 1.5 mM $MgCl_2$, 25 mM KCl, 250 mM sucrose. Nuclei and cell debris were pelleted at 3,000g for 10 min and the supernatant was removed and centrifuged at 30,000 r.p.m. (100,000g) for 20 min in a Beckman SW 41 rotor. The resultant microsomal pellet was dissolved in 2 volumes 5 mM Tris buffer, 3% SDS, at room temperature and immediately extracted twice on ice with 1 volume of phenol and subsequently 1 volume chloroform. The RNA was precipitated from the aqueous phase by addition of ethanol, washed, dissolved in water and layered on four 5%–30% sucrose gradients made in 0.1 M NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.5% SDS. Gradients were centrifuged at 25,000 r.p.m. in a Beckman SW 41 rotor at 25 °C for 16 h, and then were fractionated by displacement from the bottom with 40% sucrose, while the fractionation was monitored with an Isco UA-4 (see trace). The fractions were pooled as indicated and the RNA in each pool was collected by ethanol precipitation, washed, and purified on oligo dT-cellulose (T-3; Collaborative Research) at room temperature in the presence of 10 mM Tris buffer and 0.5% SDS. The poly-A⁺ RNA, as well as the poly-A⁻ RNA, from each gradient were collected and washed as above, re-dissolved in water and stored at -20 °C.

a column of oligo dT-cellulose to separate the polyadenylated (poly-A⁺) RNA, presumably mRNA, from the non-polyadenylated (poly-A⁻) RNA. The HMW pool of Fig. 2 yielded 80 μ g poly-A⁺ and 2.1 mg poly-A⁻ RNA; the LMW pool yielded 18 μ g poly-A⁺ and 330 μ g poly-A⁻ RNA.

Three of these RNA fractions were analysed in 250 μ l rabbit reticulocyte lysates for their ability to stimulate protein synthesis *in vitro*. HMW poly-A⁺ RNA (25 μ g), LMW poly-A⁺ RNA (15 μ g), and HMW poly-A⁻ RNA (50 μ g), added to the assay system stimulated the incorporation of labelled amino acid to levels of 5,470, 3,031, and 708 c.p.m. above background (background=1,291 c.p.m.) per 2 μ l aliquot. Each of the incubated lysates was precipitated with antiserum to human μ chain. Only the HMW poly-A⁺ RNA stimulated lysate contained specifically immuno-



(Miles). After incubation at 37 °C, for 90 min each sample was layered over a discontinuous sucrose gradient (100 μ l 1 M sucrose + 50 μ l 0.5 M sucrose) in a 400 μ l Beckman microfuge tube and centrifuged at 16,000g for 5 min. The tubes were frozen at -80 °C and the tip of each was cut off in order to collect the antibody precipitates, which were then dissolved, reduced, monitored for radioactivity and electrophoresed in 10% polyacrylamide disc gels as described in the legend to Fig. 1. The gel patterns shown represent the anti- μ -chain immunoprecipitates obtained from lysates containing 60 μ g ml⁻¹ LMW poly-A⁺ RNA (a), 200 μ g ml⁻¹ HMW poly-A⁺ RNA (b), and 100 μ g ml⁻¹ HMW poly-A⁺ RNA (c). The gel pattern (not shown) of the anti-ovalbumin immunoprecipitate from a lysate containing 100 μ g ml⁻¹ HMW poly-A⁺ RNA is identical to patterns (a) and (b). The gel pattern (not shown) of the anti-ovalbumin immunoprecipitate from a lysate containing 200 μ g ml⁻¹ oviduct polysomal RNA shows one labelled peak well separated from the μ -chain position and co-migrating with the unlabelled ovalbumin marker.

Fig. 3 Polyacrylamide gel electrophoresis demonstrating Ig μ -chain mRNA from human lymphocytes translated *in vitro*. RNA samples were analysed in 250 μ l rabbit reticulocyte lysates prepared by the method of Palmiter⁹, but buffered with 14 mM HEPES, pH 7.6, stored at -80 °C, and pretreated with micrococcal nuclease by the methods of Pelham and Jackson¹⁰. After addition of 22 μ Ci ³⁵S-methionine and the RNA to be tested, each lysate was incubated for 90 min at 26 °C. Aliquots of 2 μ l were precipitated with trichloroacetic acid and precipitates were washed, dried and monitored for radioactivity as a measure of total protein synthesis. To determine the quantity of μ chain synthesised, the remainder of each 250- μ l lysate was immunoprecipitated as follows: lysates were each mixed with 50 μ l 50 mM methionine, 25 μ l 10% Triton X-100, 25 μ l 10% sodium deoxycholate, and 4 μ l human IgM (8.7 mg ml⁻¹) or, as a control, 9 μ l ovalbumin (1 mg ml⁻¹) and centrifuged at 16,000g for 5 min. From each sample the top 225 μ l was removed and added to 50 μ l anti-human μ -chain antiserum (Miles) or 50 μ l anti-ovalbumin antiserum

precipitable protein. The amount of radioactivity recovered in the immunoprecipitate (multiplied by 3 since only one-third of the HMW poly-A⁺ RNA was tested in the assay) equals 0.7% of the total protein synthesised in response to the three RNA fractions (obtained as the sum of the correspondingly normalised activities). Gel electrophoresis of the immunoprecipitates in Fig. 3 shows that the protein precipitated by the μ -chain antiserum is homogeneous, and identical in mobility to human μ -chain marker. These results indicate the presence of intact, translatable μ -chain mRNA in the HMW poly-A⁺ fraction of RNA prepared from human lymphocytes.

The cells used in the experiments described above were freshly collected, but this does not seem to be essential. The entire procedure has been repeated using a similar quantity of cells which had been rapidly frozen in liquid N₂ as a cell pellet and stored at -80 °C before RNA preparation. The results of the second experiment, including yields of RNA, the sedimentation pattern of the RNA, the yields of poly-A⁺ and poly-A⁻ RNA and the ability of the HMW poly-A⁺ fraction to stimulate the *in vitro* synthesis of anti- μ chain precipitable protein, were essentially identical to the results with freshly collected cells illustrated in Figs 2 and 3. The second preparation yielded a percentage of anti- μ -chain precipitable protein of 3.4%, which approaches the level of 5%–10% estimated for *in vivo* synthesis.

In this second experiment, the 18S peak from the sucrose gradient was pooled separately from the rest of the HMW fraction. Immunoprecipitation of the *in vitro* translation products showed that more than 90% of the μ -chain mRNA is located in the 18S fraction, indicating that this mRNA has a sedimentation coefficient of around 18S. This result may be compared with reports that, in mouse, the α -heavy chain mRNA (from MOPC 315 plasmacytoma) sediments at

16–19S (ref. 3), and γ -chain mRNA (from MOPC 21 tissue culture cells) at 17S (ref. 1), and suggests that the human μ -heavy chain mRNA may be of similar size. Work is now in progress to prepare large quantities of RPMI 1788 RNA to be used for the further physical and chemical characterisation of the Ig mRNA.

We thank Dr Brian Richards, Dr John Birch and Mr David Edwards of Searle Research Laboratories for support and collaboration, Dr Ru Chih Huang for advice, and Mr Paul Singer for help with preliminary experiments. This work was supported by MRC grant G969/509/B, USNIH postdoctoral fellowship 1-F32 GM 05558-01 (C.K.K.), and a King's College tutorial fellowship (F.C.).

CAROL K. KLUKAS

FRANS CRAMER

HANNAH GOULD

Department of Biophysics,
King's College,
26–29 Drury Lane,
London WC2, UK

Received 31 May; accepted 27 July 1977.

- Cowan, N. J. & Milstein, C. *Eur. J. Biochem.* **36**, 1–7 (1973).
- Cowan, N. J., Secher, D. S. & Milstein, C. *Eur. J. Biochem.* **61**, 355–368 (1976).
- Green, M., Graves, P. N., Zehavi-Willner, T., McInnes, J. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 224–228 (1975).
- Green, M., Zehavi-Willner, T., Graves, P. N., McInnes, J. & Pestka, S. *Archs Biochem. Biophys.* **172**, 74–89 (1976).
- Bedard, D. L. & Huang, R. C. C. *J. biol. Chem.* **252**, 2592–2598 (1977).
- Moore, G. E., Gerner, R. E. & Franklin, H. A. *J. Am. med. Ass.* **199**, 519 (1967).
- Premkumar, E., Potter, M., Singer, P. A. & Sklar, M. D. *Cell* **6**, 149–159 (1975).
- Premkumar, E., Singer, P. A. & Williamson, A. R. *Cell* **5**, 87–92 (1975).
- Palmiter, R. D. *J. biol. Chem.* **248**, 2095–2106 (1973).
- Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).

Identification of chromosomal location of yeast DNA from hybrid plasmid *pYeleu10*

THERE is strong evidence to suggest that some genes of baker's yeast *Saccharomyces cerevisiae* can be expressed in *Escherichia coli* when cloned on plasmid or phage vectors^{1,2}. This conclusion is based on the observation that hybrid plasmids and bacteriophage carrying specific fragments of yeast nuclear DNA can complement certain auxotrophic mutations in the *E. coli* genome. Complementation alone, however, is not sufficient to prove that the cloned DNA contains a yeast gene equivalent to the defective bacterial gene. One aspect of the identification of any cloned DNA is the identification of its chromosomal origin. We present here a method for the identification of the chromosomal origin of cloned yeast DNA using hybridisation to DNA from yeast strains aneuploid for a single chromosome. Using this method we have confirmed the chromosomal location of the DNA of a yeast hybrid plasmid (*pYeleu10*) presumed to contain sequences coding for the leucine biosynthetic enzyme β -isopropylmalate dehydrogenase, the *leu2* gene of yeast.

Ratzkin and Carbon¹ attempted to isolate a hybrid plasmid containing a functional yeast *leu2* gene by transformation of a *leu*⁻ bacterial strain (JA199; *leuB6*, *trpE5*, *lacY*⁻ *hsdR*⁻ *hsdM*⁻/*F*⁺) defective in β -isopropylmalate dehydrogenase to *leu*⁺ using randomly sheared yeast DNA cloned on the plasmid vector *ColE1*. These workers isolated several independent clones which contained plasmids capable of suppressing the *leuB*⁻ defect in the host cell. Analysis of the restriction patterns of the plasmid DNA from these clones gave the surprising result that at least four distinct yeast DNA sequences were represented among these plasmids. As only one locus (*leu2*) is known in yeast to code for the enzyme β -isopropylmalate dehydrogenase, this result meant that in at least some cases suppression of the host

leuB mutation could occur by means other than enzymatic complementation. This result emphasises that complementation does not provide clear evidence for the presence of a specific functional gene or a unique segment of DNA corresponding to the yeast *leu2* region. Further genetic tests by these workers showed that one plasmid, *pYeleu10*, complemented several *leuB*⁻ alleles while the rest were specific for the original allele, *leuB6*. For this reason it seemed likely that plasmid *pYeleu10* might be expressing the cognate yeast gene, *leu2*, which is located on the left arm of chromosome III, near the centromere.

To determine unambiguously the chromosomal location of the yeast DNA carried by *pYeleu10*, we have analysed the hybridisation of labelled plasmid DNA to yeast DNA from strains aneuploid for chromosome III. Hybridisation was carried out after transferring restriction digests of the yeast DNA to nitrocellulose filters by the Southern blotting procedure³. The rationale for the experiment was that in the conditions used (that is, excess probe) the amount of label bound should be directly related to the amount of DNA originally present on the filter, and therefore that the degree of hybridisation to bands from chromosome III should reflect the aneuploid condition. The yeast strains used are described in Table 1 and include a normal haploid, a normal diploid, a diploid monosomic for chromosome III ($2n-1$) and a haploid disomic for chromosome III ($n+1$). Thus, the amount of chromosome III DNA per haploid genome (n) in each strain varied from $n/2$ (H151-2A) to n (+D4, 8481) to $2n$ (H159/1). It should be emphasised that the success of this and similar experiments depends on an accurate determination of the genotype of the aneuploid strains at the time of DNA isolation. Aneuploidy in most organisms, including yeast, is an unstable condition and strains may frequently revert to euploidy during culture. Genetic assays for aneuploidy in strains H159/1 and H151-2A are described in the legend to Table 1. Methods used for isolation and labelling of DNA along with conditions for hybridisation are described in the legend to Fig. 1. The hybridisation probe consisted of a mixture of ³²P-labelled

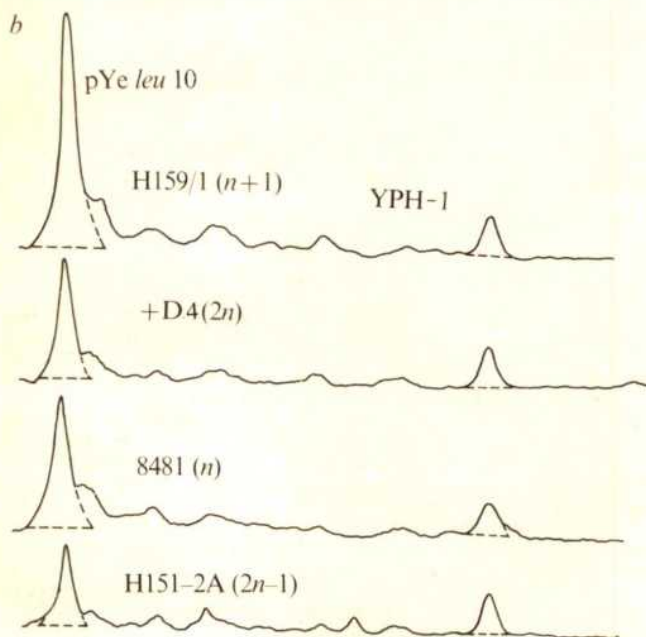
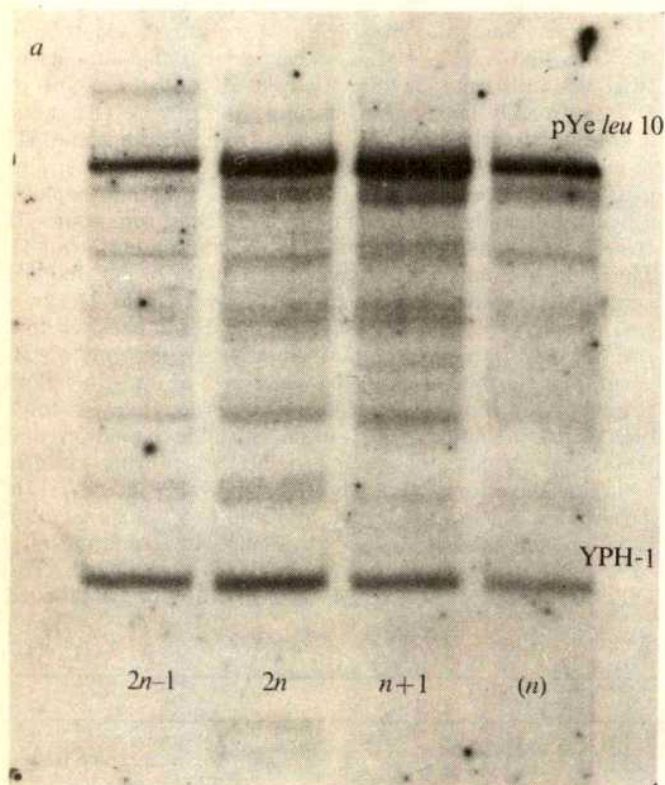
Table 1 Yeast strains used

Strain	Chromosome no.	Genotype	Copies of chromosome III per haploid genome
A364a + D4	$2n$	<i>ura1 ade1 ade2 a</i> <i>ura1 ade1 ade2 a</i>	1
8481	n	<i>his4-260 a HOL1-1</i>	1
H159/1	$n+1$	<i>his4-38 leu2-1_{oc} a</i> + <i>lys1-1_{oc} can1-100_{oc} met8-1</i> <i>his4-280 leu2-1_{oc} a SUP61</i>	2
H151-2A	$2n-1$	<i>his4⁻ leu2-1 a thr4 mal2</i> <i>lys1-1 ade2-1</i> + +	$\frac{1}{2}$
S36	$2n-1$	<i>a ino1-13 ino4-8</i> <i>ino1-13 ino4-8</i>	$\frac{1}{2}$

Strains were constructed in this laboratory by standard genetic techniques⁶ with the exception of A364a + D5 which was obtained from Lee Hartwell. The media used were described previously⁷. Cultures of H159/1 and H151-2A used for preparation of yeast DNA were tested for aneuploidy immediately before collecting as follows. H151/1: the culture was diluted and plated on YEPD (complete) agar for single colonies and tested for expression of genetic markers by replica plating. Because of the presence of the ochre suppressors *SUP61* (Hawthorne, personal communication) the phenotype of a colony arising from an aneuploid cell of the genotype shown in this Table will be *Leu*⁺ *Lys*⁺ *His*⁻ *Met*⁻ *canavanine* sensitive (*Can*^s) and non-mating (*a/a*). But, because of the spontaneous loss of the chromosome containing the suppressor, some cells in the colony exhibit an altered phenotype, *Leu*⁻ *Lys*⁻ *His*⁻ *Met*⁻ *Can*^r and mating type *a*. Such segregants can be detected by plating on medium containing canavanine and subsequently testing the *Can*^r segregants for the other nutritional markers and mating type. The presence of these segregants in a clone provides evidence that the parent cell was aneuploid. All 289 colonies tested exhibited all aspects of this complex phenotype and the culture was assumed to contain virtually 100% aneuploid ($n+1$) cells. H151-2A: an aliquot of this culture was mated with cells of strain S36 on YEPD agar, then transferred to SPOR agar to induce meiosis and sporulation. Tetrads were dissected and assayed for each of the genetic markers in the cross. Because the two strains were mated *en masse*, resulting in the formation of thousands of independent zygotes, it was assumed that each tetrad was the result of an independent mating event and thus reflected the genotype of an individual cell from the H151-2A culture. Tetrads resulting from matings involving aneuploid H151-2A cells would be expected to exhibit 2:2 segregation for each of the alleles located on chromosome III. But, tetrads exhibiting 4:0 or 3:1 segregation for mating type, cryptopleurine resistance (*Cry*^r) or maltose fermentation would indicate that two copies of each of these alleles were present and that parent H151-2A cell had reverted to euploidy. Of 83 tetrads, 70 scored were of the former type (2:2) indicating that the culture of H151-2A from which DNA was isolated contained at least 80% aneuploid ($2n-1$) cells. *Lys1-1* and *ade2-1* exhibited normal tetraploid segregation.

DNA from *pYeleu10* and *YPH-1*, a hybrid plasmid created in this laboratory containing an unidentified segment of yeast DNA. *YPH-1* was used as an internal standard for the extent of hybridisation. Although its chromosomal location is not known, it is presumed not to be located on chromosome III because hybridisation of *YPH-1* to whole yeast DNA is not affected by aneuploidy of chromosome III (J.H., unpublished). *Hind* III was used to digest the yeast DNA because neither *pYeleu10* nor *YPH-1* are cut by this enzyme and each should thus be represented by a single band in a yeast *Hind* III digest.

The results of one hybridisation experiment are shown in Fig. 1. The degree of hybridisation in each of the two main bands was assayed by cutting out and weighing the two main peaks in the densitometric tracings. As shown in Table 2, the ratio of the peak areas in each track varies



directly with the ratios of chromosome III per haploid genome. This result was confirmed by cutting the bands out of the filter and assaying radioactivity in a scintillation counter (not shown). From these data we conclude that *pYeleu10* carries sequences located on chromosome III. This experiment has been repeated with similar results (not shown).

An intriguing feature of the radioautograph obtained by this procedure is that faint bands are observed in each track in addition to the single main band associated with each plasmid. These are present even when *pYeleu10* alone is used as probe and have been frequently observed by other workers (K. Struhl and J. Carbon, personal communication). It is not likely that they are the result of incomplete digestion of the yeast DNA since some of them are smaller than the smallest *Hind* III fragment which could correspond to *pYeleu10*, that is, a fragment equal in size to *pYeleu10* itself since *pYeleu10* contains no *Hind* III restriction sites. It is tempting to speculate that these 'ghost bands' might represent partially homologous sequences scattered throughout the genome which have a common regulatory or structural function.

We conclude that plasmid *pYeleu10* contains sequences from chromosome III of yeast, probably including the *leu2* locus. Using this plasmid as a probe it will be possible to locate sequences flanking the *leu2* region among collections of clones containing yeast DNA and by repeating that process to 'walk' down the chromosome to other regions of

Fig. 1 *a*, Radioautograph of ^{32}P -labelled DNA from plasmids *pYeleu10* and *YPH-1* hybridised to a 'Southern blot' (ref. 3) containing *Hind* III restriction digests of yeast DNA. *b*, Densitometer tracings of the individual tracks in the radioautograph. Yeast DNA was prepared by the method of Cryer, Eccleshall and Marmur⁸. Approximately 2 μg of DNA from each strain was digested with restriction endonuclease *Hind* III (New England Biolabs; 2 units per μg DNA, 37 °C for 4 h) and subjected to electrophoresis on a horizontal 0.8% agarose slab gel (1.3 \times 15 cm tracks) for 12 h at 4 mA per track. The restriction digest in the gel was then transferred to a nitrocellulose filter (Millipore HAWP) by the Southern blotting procedure⁹. DNA from plasmids *pYeleu10* and *YPH-1* was isolated by a modification of procedures described by Clewell⁹ and Guerry *et al.*¹⁰. Cultures were grown in supplemented synthetic medium to mid-log phase ($2\text{--}4 \times 10^8$ cells per ml) at which time chloramphenicol was added to a concentration of 250 mg l^{-1} and the cultures were shaken at 37 °C for 25 h to allow amplification of the plasmid⁹. The culture was divided into 200-ml aliquots for further processing. Cells were collected by centrifugation, washed in 10 mM Tris-1 mM EDTA, pH 8.0 and resuspended in 2 ml 50 mM Tris-25% sucrose, pH 8.0 plus 2 mg lysozyme (Worthington). The mixture was incubated 5 min at 0 °C before addition of 0.4 ml EDTA, pH 8.0 and further incubation for 10 min at 0 °C. Lysing buffer (1% Triton X-100, 1 mM EDTA, 50 mM Tris, pH 8.0) 4 ml was added followed by incubation for an additional 15 min at 0 °C. The lysate was then centrifuged for 90 min at 18,000 r.p.m. in a Sorvall SS-34 rotor to pellet cell debris and most of the chromosomal DNA. The supernatant was then carefully decanted away from the loose pellet, volume was adjusted to 8.0 ml with 0.5 M EDTA and 0.3 ml ethidium bromide (10 mg ml^{-1}) was added. Protein precipitated by the CsCl-EtBr was removed by centrifugation at 30,000g and the cleared supernatant centrifuged for 40 h at 40,000 r.p.m. in a Beckman 65 rotor. The plasmid DNA band was extracted from the density gradient as described by Clewell⁹. Ethidium bromide was removed by three extractions with isopentyl alcohol followed by dialysis against 10 mM Tris-1 mM EDTA pH 7.5. Plasmid DNA was labelled with ^{32}P by nick translation using DNA polymerase I (Boehringer-Mannheim Grade I) according to the method of Maniatis *et al.*¹¹. Specific activity of the labelled plasmid DNA was 1.1×10^6 Ci μg^{-1} . After denaturation of the probe DNA in 0.2 M NaOH at 65 °C, hybridisation to the filter-bound DNA was accomplished in 10 ml of $4 \times \text{SSC} + 0.1\%$ sodium dodecylsulphate at 65 °C for 44 h. The filter was then exhaustively washed in 3 mM Tris base (M. Botchan, personal communication) and exposed to Kodak No-Screen X-ray film (NS2T) for 3h. The exposed film was then scanned using a densitometer. The sensitivity of the densitometer was adjusted for each scan to normalise approximately *YPH-1* peaks.

Table 2 Comparison of the peak areas in the densitometric tracings in Fig. 1

Strain	Weight of cut-out peak (mg) <i>pYeleu10</i>	Weight of cut-out peak (mg) <i>YPH-1</i>	Ratio: <i>pYeleu10</i> / <i>YPH-1</i>	Copies of chromosome III per haploid genome
H151-2A	17.1	10.2	1.4	$\frac{1}{2}$
8481	36.7	11.7	3.1	1
+D4	32.7	10.2	3.3	1
H159/1	66.8	11.6	5.8	2

Areas were measured by reproducing the densitometer tracings onto heavy paper, cutting out two copies of each peak (as indicated by the dotted lines) and weighing each one. Weights shown are the average of two measurements.

interest. It should further be possible to identify cloned DNA from specific genetic loci by hybridisation to restriction digests of DNA from yeast strains carrying genetically defined deletions which enter these loci. Southern blots of DNA from such strains should exhibit altered hybridisation patterns relative to wildtype DNA. Deletions exist which have endpoints in or near the *his4* locus⁴, the centromere (H. Klein and G. F., unpublished), and the mating type locus⁵. Attempts to isolate the DNA from these loci by this approach are under way.

We thank Barry Ratzkin and John Carbon for allowing us to use the *pYeleu10* plasmid and Dick Hallberg for informative discussion. This work was supported by NIH grant GM15408 (G.R.F.) and an NIH Post-doctoral Service Award (J.B.H.). All work involving living cells containing recombinant DNA was performed in accordance with the NIH Guidelines for Recombinant DNA Research¹².

JAMES HICKS
GERALD R. FINK

Section of Genetics, Development and Physiology,
Cornell University,
Ithaca, New York 14853

Received 31 May; accepted 18 July 1977.

- ¹ Ratzkin, B. & Carbon, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 487-491 (1977).
- ² Struhl, K., Cameron, J. R. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1471-1475 (1976).
- ³ Southern, E. J. *molec. Biol.* **98**, 503-518 (1975).
- ⁴ Fink, G. R. & Styles, C. A. *Genetics* **77**, 231-244 (1974).
- ⁵ Hawthorne, D. C. *Genetics* **48**, 1727-1729 (1963).
- ⁶ Mortimer, R. K. & Hawthorne, R. C. in *The Yeasts* (eds Rose, A. H. & Harrison, J. S.) 385-460 (Academic, New York, 1969).
- ⁷ Hicks, J. & Herskowitz, I. *Genetics* **83**, 245-258 (1976).
- ⁸ Cryer, D. R., Eccleshall, R. & Marmur, J. *Meth. Cell Biol.* **12**, 39-44 (1975).
- ⁹ Clewell, D. B. *J. Bact.* **110**, 667-676 (1972).
- ¹⁰ Guerry, P., LeBlanc, D. J. & Falkow, S. *J. Bact.* **116**, 1064-1066 (1973).
- ¹¹ Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1185-1188 (1975).
- ¹² *Fed. Regist.*, **41**, (no. 131) (1976).

Deuterium NMR study of lipid organisation in *Acholeplasma laidlawii* membranes

THE most convincing studies of molecular order in model membranes are undoubtedly those involving deuterium nuclear magnetic resonance (NMR) of labelled lipids in the lamellar liquid crystalline phase¹⁻⁶. The relevance of these results to biological membranes has not yet been established, however; there is no *a priori* reason to expect the state of organisation of lipids in the lamellar liquid crystal to resemble closely that of a natural membrane. We report here the first detailed study of acyl chain order in the lipids of a natural biological membrane—the plasma membrane of the microorganism *Acholeplasma laidlawii*. Specifically deuterated fatty acids were incorporated biosynthetically into the membrane lipids as described previously⁷. The ²H-NMR spectra of the membrane demonstrate the co-existence of at least two different kinds of lipid. Signals

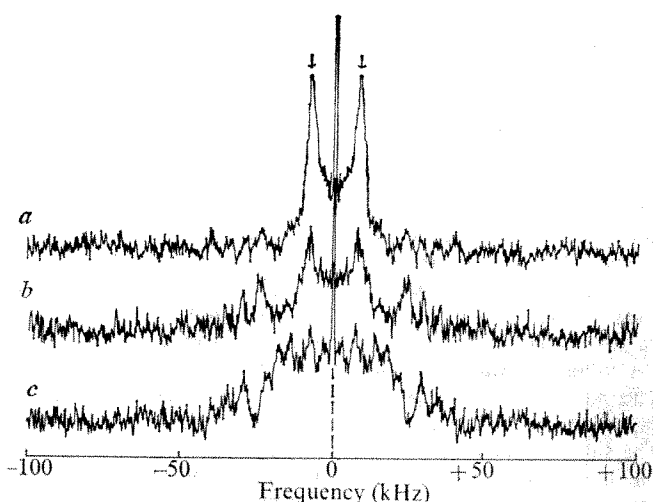
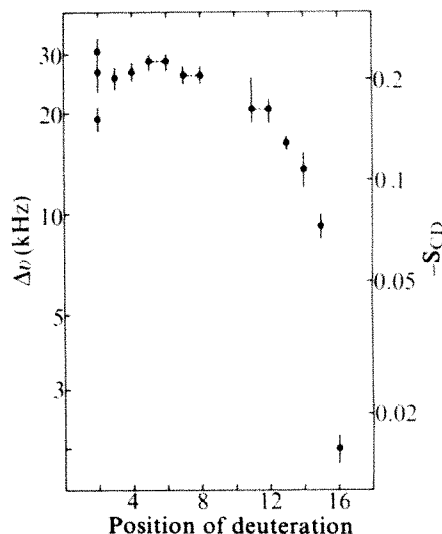


Fig. 1 ²H-NMR spectra, at 13.8 MHz, of *Acholeplasma laidlawii* membranes containing palmitate-13d₂. At 42 °C (a) the spectrum is dominated by the fluid phase with the splitting indicated by the two arrows. As the temperature is lowered (b, 30 °C; c, 25 °C), the spectrum appears more characteristic of a gel. All the spectra were recorded at a repetition rate of 4 scans per s. The number of scans was 120,000 at 42 °C and 40,000 at 30 °C and 25 °C. The spectra were symmetrised about the Larmor frequency which is represented by zero.

from the more fluid lipid are most prominent at temperatures near and above the growth temperature (37 °C). The more solid-like lipid, which predominates at lower temperatures, may be similar to the gel state of lamellar phases and could include lipid associated with the membrane protein. For the more fluid phase, the plot of order parameter against position of deuteration strongly resembles that for egg yolk² and dipalmitoyl³ phosphatidylcholine. Incorporation of cholesterol into the membrane of *A. laidlawii* increases the average acyl chain order and also increases the spread of order parameters, similar to its effect on egg yolk phosphatidylcholine⁸. These results represent a major justification for the use of lamellar liquid crystals as models for the lipid in biological membranes.

The NMR samples consisted of 0.5–1.3 g of lyophilised *A. laidlawii* membranes which were hydrated with 1–3 ml of distilled water. The average acyl chain composition of the membrane lipids was as follows: 12:0 = 6.6% (1.2); 14:0 =

Fig. 2 The distance between the peaks in the powder pattern, $\Delta\nu$, against position of deuteration of the palmitate chains at 42 °C. Points connected by dotted lines were obtained from samples labelled in two adjacent positions for which the two powder patterns are not resolved. Error bars are estimated from the spectra and do not include possible effects due to variation in membrane composition.



18.1% (2.3); 16:0 = 68.8% (3.6); minor components = about 6.5% as determined by gas-liquid chromatography. The values given represent mean compositions for all 10 specifically labelled samples. The numbers in parentheses are average deviations from the mean. Thus the membrane lipid chains were enriched to about 70% with specifically deuterated palmitate. The small random variation in chain composition, which seems to be unavoidable in this experiment, is independent of the position of deuteration in the palmitate chain.

The ^1H -NMR spectra of labelled biological membranes are characterised by quadrupolar 'powder' spectra spanning a wide frequency range and with residual quadrupolar splittings of 10^4 – 10^5 Hz (refs 2, 7). A quadrupolar spin echo technique ('solid echo') was used here to obtain undistorted spectra. The merits of this simple pulse technique, necessary to these experiments, have been discussed in detail by Davis *et al.*⁸. The signals were recorded at the ^1H Larmor frequency so that the low field half of the symmetrical quadrupolar powder spectrum appears folded and superimposed on the high field half, thus increasing the detection sensitivity by a factor of $\sqrt{2}$. To facilitate visual interpretation of these spectra, we plotted sample spectra reflected about the symmetry axis to simulate the appearance of a complete spectrum and show the quadrupolar splitting (Fig. 1). The reader should not be misled by the apparent perfect symmetry of these spectra.

As shown in Fig. 1, above the growth temperature of 37 °C, the ^1H -NMR spectra are dominated by a quadrupolar powder spectrum which resembles strongly those observed for simple liquid crystalline phospholipids^{2,3}. At lower temperatures, signals characteristic of a more solid-like phase appear. This behaviour is entirely consistent with the extremely broad thermal phase transition reported previously for *A. laidlawii* membranes derived from palmitate-supplemented media⁹. The quadrupolar splittings and related order parameters S_{CD} were measured as a function of the position of the deuterium label in the palmitate chain for the fluid phase of *A. laidlawii* membranes at 42 °C. A plot of the deduced order parameters S_{CD} against position of deuteration is shown in Fig. 2. This plot has the same characteristic shape as those found for lamellar liquid crystalline phases of single phospholipids^{2,3}. The 'plateau' region of constant order extends from carbon

2 to at least carbon 8 as for the lamellar phases, and the actual values for S_{CD} are comparable with those found for egg yolk and dipalmitoyl phosphatidylcholine^{2,3}. The important consequence of these findings is that inferences concerning acyl chain conformation and bilayer geometry, and their relationship to biological function, made previously for the lamellar phases^{2,3} apply also to *A. laidlawii* membranes.

The spectrum for membranes containing 2,2-dideuterio-palmitate is considerably different from that for any other label position. The unusual shape of the line suggests the presence of possibly three overlapping powder doublets. The quadrupolar splitting for this position is expected to be very sensitive to differences in the initial conformation of the two acyl chains¹⁰, and also to differences in the head groups (*A. laidlawii* lipids contain several different head groups).

The effect of incorporation of cholesterol into *A. laidlawii* membranes was investigated using fully deuterated palmitate chains. While this technique was highly successful in probing the effect of cholesterol on egg yolk phosphatidylcholine⁸, much less resolution of the NMR signals for individual chain positions was obtained for the membranes (see Fig. 3). Nevertheless, the general shape of the envelope due to numerous overlapping powder doublets can provide useful information⁸. The sharp edges of the envelope are characteristic of a system having a plateau region in the ordering profile¹¹ (Fig. 2). The effect of cholesterol is to spread the intensity of the NMR spectrum over a wider frequency range. This is consistent with the much greater ordering effect of cholesterol on the plateau region compared with the methyl end of the chain, as found previously for egg yolk phosphatidylcholine⁸. The increase in the quadrupole splitting from 30 to 50 kHz for the plateau region with 2:1 lipid:cholesterol ratio is very similar to that found for egg phosphatidylcholine⁸.

With the increased sensitivity of contemporary NMR techniques and the greater availability of deuterium-labelled lipids, ^1H -NMR provides an excellent method to probe the fine detail of molecular organisation in a biological membrane with a minimum of perturbation or approximation.

The research at UBC was supported by the National Research Council of Canada and by a special Killam-Canada Council Interdisciplinary Grant. J. H. D. is the holder of a NRC Postdoctoral Fellowship.

GERALD W. STOCKTON
KENNETH G. JOHNSON
KEITH W. BUTLER
A. P. TULLOCH
YVAN BOULANGER
IAN C. P. SMITH

Division of Biological Sciences,
National Research Council of Canada,
Ottawa, Ontario, Canada

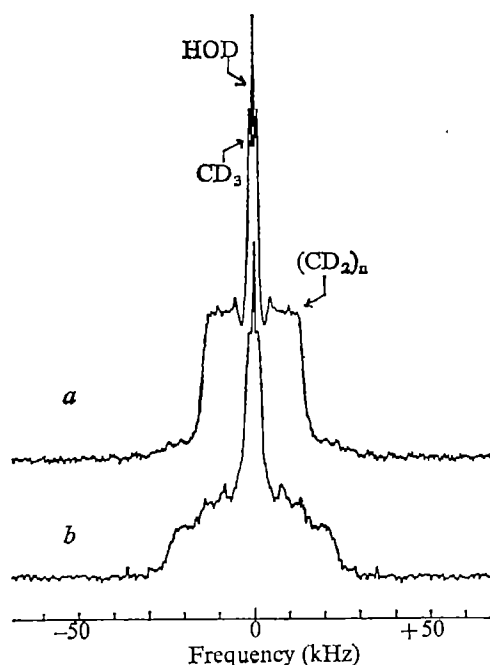
JAMES H. DAVIS
MYER BLOOM

Department of Physics,
University of British Columbia,
Vancouver, British Columbia, Canada

Received 21 April; accepted 11 July 1977.

- ¹ Oldfield, E., Chapman, D. & Derbyshire, W. *FEBS Lett.* 16, 102–104 (1971).
- ² Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F. & Smith, I. C. P. *Biochemistry* 15, 954–966 (1976).
- ³ Seelig, A. & Seelig, J. *Biochemistry* 13, 4839–4846 (1974).
- ⁴ Mely, B., Charvolin, J. & Keller, P. *Chem. Phys. Lipids* 15, 161–173 (1975).
- ⁵ Marcelja, S. *J. chem. Phys.* 60, 3599–3604 (1974).
- ⁶ Stockton, G. W. & Smith, I. C. P. *Chem. Phys. Lipids* 17, 251–263 (1976).
- ⁷ Stockton, G. W. *et al. Biochim. biophys. Acta* 401, 535–539 (1975).
- ⁸ Davis, J. H., Jeffrey, K. R., Bloom, M. & Valic, M. I. *Chem. Phys. Lett.* 42, 390–394 (1976).
- ⁹ Stein, J. M., Tourtellotte, M. E., Reinert, J. C., McElhaney, R. N. & Rader, R. L. *Proc. natn. Acad. Sci. U.S.A.* 63, 104–109 (1969).
- ¹⁰ Seelig, A. & Seelig, J. *Biochim. biophys. Acta* 406, 1–5 (1975).
- ¹¹ Davis, J. H. & Jeffrey, K. R. *Chem. Phys. Lipids* (in the press).

Fig. 3 ^1H -NMR spectra, at 13.8 MHz and 42 °C, of *A. laidlawii* membranes containing perdeuteriopalmate; a, without cholesterol and b, with 2:1 lipid:cholesterol ratio. As in Fig. 1, the spectra were made symmetrical about the Larmor frequency.



reviews

Energy crisis

John Chesshire

Energy: The Continuing Crisis. By Norman Metzger. Pp. 242. (Thomas Y. Crowell: New York, 1977.) \$12.95.

AS if to anticipate the reviewer, the author commences his study by questioning the need for yet another book on the "energy crisis". The justification lies in his belief that since 1973, energy consumers have been lulled into a dangerous complacency: new finds of fossil fuels are reported daily, and the nuclear and non-conventional technology lobbies sing in high praise of the potential of their systems. This appearance of plenty allows consumers the luxury of using yet more fuel while putting up stern and vocal criticism to planning applications for new nuclear stations, oil refineries and coal mines. Meanwhile, their elected representatives, whether in the US Congress or in the British House of Commons, point to the political implications of tough conservation measures, especially any which might bring about the transition of fuel prices to the long-term marginal costs of replacements. Thus, the "continuing crisis", and the need for another book.

The author adopts the now-common, fuel-by-fuel approach in his analysis, and focuses on the US situation. He points to the steady demise of coal this century, and to the massive injection of funds now required to raise production by providing new capacity and efficient mining, conversion and utilisation technologies. He concludes by arguing that the scale of the resource requirement is such that the vast expansion of the coal industry is by no means the certainty it seems to be in current energy policy planning.

Critical to his review of the oil sector is an analysis of the likelihood of continued cohesion of the OPEC cartel (a fixation with many American commentators). The absence of any discussion of the role of the state in determining alternative depletion policies is a weakness here, as in the succeeding chapter on natural gas. His useful arm's-length account of the "Troubled Youth of Nuclear Power" includes an assessment of recent American action and reaction in the build-up of nuclear generating capacity, and a damning indictment of the lack of attention to waste disposal

and storage, and leads him to the whimsical (but incisive) view that "maybe the trouble with nuclear reactors is that they don't have belching smokestacks".

Of the unconventional technologies, he anticipates that the major contribution will come from the fusion, solar and geothermal routes, and he highlights the critical uncertainties regarding the cost, scale and timing of their contribution to future energy supplies.

On balance, the author is to be commended for his realistic evaluation of the many exaggerated claims that have

been made about both fossil and novel energy supplies, and for his sensitive analysis of the constraints on change imposed by the power and vested interests of the energy corporations and the regulatory functions of the state. It is unfortunate that he favours a supply—rather than a demand—oriented approach, thus neglecting conservation policies and technologies and that he adopts a relatively short-term US-centred view of the world. □

John Chesshire is a Fellow of the Science Policy Research Unit, University of Sussex, UK.

Tracing the history of life

The Phylogeny of the Vertebrata. By Søren Løvtrup. Pp. xii+330. (Wiley: London and New York, 1977.) £15; \$29.50.

COMPARATIVE BIOLOGY has been revitalised in recent years under the stimulating influence of Willi Hennig's *Phylogenetic Systematics*. No longer can it be regarded as the feeble survivor of a primitive descriptive phase of biology, for it is evidently central to evolutionary biology and gives meaning and direction to research in fields as diverse as embryology, population genetics, biogeography and behaviour. It is based on a belief in the objective existence of a true path of descent for all living organisms which may be reconstructed by the empirical testing of well formed hypotheses. Nevertheless, despite its already long life, comparative biology is not yet a mature science with well understood philosophical and theoretical foundations. Søren Løvtrup's book attempts to remedy this defect, using the *Vertebrata* to exemplify his ideas.

The book opens by setting out its Popperian research programme and then immediately takes up the "Logic of Phylogenetics". By excluding fossils and thereby avoiding argument about what is in any case a practical rather than a theoretical difficulty, Løvtrup is able to treat classification and phylogeny reconstruction as the same process. Thus, of the two levels of taxonomic activity differentiated by Gregg—taxonomy proper and method-

ological taxonomy—Løvtrup attacks the latter. Taxonomy at the higher level is a primitive science. As Gregg says, "Taxonomists engaged in methodological research are [thus] without adequate linguistic resources to support their investigations. They must rely almost entirely upon unsuitable prescientific idioms borrowed from everyday language". Gregg's own solution was the use of set theory, but this had no hope of acceptance in a pre-Hennigian intellectual climate that deliberately depressed rigour and allowed artifice and authority to flourish. Twenty-five years later, Løvtrup is in a more favourable position, though it is perhaps unfortunate that his "language" is a series of definitions, axioms and theories, rather than the symbolic logic of Gregg.

The heart of Løvtrup's book comprises two long chapters on the ancestry and divergence of vertebrates. These are more easily approached than other chapters and will no doubt readily attract attention because of their heterodox conclusions. However, Løvtrup has more important things to say elsewhere in this book, and as he deviates so disastrously from his professed deductive approach, I am disinclined to give much weight to his conclusions. The last section of the book deals with the mechanism of evolution. Comparative biology has not benefitted greatly at a practical level from work in this field, and Løvtrup tries to redress the balance with an axiomatised version of his "comprehensive" theory. This is

distinguished by the importance it attaches to large step changes and the pressure of macromutations, over and above natural selection.

How well has Løvtrup succeeded in clarifying these important and difficult issues? Reluctantly, I must suggest that in large measure he has been unsuccessful, though whether this is due to the unsuitability of the methods adopted, or to the way these are used, is not a question that can be answered here. His arguments are based on formal language but are often illogical and imprecisely formulated. He purports to follow Popper but his theories are often unfalsifiable in principle or protected by phrases such as "Other things being equal". Large sections of the argument about relationships are indistinguishable from induction by enumeration and from the search for confirmation, as is shown by the repeated use of statements such as, "If we want to find support for the classification in Figure 4.12, we must look for characters in common between *Caudata* and *Salientia*".

The section which purports to confront his theory of vertebrate interrelationships with the facts of the fossil record, seems to rely heavily on the interpretation of the facts in the light of the theory they are said to support. Yet even this will not dispose of the Placodermi, which are therefore dismissed with an astonishingly vacuous quotation from Romer, which is said to seem "to be quite a close approximation to the truth". Unfortunately, the form of the argument is also imprecisely handled and this hinders comprehension. Some axioms are either self-contradictory or ambiguously stated, others are stated and then immediately refuted, and yet others introduced with an exception that is not defined until much later. At least one theory is a paraphrase of the definition from which it is said partly to be derived, and another is incompatible with the theory from which it is said to be deduced.

These are harsh criticisms of an honest attempt to deal in an explicit way with a number of difficult problems. I share Løvtrup's views on the importance of criticism and on the seriousness of the failure of many comparative biologists to make their assumptions, arguments and conclusions clear. Therefore, I recommend this book, particularly to those involved in tracing the history of life. For, despite its failures, it is full of challenging ideas, and I unreservedly commend the attitudes and hopes that motivated its writing.

R. S. Miles

R. S. Miles is Head of the Department of Public Services at the British Museum (Natural History).

Ellipsometry and polarised light

Ellipsometry and Polarized Light. By R. M. A. Azzam and N. M. Bashara. Pp. xvii+529. (North-Holland: New York, Amsterdam and Oxford, 1977.) Dfl.180; \$37.50.

ELLIPSOmetry is a technique for examining the optical properties of surfaces and thin films with polarised electromagnetic radiation. The technique is intrinsically very sensitive and deceptively simple in principle. Thus, it can readily be explained at a superficial level to students with only a minimal grasp of optics. Scientists who apply ellipsometry to investigate a wide range of phenomena, however, are aware of the need to take account of many possible sources of systematic error and of the consequent notorious difficulties in interpreting ellipsometric observations. The publication of a book of five hundred pages on ellipsometry will therefore not surprise them. Indeed, English-speaking scientists will welcome the first book of its kind written in their language by authors with international reputations for contributions to the development and use of ellipsometry.

The first two chapters are devoted to descriptions of well established and predominantly matrix methods of representing polarised radiation and the operation of optical elements with polarising properties. In chapter three the authors give an analysis of the main ellipsometric arrangements and a well ordered discussion of associated systematic errors; essentially the core

material of the book. The procedures for relating observable optical functions to the macroscopic optical constants of the materials of thin films and surfaces are presented in chapter four. Much of its contents can be found in other texts on thin film optics, but the account has been updated by references to recent work on anisotropic crystalline materials, as well as by a discussion of numerical inversion procedures. An account of instrumentation is given in chapter five with emphasis on imperfections and automation. In the final chapter, the authors provide a necessarily brief survey of the very wide range of physical surface investigation which has prompted the application of ellipsometry. Examples are taken from optics, physical chemistry, electrochemistry, particle radiation physics and biology.

The book is well written, with clear diagrams and few typographical errors. A pleasing feature of style are the separate introductions to each chapter, which provide the reader with a general preview of what is to follow. On the other hand, the balance of the material, which presumably reflects the authors' particular interests, will not be to the taste of some readers. For instance, the mathematical representations of polarised radiation are treated exhaustively at the expense of explanations of the underlying physical phenomena. A prominent example of inadequate emphasis concerns the ultimate sensitivity of ellipsometers; an important matter dismissed in a single paragraph.

P. H. Lissberger

P. H. Lissberger is Professor of Physics at the Queen's University, Belfast, Northern Ireland.

Ethological dictionary

Ethological Dictionary. By Armin Heymer. Pp. 237. (Paul Parey: Berlin and Hamburg, 1977.) DM28.

A dictionary in three languages (German, English, French) such as this one is useful to both students and research workers if it contains a wide enough selection of terms. I carried out a quick test by opening a recent textbook of animal behaviour at two randomly chosen pages. The Dictionary scored only a 40% success rate on the first ten technical terms encountered. Territoriality, echolocation, circadian rhythm and orientation were included and adequately defined, but habituation, phonoresponse, species recognition, arousal, sexual selection and selective attention were not listed.

Admittedly this is a small sample, but several of the missing terms are very common in ethological literature.

The dictionary does, however, score well in including some obscure terms with which I was not familiar. "Night dancers" are "bees that dance at 3.22 a.m. with only 12.5° error in the waggle dance", and equally intriguing "shag time" is apparently slang for "breeding season in hares and rabbits".

In general, the technical terms included in the dictionary and the way in which they are defined very much reflect the Lorenzian school of ethology, so that the dictionary might prove useful to those wishing to delve into the earlier German literature. Other users may find the range of terms covered too limited for the book to be worth buying as an all round aid to translation.

John Krebs

John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, Oxford, UK.

Ecological stock-taking

A Nature Conservation Review: The Selection of Biological Sites of National Importance to Nature Conservation in Britain. Edited by D. A. Ratcliffe. Vol. 1. Pp. xvi+401. £35. Vol. 2. Pp. viii+320. £25. (Cambridge University: London, Cambridge and New York, 1977.)

THE many demands made on land in Britain are causing a widespread and significant reduction in wildlife. The purpose of *A Nature Conservation Review* has been to take stock of the nation's species and habitats and to identify those sites of national importance, so that new measures to safeguard them shall follow. The work was begun by the Nature Conservancy in 1965 and involved appraisal of several thousand sites. The process and its results are now published in two volumes.

Volume 1, which is complete in itself, explains the *Review's* purpose and methods, lists those sites which qualify as being of national importance and assesses how far their protection would ensure the survival of our total variety

of flora and fauna. It also provides a concise and detailed examination of British habitat types and their wildlife; this makes fascinating reading and will be invaluable for general reference.

Careful consideration is given to possible shortcomings in the *Review's* scope and methodology. This is of more than intrinsic value. There is a tendency not only in industry but amongst a few planning authorities and statutory agencies (who should know better) to doubt the Nature Conservancy Council's objectivity. *A Nature Conservation Review* will reassure them.

Volume 2 comprises descriptions of the 735 key sites, outlining the features which justify their selection from a total of some 3,500 scheduled over the years as Sites of Special Scientific Interest. Although many of the chosen sites contain rare species, good examples of typical habitats or wildlife communities are also included if the continued survival of the type is uncertain.

Obviously, there is room for discussion on whether the right sites were selected in every case but, accepting that new key sites may yet remain to be found and that others will change in merit, there can be little doubt that

the choice is largely correct. A pity then that, having taken ten years to publish the results of the survey, there has been some failure to amend it in the light of the greatly increased amount of information available in the mid-1970s. This is particularly evident where references occur to some rare birds whose recent status has changed notably.

It is particularly important that *A Nature Conservation Review* is read widely outside the nature conservation movement. Criticisms of details apart, it is a remarkable work which can do the NCC's standing nothing but good. It is also a means to an end: protection of the key sites must be achieved somehow and, as the NCC recognises, there is also a need to pursue more general measures for wildlife conservation throughout the countryside. At least volume one should be seen by all concerned with or by the use of land. It therefore passes comprehension that it has been priced so highly that it may fail to reach much of the readership at which it is aimed.

J. H. Andrews

J. H. Andrews is Head of the Conservation Planning Department at the Royal Society for the Protection of Birds.

Mammals of Pakistan

The Mammals of Pakistan. By T. J. Roberts. Pp. 361. (Ernest Benn: London and Tonbridge, 1977.) £35.

PAKISTAN lies astride the interface between the Palaearctic and Oriental faunal regions and, in addition, its fauna includes a small element from the Ethiopian region. The country is a mosaic of diverse habitats from the high montane snowlands of the Himalaya to the hot deserts east of the Indus. These factors are reflected in the richness of the mammal fauna, which includes over 150 species of recent mammals. Recent advances in agriculture and the increase in population have led to a rapid decline in numbers of the larger species and to an increased awareness of the role of smaller animals as pests and public health problems. An assessment of the current distribution and status of the mammals of Pakistan is therefore timely.

Tom Roberts has produced a comprehensive account of the mammals of the area which will be the standard work for years to come. The volume begins with two chapters on the zoogeography and habitats, and a chapter

on the adaptation of mammals to desert survival. The remainder of the book is devoted to a systematically arranged account of the species with information on description, distribution, status and biology. Keys are provided for the identification of each taxon. Maps, plates and drawings are used extensively to supplement the text. Useful appendices on field methods, a bibliography and a gazetteer are provided, and the book is fully indexed.

There are some weaknesses in the taxonomy which reflect the current lack of knowledge of Pakistan mammals and which cannot be attributed to any failure on the part of the author. The strength of the book lies in the author's painstaking compilation of data on distribution and status and his first-hand knowledge of the biology of the mammals based on his own field experience. The detailed treatment of each species surpasses the level expected in such a comprehensive volume.

The book should be of great value to the increasing number of mammalogists involved in agricultural, medical and conservation research in the area, as well as to the naturalist interested in the fauna of Pakistan and neighbouring regions.

I. R. Bishop

I. R. Bishop works in the Department of Zoology at the British Museum (Natural History).

**tins
will
change
your
reading
habits**

from July, 1978

**ELSEVIER/NORTH HOLLAND
BIOMEDICAL PRESS**

Circle No. 23 on Reader Enquiry Card.

Acridologists' vademecum

Grasshoppers and Locusts. Vol. 2. By Boris Uvarov. Pp. 613. (Centre for Overseas Pest Research: London, 1977.) £16.

ON retirement, Sir Boris Uvarov undertook the preparation of "a synthesis of present-day knowledge of the theory and practice of acridology", and this publication sees the eventual fulfilment of that task after his death in 1970. The first volume covering anatomy, physiology, development, phase polymorphism and an introduction to taxonomy came out in 1966 (Cambridge University Press). This second, posthumous volume covers behaviour (10 chapters) and ecology and biogeography (3).

The longest single chapter discusses the dynamics of grasshopper and locust outbreaks and plagues. Only in this chapter and an earlier section on swarm migrations is the information arranged by species; otherwise, it is organised by topics and truly comprehensive in scope. The book will thus be useful to many people who do not see themselves as 'acridologists' but wish to draw on the large store of information available from the research on these insects. They will not find all they want on any one topic within these 600 pages, 270 text-figures, 95 tables and 24 double-column pages of index, but they will get the gist of it along with a guide to the rest supported by 1,700 references.

Uvarov's colleagues Z. Waloff, R. F. Chapman and N. D. Jago, who edited the text after his death, have added supplementary material to his drafts but have not attempted to write the planned chapters he had not yet drafted, on economic aspects and the principles of control. Instead, they have rounded the book off with what amounts to a testament, a late review paper of Uvarov's on "Current and future problems of acridology", which sets forth his matured views on population dynamics, control and research.

The book may be fairly described as a monument to the author in more ways than one. The huge edifice of modern acridology was built on his inspiration and drive. The book is also very much the man, in its detailed attention to the facts, and to gaps in them, in its exceptional biological span, its scepticism, criticism and proposals for research, and in its sense of continuity between science and practice that Uvarov always displayed, not least in the unconventional naming of his research organisation, the "Anti-Locust Research Centre". As a more important illustration of that same point, the book incidentally goes some

way toward rehabilitating Uvarov's original discovery of phase transformation in locusts as the basis for a strategy of concentrating control operations on 'outbreak areas' where the swarming phase is produced from non-swarming precursors. Both ideas came under a cloud of criticism in the 1950s and 1960s, not least because it turned out that the most notorious species, the Desert Locust, was highly mobile in both phases and did not have just a few, permanent, outbreak areas like the others. By the time this volume was written, however, enough new information had accumulated to show that even in this species phase change or "gregarisation" is crucial in plague development and occurs in geographically identifiable areas, which are indeed larger and less fixed than the outbreak areas of other species but still far smaller than the

vast region subsequently over-run by the swarms.

Uvarov surely went too far in his reiterated hope that the outbreak areas would one day be deliberately altered ecologically for the sole purpose of preventing locusts from swarming, once and for all. Nevertheless, for the more pressing purposes of human settlement, the necessary ecological changes have already occurred in some regions such as North America, and they will doubtless occur in later-developing countries in due course. If the expected result is an endemic grasshopper problem replacing the locust invasions, we are fore-armed with this book.

J. S. Kennedy

J. S. Kennedy is External staff member, Agricultural Research Council, and Professor of Animal Behaviour at the Imperial College of Science and Technology, University of London, UK.

Calculating electronic structure

Semiempirical Methods of Electronic Structure Calculation. Part A (vol. 7) Techniques. Pp. 274. Part B (vol. 8): Applications. Pp. 274. Edited by G. A. Segal. (Plenum: New York and London, 1977.) \$47.40 each part.

WITH so many books available on molecular orbital theory it is hard to imagine many gaps in the literature. Nonetheless, with calculations being run increasingly by non-professional theoreticians there has been a need for a consumer guide to the various alternative semiempirical methods. To some extent the two volumes edited by Segal provide this service.

Most of the variants of molecular orbital techniques are the subjects of monographs but here in the first of the companion volumes several methods are summarised, each in about thirty pages. The individual contributors are distinguished advocates of the methods they describe; in particular the chapters on the PCIO and X α methods will be welcomed as providing more convenient summaries than those formerly available. One would wish, however, that for the price the coverage had been more comprehensive. Although neglect of differential overlap and the methods based on this approximation are the subject of one chapter, it would have been helpful to have a full chapter on the MINDO method even if the editor (as is clear from his preface) does not approve of this variant.

The tone of part A is serious and the theoretical basis for semi-empirical theories is discussed in the longest

chapter by Karl Freed. Many users of semi-empirical methods, on the other hand, only seek justification in the quality of predictions made by their calculations. Some of these applications are the subjects of the chapters in Part B.

Again, some well known names contribute. The topics to which the calculations are applied include thermochemistry; excited states of organic molecules; photochemistry; inorganic complexes; spin resonance parameters; solid-state problems; and electron scattering. Any non-specialist with a problem in one of these areas would be very much helped in choosing the most sensible approximation to select and given an honest indication of the quality of results he might expect.

In one respect the two volumes do not quite provide the complete consumer's guide. Many chemists are not controlled by the technique they use but by the molecules they are studying. They are interested in several properties of the same group of molecules, perhaps the vibration frequencies, the pK_a, the conformation and the nmr chemical shifts. A question one is often posed is, "Which method will answer these questions for me?" and at the same time "Just how much computer time is likely to be required?". A complete guide for the non-specialist would contain a set of 'benchmark' problems on model systems with the results from the various approximations compared and timed. Despite this omission the volumes will not rest unused on library shelves.

Graham Richards

Graham Richards is Lecturer in the Physical Chemistry Laboratory and a Fellow of Brasenose College, Oxford, UK.

announcements

On the Move

Professor F. Buchthal, Institute of Neurophysiology, Copenhagen, Denmark, to University Hospital (Rigshospitalet), Research Building, Copenhagen, from 1 September 1977.

Professor H. G. Schweiger, Max-Planck Institut für Zellbiologie, Wilhelmshaven, Germany, to Max-Planck Institut für Zellbiologie, Ladenburg, from 1 August 1977.

Dr J. Lawrence Fox, Associate Professor of Zoology, University of Texas at Austin, to Max-Planck Institut für Biochemie, Martinsried, Germany for one year sabbatical from 1 September 1977.

Dr B. P. Dash, Department of Geology, Imperial College of Science and Technology, University of London, to Chair of Applied Geophysics, University of Ife, Ife-Ife, Nigeria for two years from 1 September 1977.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Appointments

Professor A. Lazenby, to Director of the Grassland Research Institute from 1 August 1977.

Dr R. J. Slater, to Director of Medical Programs for The National Multiple Sclerosis Society from 1 July this year.

Awards

Drs J. K. Burdett and **M. Poliakoff** are joint recipients of the Meldola Medal, awarded by the Royal Institute of Chemistry and Society of Maccabaeans for their work on laser chemistry and chemical effects of light.

The Actonian Prize of the Royal Institution has been awarded to **Professor R. L. Wain**, Professor of Agricultural Chemistry at Wye College, University of London and Honorary Director of the Agricultural Research Council Unit on Plant Growth substances and Systemic Fungicides, for his work on the chemical control of plant growth and the chemical basis of disease resistance in plants.

Dr F. Greaves, of the Imperial Cancer Research Fund has been awarded the Paul-Martini Prize 1977 (20,000 DM) for his work on a new diagnostic method for the specific proof of acute lymphoblastic leukaemia cells.

Meetings

14–16 September, **3rd National Quantum Electronics Conference**, Southampton (G. C. Thomas, Department of Electronics, University of Southampton, Highfield, Southampton, UK).

16–17 September, **Joint Radiological Meeting**, Guildford (The British Institute of Radiology, 32 Welbeck Street, London W1, UK).

19–23 September, **Application of Microprocessors**, Newcastle (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

Person to Person

A cooperative programme 'Species Pollution at World Scale' is trying to assess the real situation of the problem of introduced species. Botanists with experience in floristics are kindly requested to send the following information. (1) Place and area surveyed, (2) number of native species, (3) number of introduced species, including ephemerals and (4) a complete bibliographical reference for quotation, to E. H. Rapoport, Fundación Bariloche, c.c. 138, Bariloche 8400, Argentina. Collaborators will receive the printed map.

Exchange 4-bedroom furnished house in Montreal for similar in London area July 1978–August 1979. Car also considered. Contact D. Denhardt, 4338 West Hill Avenue, Montreal, Quebec H4B 2S9, Canada.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

21 September, **Modern Trends in Fluorescence Techniques**, Guildford (D. Irish, Hon. Secretary, UV Group, c/o Pye Unicam Ltd, York Street, Cambridge, UK).

21–23 September, **The Safe Use of Materials**, Nottingham (R. H. Biddulph, Borax Consolidated Ltd, Cox Lane, Chessington, UK).

22 September, **Heterotechnologies for the Mail Service**, London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

26 September, **Surface Expression of Orebodies**, Swansea (The Institution of Mining and Metallurgy, 44 Portland Place, London, UK).

25–27 September, **Conference on Engineering for Health in Hot Countries**, Loughborough (R. Steele, University of Technology, Loughborough, UK).

25 September–11 October, **2nd International Kimberlite Conference**, Santa Fe, New Mexico (Kimberlite Conference, Sylvia-K Inc., 5671 Blue Sage Drive, Littleton, Colorado 80123).

26–28 September, **International Conference on Distributed Computer Control Systems**, Birmingham, UK (The Institution of Electrical Engineers, Savoy Place, London, UK).

26–28 September, **1st German Solar Energy Forum**, Hamburg (Congress Centrum Hamburg, Am Dammtor, PO Box 302360, D-2000 Hamburg 36, Germany).

26–29 September, **The 1st BOC Priestly Conference on Heterogeneous Oxidation**, Leeds (J. T. Gleave, Special Courses Division, Department of Adult Education and Extramural Studies, University of Leeds, Leeds, UK).

27–29 September, **International Conference on Power Electronics—Power Semiconductors and their Applications**, London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

28 September, **Applications of Broad-Band Tuneable Lasers**, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

28–30 September, **Symposium on Platelets: A Multidisciplinary Approach**, Florence, Fondazione Internazionale Menarini, Piazza del Carmine, 4-20121 Milan, Italy).

Reports and Publications Other countries—June

Annals of the South African Museum. Vol. 72, Part 12: The Validity of *Malacoraja* Stehmann, 1970, (Chondrichthyes, Batoidea, Rajidae) and Its Phylogenetic Significance. By P. Alexander Hulley and Matthias Stehmann. Pp. 227–237. R.2. Vol. 72, Part 13: New Records of Marine Crustacea Isopoda from South Africa. By Brian Kensley. Pp. 239–265. R.2.90. (Cape Town: South African Museum, 1977.) [216]
Office des Recherches sur les Pêcheries du Canada. Rapport Annuel 1976. Pp. 16. (Ottawa: Ministère des Pêches et de l'Environnement, Service des Pêches et de la Mer, 1976.) [226]
SRI. People at Work: Annual Report 1976. Pp. 32. (Menlo Park, California: SRI, 1977.) [226]
United States Department of the Interior: Geological Survey, Bulletin 1397-B: Mineral Resources of the Laramie Peak Study Area, Albany and Converse Counties, Wyoming. By Kenneth Segerstrom and

Robert C. Weisner. Pp. vi+35+plate 1. Water-Supply Paper 2044: Modeling Chloride Movement in the Alluvial Aquifer at the Rocky Mountain Arsenal, Colorado. By Leonard F. Konikow. Pp. v+43. (Washington, DC: US Government Printing Office, 1977.) [276]

Proceedings of the Nutrition Society of Australia, Vol. 1: First Annual Conference, Melbourne, Victoria, August, 1976. Pp. 41. (Blacktown, NSW: Nutrition Society of Australia, Dr. J. L. Black (Hon. Secretary), c/o CSIRO, P.O. Box 239, 1977.) [273]

Space—Part of Europe's Environment Pp. 40. (Paris: European Space Agency, 8/10 rue Mario Nikis, 1977.) [276]

Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. Tätigkeitsbericht 1976. Pp. 360. (Jahresbericht Band 1). Programme und Project 1976. pp. 832. (Jahresbericht Band 2.) (Bonn-Bad Godesberg: Deutsche Forschungsgemeinschaft, 1977.) [286]

World Health Organization, Technical Report Series No. 606: The Role of Immune Complexes in Disease: Report of a WHO Scientific Group. Pp. 58. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 8; \$3.20. [286]

Harvard University: School of Public Health, Dean's Report, 1976. Pp. 56. (Boston, Mass.: Harvard University, School of Public Health, 1977.) [286]

Smithsonian Contributions to Zoology, No. 221: Polychaetes from Intertidal Areas in Panama, with a Review of Previous Shallow-Water Records. By Kristian Fauchald. Pp. iii+81. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [286]

Fish Kills at Lake Lanier Hatchery: Investigations and Report of Findings. By W. C. Noell and G. B. Oglesby. Pp. iii+123. (Atlanta, Georgia: Department of Natural Resources, Environmental Protection Division, 270 Washington Street, SW, 1977.) [286]

Smithsonian Contributions to Zoology, No. 238: A Systematic Monograph of the Tongue Soles of the Genus *Cynoglossus* Hamilton-Buchanan (Pisces: Cynoglossidae). By A. G. K. Menon. Pp. iv+129 (21 plates). (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [296]

European Space Agency, ESA Annual Report 1976. Pp. 201. Report presented by the European Space Agency to the 20th COSPAR Meeting, Tel Aviv, Israel, June 1977. Pp. 268. (Paris: ESA, 1977.) [306]

Reports and Publications

UK & Ireland—July

Fulmer Annual Review, 1976. Pp. 8. Stoke Poges, Bucks: Fulmer Research Institute, Ltd., 1977.) [17]

Smith Kline and French Foundation, Fourteenth Annual Report, 1976. Pp. 8. (Welwyn Garden City, Herts.: Smith Kline and French Foundation, 1977.) [47]

University of Oxford. Annual Report of the Curators of the Bodleian Library for 1975-76. (Supp. No. 4 to the *University Gazette*.) Pp. 48. (Oxford: The University, 1977.) £1.75. [47]

Microbiological Research Establishment. Abstracts of Work Published in 1976. Pp. 22. (Porton Down, Salisbury, Wiltshire: Microbiological Research Establishment, 1977.) [57]

National Radiological Protection Board, NRPB-R52: Human Exposure to Radiation Following the Release of Radioactivity from a Reactor Accident—a Quantitative Assessment of the Biological Consequences. By H. Smith and J. W. Stather. Pp. ii+39. NRPB-R60: Measurement of Activity of Surfaces Contaminated by Beta-emitting Nuclides and ^{59}Fe . By W. J. Iles, P. H. Burgess and D. F. White. Pp. 13. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1977.) [57]

Chemical Industries Association. Labelling Chemicals: a Guide to the Regulations for the Labelling and Marking of Containers and Vehicles. Pp. 133. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, SE1, 1977.) [57]

Wye College: Department of Hop Research—Annual Report from 1 April 1976 to 31 December 1976. (University of London.) Pp. 68. (Nr. Ashford, Kent: Wye College, 1977.) 80p. [67]

Tropical Storage Abstracts, No. 1, 1977. Pp. 14. (Slough: Tropical Stored Products Centre (Tropical Products Institute), London Road, 1977.) [67]

Third Special Report from the Select Committee on Science and Technology, Session 1976-77. Pp. ii+2. (London: HMSO, 1977.) 15p net. [67]

Philosophical Transactions of the Royal Society of London, A: Mathematical and Physical Sciences, Vol. 286, No. 1334: Turbulence and Mixing in a Scottish Loch. By S. A. Thorpe. Pp. 125-181+plate 1. (London: The Royal Society, 1977.) UK £3.60; Overseas £3.80. [67]

Office of Population Censuses and Surveys. Population Trends 8, Summer 1977. Pp. 62. (Government Statistical Service.) (London: HMSO, 1977.) £2 net. [77]

An Appraisal of Various Approaches to the Fractionation of Tobacco Smoke Condensate. By J. K. Whitehead. (Research Paper 13.) Pp. 56. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1977.) [77]

Grassland Research Institute. Annual Report for 1976. Pp. xvi+172. (Hurley, Maidenhead, Berkshire: The Grassland Research Institute, 1977.) £2.50. [77]

Building Research Establishment. Research Programme 1977-78. Pp. 48. (Garston, Watford: Building Research Establishment, Department of the Environment, 1977.) [77]

Freshwater Biological Association. Forty-Fifth Annual Report for the year ended 31 March 1977. Including Reports of the Council and of the Director, and Accounts for 1976-77, with Lists of Officers, Council and Staff. Pp. 128+4 plates. (Ambleside,

Cumbria: Freshwater Biological Association, 1977.) £2. [87]

Scottish Plant Breeding Station, Fifty-Sixth Annual Report, April 1976 to March 1977, and the Report of the Scottish Society for Research in Plant Breeding. Pp. 132. (Pentlandsfield, Roslin, Midlothian: Scottish Plant Breeding Station, 1977.) [87]

Computer-Based Packages for Teaching Earth Science. Report No. 5: Programs in BASIC for Simple Correlation and Regression (With Worked Examples). By Roger Till (with programs by Sue Watts). Pp. 62. (Reading University: Geological Reports, No. 11.) (Reading: Geology Department, The University, 1977.) 75p. [87]

Natural Environment Research Council: Institute of Terrestrial Ecology, Culture Centre of Algae and Protozoa—List of Strains 1976. Pp. ii+120. £1 net. A Coded Checklist of Animals Occurring in Fresh Water in the British Isles. By Dr. P. S. Maitland. Pp. 76. £1.50 net. Ecology of Red Deer: a Research Review Relevant to Their Management in Scotland. By Brian Mitchell, Brian W. Staines and David Welch. Pp. vii+74. £2 net. Atlas of the Non-Marine Mollusca of the British Isles. Edited by M. P. Kerney. Pp. v+199. £3. (Cambridge: The Institute of Terrestrial Ecology, 68 Hills Road, 1976 and 1977.) [87]

Safety Representatives and Safety Committees. (Health and Safety at Work.) Pp. 46. (London: Health and Safety Commission, 1977. Available from HMSO.) 35p. [117]

Department of Health and Social Security. Welsh Office. Residential Homes for the Elderly: Arrangements for Health Care. (A Memorandum of Guidance.) Pp. 20. (London: DHSS, 1977.) [117]

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research, Fisheries Radiobiological Laboratory. Technical Report FRL 12: Radioactivity in Surface and Coastal Water of the British Isles, 1975. By N. T. Mitchell. Pp. 32. (Lowestoft: Fisheries Radiobiological Laboratory, 1977.) [147]

Queen Mary College, University of London. Annual Report, Session 1975-76. Pp. 63. (London: Queen Mary College, Mile End Road, E1, 1977.) [147]

Royal Greenwich Observatory Bulletins, No. 182: The Galaxy and the Local Group. (Tercentenary Symposium held at Herstmonceux Castle, 1975, July 22-25.) Edited by R. J. Dickens and Joan E. Perry. Pp. xi+293. £7.50 net. Report 1975/76. Pp. 64. £1 net. (Herstmonceux: Royal Greenwich Observatory, 1976 and 1977.) [147]

Council for National Academic Awards. Annual Report for 1976. Pp. 71. (London: Council for National Academic Awards, 344/354 Gray's Inn Road, WC1, 1977.) [147]

The Radiochemical Centre, Amersham. Radiochemicals 1977/8. UK edition. Pp. 143. (Amersham: The Radiochemicals Centre, 1977.) [147]

Laboratory of the Government Chemist. Report of the Government Chemist 1976. Pp. iv+166. (London: HMSO, 1977.) £3 net. [157]

Science Research Council. The Work of the Rutherford Laboratory, 1976. Edited by Gordon Fraser. Compiled by the Laboratory Information Panel. Pp. xii+127. (Chilton, Didcot, Oxon: Science Research Council, Rutherford Laboratory, 1977.) [187]

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research, Fisheries Radiobiological Laboratory. Technical Report FRL 12: Radioactivity in Surface and Coastal Water of the British Isles, 1975. By N. T. Mitchell. Pp. 32. (Lowestoft: Fisheries Radiobiological Laboratory, 1977.) [187]

Chinese Astronomy, Vol. 1, No. 1, June 1977. (A selected translation of *Acta Astronomica Sinica*.) Pp. 1-196. Published Bi-Annually. Annual subscription: \$105. (Oxford and New York: Pergamon Press, 1977.) [187]

Nuclear Track Detection, Vol. 1, No. 1, 1977. Pp. 1-80. Editor-in-Chief: S. A. Durrano. Published Quarterly. Annual subscription: \$55. (Oxford and New York: Pergamon Press, 1977.) [187]

Philosophical Transactions of the Royal Society of London, B: Biological Sciences, Vol. 279, No. 969: A Physiological, Biochemical and Histological Study of Goose Tracheal Mucin and Its Secretion. By R. J. Phipps, P. S. Richardson, A. Corfield, J. T. Gallagher, P. K. Jeffrey, P. W. Kent and M. Passatore. Pp. 513-543+plates 1-4. (London: The Royal Society, 1977.) UK £2.90; Overseas £3. [207]

The Royal Scottish Museum Triennial Report, 1974-76. Pp. 74+12 plates. (Edinburgh: The Royal Scottish Museum, 1977.) £4 net. [227]

Indexed Bibliography of Publications on Water and Waste Engineering for Developing Countries. Edited by John Pickford. Pp. 52. (Loughborough: WEDC Group, Department of Civil Engineering, University of Technology, 1977.) £7. [257]

National Coal Board. Report and Accounts, 1976/77. Pp. 76. Statistical Tables, 1976/77. Pp. 12. (London: National Coal Board, 1977.) [257]

Philosophical Transactions of the Royal Society of London, A: Mathematical and Physical Sciences, Vol. 286, No. 1335: Steep Gravity Waves in Water of Arbitrary Uniform Depth. By E. D. Cokelet. Pp. 183-230. (London: The Royal Society, 1977.) UK £3.20; Overseas £3.35. [257]

Department of Industry, Industry, Education and Management—a Discussion Paper, Pp. ii+78. (London: Department of Industry, 1 Victoria Street, SW1, 1977.) [257]

British Nuclear Fuels Limited. Sixth Annual Report and Accounts, 1976/1977. Pp. 31. (Risley, Warrington: British Nuclear Fuels Limited, 1977.) [267]

Making Success Happen: Enterprise and Change in British Chemicals, Pp. 8. (London: Chemical Industries Association Limited, 93 Albert Embankment, SE1, 1977.) [267]

Chemical Crosswords: a Learning Aid for Chemistry

Students, 20 crosswords. By A. G. Hudson and S. Hind. (Tettenhall, Wolverhampton: Sigma Technical Press, 23 Dippons Mill Close, 1977.) £1. [267]

British Gas Corporation. Annual Report and Accounts, 1976/1977. Pp. 72. (London: British Gas Corporation, 59 Bryanston Street, W1, 1977.) [267]

The Association of the British Pharmaceutical Industry. Annual Report, 1976/1977. Pp. 44. (London: The Association of the British Pharmaceutical Industry, 162, Regent Street, W1, 1977.) [267]

Proceedings of the Royal Society of London, A: Mathematical and Physical Sciences, Vol. 355, No. 1683: A Discussion on New Particles and New Quantum Numbers. Organized by R. H. Dalitz, FRSE, B. Richter, B. Wiik and W. T. Toner. Pp. 441-637+1 plate. (London: The Royal Society, 1977.) UK £4.85; Overseas £5. [277]

Social Science Research Council. Energy Topics in the Social Sciences: a Report to the Research Initiatives Board of the Social Science Research Council. Pp. 13+ix. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1977.) [277]

The Macaulay Institute for Soil Research. Annual Report for 1975/1976. (No. 46.) Pp. 134. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1977.) [277]

Memoirs of the Royal Astronomical Society, Vol. 84, Part 1: Accurate Position Measurements and Optical Identifications for Radio Sources Selected at 966 MHz. By A. M. Cohen, R. W. Porcas, I. W. A. Browne, E. J. Dainton and D. Walsh. Pp. 1-44. (Oxford and London: Blackwell Scientific Publications, 1977. Published by The Royal Astronomical Society.) [287]

Reports and Publications

Other countries—July

Prospective et Santé, No. 1. Revue Trimestrielle. Pp. 1-141. Rédacteur en Chef: Michael Salomon. (Paris: *Prospective et Santé*, 5, rue Clément Marot, 1977.) [17]

Smithsonian Contributions to Zoology, No. 232: The Pierid Butterflies of the Genera *Hypochilota* Ureta, *Phulia* Herrich-Schäffer, *Ingraphula* Field, *Pierphulia* Field, and *Piercolitis* Staudinger. By William D. Field and José Herrera. Pp. iii+64. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [17]

United States Department of the Interior: Geological Survey. Professional Paper 880: Geology of the Apollo 14 Landing Site in the Fra Mauro Highlands. By G. A. Swann, et al. Pp. vii+103+plates 1-7. (Washington, DC: US Government Printing Office, 1977.) [47]

National Research Council Canada. NRS Associate Committee on Scientific Criteria for Environmental Quality. The Effects of Alkali Halides in the Canadian Environment. Pp. 171. (Ottawa: National Research Council Canada, 1977.) \$2. [47]

Geological Survey of Canada. Bulletin 281: Taxonomy of Upper Jurassic—Lower Cretaceous Microplankton from the Richardson Mountains, District of Mackenzie, Canada. By W. W. Brideaux. Pp. 89 (16 plates). (Ottawa: Geological Survey of Canada, 1977.) \$5. [47]

World Armaments: The Nuclear Threat. Pp. 39. (Stockholm: Stockholm International Peace Research Institute, 1977.) [57]

Development and Evaluation of a Hexokinase/Glucose-6-Phosphate Dehydrogenase Procedure for Use as a National Glucose Reference Method. By James W. Neese, Patricia Duncan, David Bayse, Mary Robinson, Teresa Cooper and Charles Stewart. Pp. vi+147. (Atlanta: US Department of Health, Education and Welfare. Public Health Service, Center for Disease Control, Bureau of Laboratories, Clinical Chemistry Division, 1976.) [57]

European Organization for Nuclear Research—CERN. Annual Report, 1976. Pp. 177. (Geneva: CERN, 1977.) [57]

Bulletin of the American Museum of Natural History, Vol. 158, Article 5: Stratigraphy and Biostratigraphy of Late Cenozoic Deposits in Central Sioux County, Western Nebraska. By Morris F. Skinner, Shirley M. Skinner and Raymond J. Gooris. Pp. 263-370. \$7.60. Vol. 158, Article 1: A Revision of the Spider Genera *Herythys* and *Scotophagus* (Araneae, Gnaphosidae) in North America. By Norman I. Platnick and Mohammad U. Shadab. Pp. 1-44. \$2.80. (New York: American Museum of Natural History, 1977.) [67]

Annals of the South African Museum, Vol. 73, Part 2: Relationships of the South African Fossil Frog *Euxenopoides reumigi* (Anura, Pipidae). By Richard Estes. Pp. 49-80. R.3. Vol. 73, Part 3: Cretaceous Deposits Near Bogefels, South West Africa. By Herbert Christian Klinger. Pp. 81-92. R.1.90. Vol. 73, Part 4: Deep-Sea Amphipods from West of Cape Point, South Africa. By Charles L. Griffiths. Pp. 93-104. R.1.90. Vol. 73, Part 5: The South African Museum's *Meiring Naude* Cruises. Part 3: Hydroids. By N. A. H. Millard. Pp. 105-131. R.2.80. Vol. 73, Part 6: The South African Museum's *Meiring Naude* Cruises. Part 4: Echinoderms. By Ailsa M. Clark. Pp. 133-147. R.2.20. (Cape Town: South African Museum, 1977.) [77]

United States Department of the Interior: Geological Survey. Professional Paper 1018: Characteristics of Thin-Skinned Style of Deformation in the Southern Appalachians, and Potential Hydrocarbon Traps. By Leonard D. Harris and Robert C. Milici. Pp. iv+40+9 plates. (Washington, DC: US Government Printing Office, 1977.) [77]

International Development Research Centre, Ottawa. Agriculture, Food and Nutrition Sciences Division: The First Five Years. Pp. 48. (Ottawa: International Development Research Centre, Box 8500, 1977.) [87]

nature

22 September 1977

Room at the top — for whom?

REPORTS on the state of British industry, its use of qualified scientists and engineers, the attitude of young people towards an industrial career and recipes for supposed improvements are now descending on the office in such profusion that we can no longer guarantee even to collect them from our harassed reception before dispatching them, bundled in their thousands, to a company warehouse somewhere outside Basingstoke, there to be pulped. Two recent arrivals, however, somehow crept under the door and so have at least been afforded a cursory glance by our jaded editorial staff. They are 'Education, engineers and manufacturing industry', a report to the British Association for the Advancement of Science by a small investigating team, and 'University-industry relations', the government's reply to some recommendations made by the Common's Select Committee about a year ago.

At that time, we were critical of several of these recommendations, which seemed rather poorly worked out, at least in the case of the proposed revival of the concept of SISTERS, Special Institutions for Scientific and Technological Education and Research, a step in the wrong direction of isolating science and technology from the broader world. The government's response is equally unenthusiastic, indeed its paper as a whole reads like a long succession of "we don't think this is a good idea", "we think this is a good idea and it is already happening", "we think this is a good idea but someone else should do something about it". The someone else is, of course, industry.

But one recommendation commended to industry is not so easily shrugged off. The Select Committee had said that industry should do something about offering attractive salaries and moving qualified personnel in to senior management with the ease that it is done in Germany, France and the United States or by their British contemporaries who are lawyers or accountants. A good generalised knockabout, but the British Association report puts it in much clearer perspective. It is fairly widely known that on an international comparison, British median salaries are low at the shop floor level and get relatively lower the more responsibility is

assumed. A new survey, released in part to the British Association, looks at the way that even these inferior salaries discriminate against the scientist and engineer in productive industry. Median figures (for October 1975) for about 22,000 professionals show that at all ages accountants and lawyers get paid the most, engineers and scientists the least. Even at 25 the accountants and lawyers are £1,000 ahead; by 40 their median is £9,500, that of arts graduates £8,500, that of scientists £7,500 whilst engineers are commanding a mere £6,600.

Maybe the average non-scientist in industry really is much smarter and earns his or her differential, but no-one yet seems to have said so explicitly. More likely the growing differential reflects the expanding opportunities open to the non-scientists as time goes on. More opportunities mean more rounded employees, soon to be fit for even more opportunities. Less opportunities mean more type-casting as narrow minded characters fit only for the backroom and the sales department.

The matter is not so easily laid only at industry's door, however. The government itself employs tens of thousands of qualified scientists and engineers. Their upward path until the age of 40 is fairly well-defined and somewhat better paid than in industry. But thereafter, stagnation. Ten years ago the Fulton report on the Civil Service, recognising the problem, proposed an 'open' structure at the top of the service to encourage some cross movement among real high fliers. In practice this has meant little. Certainly laboratory directors are at least nominally in the open structure, but when one asks about other scientists and engineers who have made the move into more general high level management, the same name or two keep cropping up. Whether the waste of intelligent human resources would be ameliorated by extending the open structure further down the service or by making it easier for the frustrated to retire earlier, who knows? But at the moment the government is in no strong position to commend industry to take more scientists and engineers into senior management when it shows so little inclination to do so itself. □

Refusnik scientists: keeping science alive

Max Gottesman, from the Laboratory of Molecular Biology at the National Cancer Institute at NIH, was one of ten Western scientists who attended the special four-day Jubilee meeting of the refusnik Sunday Seminar in Moscow earlier this year; the seminar dissolved itself for a 'summer recess' in May following official pressure, and reconvened again last Sunday under Viktor Brailovskii. Below, his views of the refusniks' position

FIVE years ago, the first of the refusnik Sunday Seminars on Collective Phenomena met in a Moscow apartment. Refusniks, unlike dissidents who wish to change Soviet Society, are Jews who wish to leave Russia, usually for Israel. In the case of refusnik scientists, visas to emigrate are often refused on the grounds that the applicant is in possession of "classified information". Not only is their emigration forbidden, but their official scientific life is terminated. They are expelled from their laboratories and denied library privileges; even their telephones are removed. The cut-off is usually very effective: none of Professor Benjamin Levich's colleagues, for example, nor any of the students he had trained over the years, spoke to him after he applied to emigrate.

In an attempt to stay alive scientifically, the refusniks meet together in someone's apartment each Sunday afternoon to discuss science. These meetings have come to attract Western scientists invited to Soviet-sponsored conferences, and they present papers; sometimes establishment Soviet scientists discretely listen to the presentations while remaining in the kitchen. But the special Jubilee meeting held to mark these five years was also accompanied by the constant presence of KGB men stationed outside Dr Mark Azbel's apartment, where the seminar was held.

They followed the Western scientists wherever we went. Usually consisting of four men in a taxi, they made no attempt to disguise the fact that we were being followed; they were there clearly to intimidate us. One afternoon, a truckload of soldiers unloaded below Azbel's window and lounged around several hours before leaving. A parked van, left with the motor running and no driver, contained the monitoring devices used by the KGB to follow the conversations in the apartment. Between the sessions, however, matters of a non-scientific nature were discussed, and from these talks and exchanges of written notes I obtained a picture of the present status of the refusnik scientists.

How the refusniks manage to survive varies from case to case. Some had accumulated savings before applying for an exit visa. Of course a long five- or six-year period of refusal cannot be prepared for in this way. During refusal, they find occasional work, sometimes as manual labourers and sometimes as tutors. Unfortunately tutoring positions tend to be transitory; a discrete call from the KGB to a family employing a refusnik tutor usually has the expected effect.

It is clear that the scientists who have entered into refusal have a common motivation: to escape the severe and worsening anti-semitism in Russia. The Russians wish to exclude Jews from the sciences and other professions. At the present time, it is difficult or impossible for a Jewish student to enter a first-rate university. An example: the medical school in Odessa, a city with a large Jewish population, has not a single Jewish student. Thus many Jews are driven to emigrate to assure their children equal educational and employment opportunities. Current Soviet anti-semitism comes after a long period of relative tolerance. A generation of Jews has been assimilated, and the refusniks find themselves without an inheritance of Jewish culture.

They are anxious to regain their heritage—and to learn Hebrew—in the face of severe pressure from the authorities. It is no accident that the two refusniks currently in prison, Joseph Begu and Anatolii Shcharanskii, were the most active in disseminating Jewish culture. And yet, in spite of the threats, there were several refusniks at the seminar I attended wearing Israeli pins in their lapels.

Why don't the Soviets just let the refusniks emigrate? In fact, some are permitted to go; but the rate is slow, and getting slower. The Soviets fear that an easy emigration policy will result in Jews as well as other national groups demanding, *en masse*, to emigrate, thus threatening the stability of the State. Also, the Jewish scientists have a special problem. The justification of the Soviet system is 'scientific'; Marxist-Leninist analysis of history and economics has proved, in a manner as certain as $E = mc^2$, that Soviet society is structured correctly. For a scientist to desire to emigrate is unthinkable. It should be mentioned that the only valid reason for leaving Russia is to rejoin one's family abroad.

What can be done? In view of the fact that the Soviets greatly value scientific contacts with the West, certain courses of action are feasible. In particular, international meetings and exchanges can be used to help the refusniks. Refusnik scientists wish to participate in these open meetings and could be invited. If such participation is denied by the authorities, Western organisers should consider either the possibility of moving the meeting from Soviet territory, or of limiting Soviet participation in meetings held in the West. In the case of the International Genetics Conference, to be held in Moscow in the summer of 1978, a precedent has been set. A prior conference was moved from the United States to Canada in the 1950s when the US State Department refused to issue visas to certain foreign scientists.

Publicity is essential to keep the refusniks from simply disappearing. Official Soviet visitors should be reminded that the treatment of refusnik scientists threatens Soviet-Western exchange programmes. Since visitors are invariably debriefed by the KGB on their return, this is an effective means of reaching the Soviet authorities. It should also be remembered that the critical decision as to whether a refusnik's work is 'classified' is made by his departmental superiors, or the chairman of his department. These are often the very scientists who are sent to Western conferences and exchange programmes.

Finally, it should be stressed that scientists visiting Russia are warmly welcomed at the Sunday Seminar, and are doing a great service to the refusniks by attending. For addresses and other information contact the Committee for Concerned Scientists, 9 East 40 Street, New York, New York, 10016. □



Sunday Seminar scientists in November 1976

Tethering the tide

T. L. Shaw examines the prospects for harnessing tidal energy in the Severn Estuary

FACED with a possible world fuel shortage, the UK Department of Energy (DEN) is taking stock of domestic energy sources. Together with industry, it has already provided money for scientific and technical studies on the contribution four 'renewable' sources—wave, solar, geothermal and wind—could make to meeting future demands. It is now looking at the part tidal power could play.

The DEN's recently-released Energy Paper number 23, which discusses three commissioned reports on harnessing tidal power in the Severn Estuary, concludes that although tidal schemes are technically feasible, cost and the long time estimated for construction may rule them out. Nevertheless, studies to identify one or more schemes for wider appraisal are now being seriously considered.

The tides have the particular advantage of far greater energy density than the other renewable energy sources. In the Severn Estuary, for example, tidal energy is thirty times denser than power derived from average waves on the UK's Atlantic seaboard. Individual tidal schemes could produce many thousands of megawatts of electricity but, for maximum benefit, they would have to be integrated efficiently with other (essentially thermal) generating plant.

This enormous potential could be harnessed by building a dam to separate part of the coastal inlet from the sea. Turbines that might resemble conventional hydro-electric propeller machines would be set in fully sub-

merged tubes passing through the barrage. A difference in water level either way across the barrage (during flood and ebb tide) would create flow through these tubes and so an electrical output from the slowly rotating turbines.

In the Severn Estuary, the UK has perhaps the best site in the world for producing electricity from tidal power. At least 10% and as much as 20% of the UK's present electrical needs could be met from a barrage either within the estuary (10%) or seaward in the Bristol Channel (20%). These percentages are comparable with those currently supplied by nuclear installations and oil and gas-fired stations respectively.

But this is not simply a case of one electrical source competing against another. Each has characteristics which influence its most efficient hence preferred use: nuclear stations, for example, should run continuously, whereas gas turbines are best run in short bursts at times of high demand. The output of aerogenerators is fairly regular over a period of hours and maybe days while that of wave generators may be consistent on an even longer scale. Tidal output suffers from being intermittent but it is accurately predictable. There is no reason, however, why each source, provided that it can be commercially developed with respect to its operating value, should not contribute to the total generating network.

Recent studies

Last year the DEN commissioned two investigations into aspects of the Severn barrage. The questions the investigations set out to answer were: is construction and closure of a barrage

technically feasible? how long would it take to build? what would be the likely cost? and what would be the effect of the barrage and its operation on tidal range? They were issued respectively to the Netherlands Engineering Consultants Foundation (NEDECO), which has great knowledge in maritime affairs especially coastal protection, and to the UK Department of the Environment's Hydraulics Research Station (HRS), which, since it was established some 30 years ago, has worked extensively on coastal hydraulics.

For their studies both NEDECO and HRS chose to consider barrages in positions between Cardiff and Weston-super-Mare, where 10% of the UK's present electrical demand could be produced. Most attention was paid to proposals by Professor Eric Wilson of Salford University (the simple single-basin, tide-related scheme) and myself (the more complex two-basin arrangement providing storage and giving power by day as required. Summaries of each study are as follows.

NEDECO

In the absence of adequate field data, NEDECO was obliged to make a number of assumptions about hydrodynamic conditions at the chosen barrage site, in particular data describing wave climate. The serious lack of adequate geological and sedimentological information was met by surveys carried out by the UK Institute of Geological Sciences. It identified exposed limestones and marls over a much greater proportion of this part of the estuary than the previous scant scientific data had suggested. Localised fine deposits, mainly sand and shingle are thought to be shallow, implying a firm foundation throughout for a barrage.

Based on "the impressive experience obtained in the planning and execution of the Delta Works (in Holland) together with the immense increase in know-how and expertise of recent years in the offshore industry," NEDECO is optimistic that civil engineering aspects of the barrage could be carried out satisfactorily, though it appears to have underestimated the magnitude of the challenge successfully met by British industry of placing concrete caissons in 150 m of water in the remote and exposed North Sea, compared with the task of siting correspondingly much smaller units in no more than 30 m of sheltered water in the Severn Estuary.

But the siting of caissons for the barrage raises some problems not met in the North Sea, in particular the strong currents that accompany the

T. L. Shaw is in the Department of Civil Engineering, University of Bristol.



high tides. The positioning and sinking of caissons in currents exceeding 3 metres per second could prove hazardous, but given proper planning they could be placed during slack water 'windows' which occur frequently and predictably.

Doubts also exist on the accuracy of NEDECO's estimates of extreme wave heights at the chosen barrage site. Unfortunately those local measurements that have been made are for such short periods that the validity of statistical extrapolation is questionable. Another factor is the apparently sheltered position of the barrage compared with that of the one long-established wave recorder 100 km seawards. In the absence of other evidence, however, NEDECO's estimates cannot yet be dismissed.

Extra cost, NEDECO claims, will have to be incurred in building offshore-dykes to protect the turbines in the single-basin barrage from direct wave attack. If a two-basin scheme is adopted, however, the problem need not arise: the second basin would essentially serve the purpose of offshore dykes.

NEDECO estimates that the civil engineering task alone for the two-basin scheme will require four years of preliminary study and 16 years to build. This could be taken as sufficient to rule out the scheme on economic grounds. But it does agree that the timescale could be shortened, though with an increase in capital cost. By contrast, the UK construction industry feels that a timescale of about eight years, similar to that of most thermal plant, is realistic.

An alternative to help save on interest charges, would be to secure some energy from the scheme long before completion, increasing it as the scheme advances. The full output capacity of the scheme then would not be suddenly available to the network, and would permit efficient integration. Hence 16 years may not be unreasonable, though the various electrical merits of phased construction of a barrage are not yet clear; studies well advanced at the University of Bristol, funded by the Science Research Council, are exploring the possibilities. The concept of a two-basin barrage scheme developed first to operate in the 'single-basin' mode, as discussed earlier, may make good sense. NEDECO's study did not consider phased integration.

Hydraulics research station

The general lack of field data also made it difficult for HRS to define its problems, especially the seaward boundary conditions for its study's two-dimensional mathematical model.

Coastal and offshore tide modelling has moved a long way forward in the past five years, since it was recognised that local boundary changes can have effects at a considerable distance depending on their extent and the local tidal behaviour. Although earlier models, including that used by NEDECO, assumed that conditions at the seaward end of the Bristol Channel would not be unchanged by a barrage, HRS included the Celtic and Irish Seas in its model.

It is perhaps unfortunate that the 'large area' model of HRS predicts that the barrage will marginally amplify the tides whereas 'small area' models suggest the opposite. The right answer must be found, not least because, as matters now stand, the accuracy of both is under question.

But these doubts hide two aspects of the work of HRS. First, that it is something of a pioneering exercise in its own right and deserves recognition as such even if deficiencies later emerge. Secondly, an increase or decrease of tidal amplitude by up to 1 metre or so is sufficiently small in real terms to conclude at this stage that a barrage operating in the region Cardiff-Weston will not destroy the natural energy it is designed to harness. Although it must be a matter of some urgency to resolve why different mathematical models give different answers, and in particular to produce a model giving the right answers, it would only be wasteful to repeat these studies without knowing where the barrage might be sited, and understanding pump and turbine operation and induced oscillations (seiches) in the enclosed basin(s).

When reporting 'The situation in 1975', Energy Paper number 23 quotes that the Secretary of State's Advisory Council on Research and Development for Fuel and Power (ACORD) and the DEN came to the opinion that "in view of the uncertainties surrounding some of the aspects of the studies (of the barrage, as previously carried out by others), a more vigorous examination should be undertaken of certain key technical questions in order to clarify the situation further".

Of the three questions posed, the work of NEDECO and HRS should have answered two to the satisfaction of ACORD and the DEN. I hope that the third, namely "what would be the environmental consequences, and could they be quantified in cost terms?", will also be answered in similarly sufficient detail by the book *An Environmental Appraisal of the Severn Barrage* (second edition).

The next step is aptly stated by NEDECO: "The chance exists that we could contribute towards the

● The future for tidal energy in Britain has at last been clarified. There have been strong calls for further studies by the House of Commons Select Committee on Science and Technology in its fourth report published last week and at the Secretary of State for Energy's 'Forum on the Severn Barrage', held in Bristol the week before.

Although the potential of the tides and the consequences of a barrage are fairly well understood, gaps in knowledge remain. The Select Committee, which has come out in support of tidal energy ahead of the other renewable sources, proposes that an independent Severn Barrage Committee, composed of technical, financial and administrative experts, be set up to assess Severn Barrage schemes and deliver a studied opinion to the Secretary of State. It highlights the unresolved problems of choosing a barrage location and method of operation and suggests that other studies should also be done. Outline costings, provisional construction methods and timescales should be sought by a "fairly early date . . . to command general agreement".

The Secretary of State's 'Forum' provided the DEN with first-hand evidence on most of the many relevant topics, for example energy cost/benefit estimates, environmental consequences and the widespread employment, industrial, commercial and recreational merits of the project. Though opposition was scarcely evident, many speakers were cautious because so much remains unclear. Summing up Mr Benn, the Secretary of State, said that all relevant interests must be represented in the next important phase of project definition and that a staged approach allowing for reappraisal of points was essential. (The case for a pilot installation has wide support.)

While the DEN may not altogether deserve the Select Committee's criticism of being "excessively timid" about tidal energy, its scale of research funding must increase 100-fold if these unified calls for action are to be answered. The national benefits at stake ought to make it easy for a cabinet to persuade the government to provide the still-modest sum now needed.

creation of a very clean type of energy production, the value of which can only increase because of its increasing scarcity on a worldwide scale". British industry is ready to contribute towards these investigations.

The capital cost estimate of up to £4,000 million quoted in Energy Paper number 23 might imply an expensive power station, but current unit costs for other plant tend to refute this. However, simple comparisons are dangerous when like objects are not being equated. It is much better that the next phase of study of the barrage should be carried out to identify its real electrical (and other) benefits before economic judgments are reached. A comprehensive but objective programme for the next 18 months is needed. □

OTS

Second chance

Last week, Europe's Orbital Test Satellite was destroyed shortly after launch. Judy Redfearn reports

EUROPE'S hope for a sophisticated system of communications satellites by the early 1980s might well have gone up in a cloud of smoke with the European Space Agency's (ESA) Orbital Test Satellite (OTS) last week, had it not been for ESA's foresight. In the wake of two mishaps by NASA earlier this year—its failure to launch Geos into the correct geostationary orbit and an accident with a Delta launcher which meant that OTS could not be launched in June as planned—ESA made sure that it went ahead with a plan to take out insurance cover for the launch of OTS and the integration of a second flight model. The insurance, to the tune of \$29 million at a premium of about \$2 million, was placed by Bowring of London throughout all the countries taking part in the project in proportion to their financial commitment to it.

As fate would have it, ESA's precaution was extremely wise. Seven miles above Cape Canaveral's main launch pad and only 54 s after a seemingly perfect launch, the Delta 3914 carrying OTS began to disintegrate and exploded just prior to a signal of destruction. The insurance cover, however, gives Europe

another chance. As with all satellite missions, ESA built a second OTS identical to the first which could be ready for launch by next March. This means that the OTS test and experimental programme could still be compressed to be completed within the original time.

A second chance is particularly important for the OTS because, unlike Geos, it has considerable commercial potential. It is Europe's first communications satellite and is the forerunner of four similar satellites which are planned for launch by Ariane, Europe's own launcher which is still being developed, between 1981 and 1990. They will carry a large part of intra-European telephone, telegraph and telex traffic and will relay European television programmes. The OTS's task is to test much of the equipment which will be used on the operational satellites and to provide a pre-operational European traffic capacity.

The decision on whether or not to go ahead with the second OTS launch early next year will probably be taken by ESA officials this week. As the only launcher available to do the job is the American Delta 3914, they are probably hoping for some assurance from the Americans that a similar accident is extremely unlikely to happen again. Although the success rate of Delta launchers is very good—134 have been launched since 1966, 122 of them

successfully—the history of the Delta 3914 is not so happy. Three of them have been launched and the OTS marks the first failure: but the launcher which was damaged in May this year and caused a three-month delay to the OTS programme was also a 3914. In that case, one of the booster rockets fell off the main Delta vehicle because of a defective bolt.

Late last week, NASA set up a seven member team of inquiry to look into the incident. It hopes to find out whether or not the fault lay in the basic Delta vehicle or the attached rockets before 13 October, the planned launch date of the International Sun-Earth Explorer (ISEE) in which ESA has a large interest. If the fault is found in the Delta 3914's strap-on rockets then it should be safe for the ISEE to go ahead as planned as the Delta 2914, on which it will be launched, uses a different rocket configuration.

Until late 1980 when the Ariane launcher is ready for use, ESA will have to launch its satellites on US vehicles. By the early 1980s, however, there may be another option if a West German company, Orbital Transport- und Raketen-Aktiengesellschaft (OTRAG), is successful in developing its own heavy launch vehicle. So far, it has spent \$30 million on the project and hopes by 1981 to be launching satellites from Zaire. ESA will not discuss the possibility of using OTRAG launchers until it knows whether they, and Ariane, will work. □

BRITAIN

Timely delay

The Council for Science and Society has recently published a report called The Acceptability of Risks. Alastair Hay reports

IN spite of a 12-month delay, the Council for Science and Society's report, *The Acceptability of Risks** published last week has arrived at an opportune moment: the explosion at Flixborough, and the release of tetrachlorodibenzo-p-dioxin at Séveso are recent memories, and the public enquiry at Windscale is still in progress. Its findings and recommendations will certainly provoke heated debate.

Risks, it claims, are very unevenly distributed; those in the UK at greatest risk are "concentrated in the homes,

communities and workplaces of manual workers and their families". In a harsh message, the report states that positive action to remove this inequality is urgently needed, and that it regrets that "hazards of all sorts have been shamefully neglected in scientific and technological research".

The report also points out the potential danger of regulatory agencies being weakened and rendered ineffective by financial constraints and inappropriate standards. Indiscriminate use of the terms 'safe' and 'acceptable' are to be avoided, it says, as both are ambiguous and subject to a wide range of individual interpretation.

Appendices deal with specific issues such as nuclear radiation, ammunition and explosives, the hazards of asbestos, and the Flixborough Court of Enquiry. In a disturbing commentary on the conduct of the Flixborough court investigation, one of the authors of the

report, Brigadier R. L. Allen, claims that the enquiry was too narrow; that some relevant evidence was not considered; and that statements by the court to the effect that "Nypro [owners of the Flixborough plant] were safety conscious" were inappropriate in the light of the evidence presented. He says that this is illustrated by Nypro's breach of licensing arrangements when it stored 43 times more inflammable fluid on site than it was entitled to do: a fact which severely hindered rescue operations after the initial explosion.

In conclusion, the report recommends areas of investigation to be considered by working groups and which could provide useful information for 'risk management' in the future. Also suggested is the possibility of the appointment of 'risk advisers' in particular occupations and localities.

A few of the abuses the report refers to have been dealt with during its delay at the printers. Few will consider this more than a minor fault. □

**The Acceptability of Risk* is available from Barry Rose (Publishers) Limited, Little London, Chichester, Sussex, £5.00.

USA

Battling against the inevitable

Years of hitherto unsuccessful effort in the US Congress have culminated in a bill providing for an increase in research on earthquakes. Chris Sherwell reports from Washington

DEVELOPMENTS now reaching a climax in Washington may not do for earthquake research what previous much-publicised efforts did for space and cancer research, but the comparison may not be entirely inappropriate. The fanfares may be absent and the vistas uncertain, but an agreed commitment in principle which also has legislative form now promises a long-awaited boost for work in an exciting area and marks an important move from disaster relief to disaster prevention.

The latest step along the tortuous path to a comprehensive coordinated programme to reduce the hazards posed by earthquakes came last week when the House of Representatives approved by a margin of 229 votes to 125 an authorisation bill on which two House committees had reported. The bill was due to go back to the Senate late last week for consideration of the minor differences from the Senate version

passed earlier this year, and was expected to find its way to President Carter's desk for signature shortly thereafter.

The bill, which when enacted will be known as the Earthquake Hazards Reduction Act of 1977, is designed to reduce the risks to life and property of future earthquakes in the United States. The logic underlying it is simple: earthquakes are inevitable, and population increases and urbanisation enhance the dangers they pose. To minimise both the risks and potential losses, a coordinated programme to increase research and to help apply its findings and generally improve the readiness of vulnerable areas for emergencies is necessary. The bill creates a \$215-million three-year federal programme to do just that.

Though expenditure is scheduled to begin at the start of the coming financial year (fiscal year 1978) beginning on 1 October, the money must still be appropriated in a separate process. In the meantime the agencies slated to use it must rely on appropriations already granted them for that period. The two major agencies involved—the National Science Foundation (NSF) and the US Geological

Survey (USGS)—expect to feel the improvement over recent years quite soon, however, because the appropriations already granted are based largely on the same formulation used in drawing up the bill.

That formulation was contained in a report of a joint advisory committee chaired by Dr Nathan Newmark and published a year ago. Entitled *Earthquake Prediction and Hazard Mitigation: Options for USGS and NSF programs*, the report outlined low, intermediate and high levels of support for earthquake research, all of which were higher than funding levels in past years. The intermediate level, which President Ford used in his proposed budget for fiscal year 1978 and which President Carter retained in his revisions, provided for expenditure of nearly \$54 million, an increase from \$22½ million the previous year. This was split approximately 52 : 48 between NSF and USGS (in favour of NSF). Under the bill passed by the House, some \$55 million would be split 50 : 50.

Going by the breakdowns given in the Newmark Report, the two agencies would each spend about 10% of their 1978 allocations on fundamental studies of the causes and mechanisms of earthquakes. NSF does not expect to see any great difference in this area, but on the engineering front, which

No long encounter with Halley's Comet

THE National Aeronautics and Space Administration (NASA) has reluctantly abandoned an ambitious and expensive plan to conduct a close-range study of Halley's Comet as it swings around the Sun in 1986. The plan would have entailed launching a spacecraft in 1982 on a complicated journey, culminating in an extended rendezvous with the comet three and a half years later.

According to NASA officials, development of the spacecraft together with a new propulsion system to manoeuvre it into Halley's orbit would have cost \$500–600 million. In particular, considerable outlays would have been needed in the next two years to make the 1982 launch date, and NASA simply doesn't have enough money in its budget.

Consequently, the agency has been forced to drop the idea of paying an extensive visit to Halley's Comet, and it is now looking into less expensive plans to launch a spacecraft to make a brief encounter with the comet soon after it emerges from behind the Sun. In addition, NASA is con-

sidering the possibility of developing a spacecraft to make a lengthy visit to the comet Encke in 1987.

The decision to forego the chance of a long encounter with Halley's Comet will be a major disappointment to many space scientists. Comets have recently come under serious study because they may provide some clues to the origins of the universe. According to one widely-held theory, they are like giant dusty snowballs, made of condensed matter from outside the solar system. As they swing past the sun, heat vaporises material from the comet's nucleus, which then forms the so-called tail.

A trip to Halley's Comet, culminating in an extensive visit, would have been a difficult proposition, however. The chief problem is that the comet orbits the Sun every 76 years in the opposite direction from the Earth, and a complicated series of manoeuvres would be required to bring a spacecraft to the same path and speed as the comet.

For the past year, NASA has been looking into two propulsion systems

which could provide the required manoeuvres. The first, a so-called solar sail, would have consisted of an immense plastic sheet, deployed from the spacecraft on booms, which would act like a sail in the solar photon stream. The second idea, an ion drive system, would involve the ionisation of mercury vapour, which would then stream out of the back of the spacecraft to provide thrust. Last week, NASA announced that it has chosen the ion drive system which will be developed for a possible mission to visit Encke in 1987 and which will also be used to power future interplanetary probes.

Though the plan to rendezvous with Halley's comet has been abandoned, NASA may still launch a spacecraft in 1985 to fly close to the comet and pass through its tail. Such a mission would not require a special propulsion system because complicated manoeuvres will not be required to match orbits. It would consequently be much cheaper, but the scientific benefits would also be much less.

Colin Norman

would take some 60% of the NSF allocation, research funding will almost double in the coming year. This effort will focus on the design, planning, construction and use of various man-made works to resist earthquakes. Another 20% of NSF funds would probably go to related socio-economic and policy areas.

On the same basis, USGS is likely to devote about half its allocation to the development of methods to predict the time, place and magnitude of future earthquakes. Another 35% or so would go on assessing earthquake hazards, while a small amount would be spent on studies of artificial earthquake inducement.

As for fiscal years 1979 and 1980, the 50:50 split between the two agencies would continue for budgets totalling \$70 million and \$80 million; the breakdown could be expected to follow broadly the same pattern. If the patterns of spending are predictable, however, the scientific results are not. Recent scientific progress in the field has probably assisted the bill's passage; reliable earthquake prediction seems more likely than ever before.

Of equal importance for the bill, however—apart from an unsubstantiated sense that more earthquakes than usual have struck in recent years—was the support given by the Carter Administration, notably by the Office of Science and Technology Policy in the White House, which is headed by the President's science adviser, Frank Press, himself a geophysicist. Indeed, the bill reflects many of the Administration's preferences. When it came out of the House Interior and Insular Affairs Committee, under which USGS falls, the important difference from the bill it received from the House Science and Technology Committee, and which put it in line with the Senate version, was that it contained no specific institutional proposals. The bill it received had suggested an Office of Earthquakes Hazards Reduction, a National Advisory Committee, and an Earthquake Prediction Evaluation Board.

Given the President's desire for flexibility while pursuing plans for government re-organisation, these suggestions were premature. Accommodatingly, the bill thus provides simply for designation of a "lead agency", and the usual establishment by the President of roles and goals.

On Capitol Hill not all reactions

Controlling technology flow

QUESTION: how does a technologically and militarily sophisticated superpower, which is committed to free trade principles, contain the contribution that its much sought-after exports might make to the military capability of potential adversaries?

Answer: it's a problem. The latest round in a recurring debate in the United States on the matter came immediately before the Labor Day weekend at the end of August, when the Secretary for Defense, Harold Brown suddenly released a five-page memorandum sent to various officials in defence-related agencies.

Cast as an interim policy statement on controls over the export of US technology, the memorandum draws on the recommendations from the Defense Science Board Task Force, published last year as the so-called Bucy Report (see *Nature*, 15 April 1976). This indicated that controls over the flow of strategic or "critical" technology to Communist countries had broken down in recent years, and suggested that the United States should refine the list of relevant technologies and apply sanctions where they were unwarrantedly passed on, directly or otherwise.

The "interim internal guidance" which the Brown memorandum provides for the Department of Defense (DOD) covers exports both to allies and to potential adversaries. It says DOD will support the transfer of critical technology to countries with which the US has a major security interest where this can strengthen collective security, contribute to NATO standardisation and enhance the return on R&D. But with both allies and other non-Communist countries, DOD will also assess the recipient's "intent and ability to prevent either the compromise or the unauthorised re-export of that technology", relying on the intelligence and security communities to help discover any breaches. Violations would result in sanctions.

Regarding exports to potential adversaries, a "presumption for recommending disapproval" will operate where these involve a revolutionary advance in defence-related technology. But where they involve end

products not of strategic importance or with virtually unextractable valuable technology, DOD will normally recommend approval. Either way, the key consideration would be the recipient's military capability.

According to the memorandum, the Department of Defense will be asking the Commerce Department to change present regulations so that exports of critical technology to all countries would require a valid licence; DOD will also recommend a streamlining of application procedures to minimise delays. In addition, DOD will suggest that the State Department negotiate new measures to control the flow of technology to Communist countries with the Consultative Group Coordinating Committee (COCOM), which is a group of NATO countries and Japan.

The primary objective in all this, of course, is to protect US lead times in the application of technology to military capabilities. The hope is that this will be more readily achieved through an emphasis on technology rather than end products, and by having the policy cover all countries using faster procedures. The provision allowing for DOD "recommendation" will help here; so too will the plan to maintain a continuously updated list of critical technologies. No indication was available, however, regarding the timetable for a fully national policy; that will bring in other government departments.

In the meantime, one minor worry remains. A covering letter to the Bucy Report described, as potentially an "area of concern", the scientific exchange agreements under which scientists move between the USA and the Soviet Union. The Brown memorandum does not take this matter up in any real detail, but says that when the potential for "inadvertent transfer" is high, DOD will recommend restrictions "on the amount, extent or kind of interpersonal exchange". Such exchanges are already subject to certain procedures and regulations, however, and the Head of the Exchanges Office at the State Department says the memorandum implies no changes.

Chris Sherwell

Colin Norman leaves Nature this week to go to the Worldwatch Institute in Washington. He has been our Washington correspondent for about six years, having previously worked in the London office. With his departure the journal loses a distinguished observer of the scientific scene.

were positive. Senator Alan Cranston of California, who has sought earthquake legislation for five years, naturally welcomed passage of the bill as a "historic step"; last year he saw his own bill, already passed by the Senate, die in the House at the end of

the session. But in the House a Maryland Republican was more caustic: a Congress which could not detect an inflationary impact in spending a quarter of a billion dollars, he suggested, "could not possibly detect an earthquake". □

PAKISTAN

Voice for science

Academics and industrialists have been discussing the role of R&D in Pakistan. Azim Kidwai reports

PAKISTAN has remained in a state of political flux since the general election last March when Mr Zulfikar Ali Bhutto's Peoples' Party won a doubtful victory. The army takeover and the introduction of martial law in early July has also had its sobering effects; but in the face of this and the prospect of another, perhaps emotional, election next October, Pakistan's R&D effort seems unusually resilient.

This is reflected in the fact that the consensus reached by important representatives from education, science, technology and industry at the National Seminar on Cooperation between Universities, Research Institutes and Industry, recently held in Karachi, may have significant effects on the promotion of R&D in Pakistan. The seminar was unique in that it was organised by the Federation of Pakistan Chambers of Commerce and Industry and was attended, among others, by three vice-chancellors, the Chairman of the Pakistan Council of Scientific and Industrial Research, the Secretary of the Ministry of Science, several heads of research institutes and many leading industrialists. It was, in fact, a sequel to a UNIDO Adhoc Expert Group meeting on the same subject, which was held in Vienna last December and in which Pakistan participated.

The Karachi moot, however, reflected new trends in Pakistan one of which was that the private sector of industry, which had hitherto been largely apathetic towards scientific and industrial research, had started showing awareness of its importance. A fruitful outcome of the three-day deliberations was the formation of a National Research Utilisation Board by the Chambers of Commerce and Industry, consisting of representatives from universities, research institutes and industry itself. It is to promote industries based on indigenous research.

The main bottleneck so far in making use of research has been the absence of any proper mechanism to rope industry into the R&D effort. The entrepreneurs in industry have largely settled for 'turn-key' jobs on proven products and for technology developed by foreign firms. The element of challenge and the calculated gamble in using products and processes developed locally has rarely been acceptable to

the industrialist.

The position now appears to have changed considerably with the tight balance of payments making it imperative to exploit indigenous raw materials and local technological talent. The dearth of foreign exchange has had its repercussions on the industrial sector. For the first time, industrialists are coming round to the view that the limited resources of the country call for an organised approach based on self-reliance and for the mobilisation of the meagre R&D resources. The technology which will be applied, however, will need to be appropriate to the physical, human and capital resources available.

Until now, there have been two weak points in the structure. The universities have been dominated by academics and have contributed almost nothing to applied research and the development of industry; and there has been a lack of interest on the part of industry in the government's R&D effort. The recommendations which came out of the seminar are for more collaboration between industry and research institutions and industry and the universities.

Another suggestion to be accepted at the seminar was that research and development centres should be gradually created within industrial enterprises and that the R&D function should be part of feasibility studies and the design of new large plants. To promote applied research in the universities, the Universities Grants Commission and the Pakistan Science Foundation are likely to give liberal financial support for collaborative research, while the Government may help by providing industry with incentives, such as tax exemption, for sponsoring research projects. Research in the universities suffers from inadequate funds; if more money were available, they could work for technological progress as well as pure knowledge.

Dr Salimuzzaman Siddiqui, a chemist, stressed the point that pure and applied research cannot easily be demarcated. So far as problem solving is concerned, priorities cannot be allocated under rigidly defined categories and there should be a massive but carefully phased multidisciplinary programme. Most of the participants felt strongly about another point raised by Dr Siddiqui, namely that Pakistan was spending barely 0.2% of its gross national product on R&D compared to 2-4% in many advanced countries: there was need for a major change. □

CHINA

Desert song

LIKE the United Nations, which organised an intergovernmental conference on desertification in Nairobi earlier this month, China has been taking an interest in the problems of desert control. Over recent years two national conferences on desert control have been held and several major works on aspects of desertification have been published, including 'An outline of Chinese deserts' and 'Summary of mass experience in China's desert control'. Two further works, 'Control of sand destructive to farmland' and 'Protecting railways against sand' are to be published soon.

Using the findings reported in these publications as guidelines, prefectures, counties and communes in six provinces and autonomous regions have drawn up their own plans to combat or even reverse desertification taking into account local conditions. The now established causes of desertification—unplanned irrigation, excessive tree felling, overcultivation, overgrazing, with drought being a condition but not a fundamental cause—are supported in ancient Chinese records. Measures taken to tame deserts include:

- planting wind resistant shrubs on the windward side of land threatened by encroaching deserts and tall trees near farmland. When the wind blows at average speeds, the length of the protected area is some twenty times the depth of the forest shelter belts. Criss-crossing tree belts in oases provides further protection against the wind.

- creating new oases by building reservoirs to store seasonal flood water from streams and lakes and using melted snow from the mountains or underground water to irrigate the reclaimed land before planting trees to protect it.

- developing pasture land for animal husbandry in deserts with fixed sand dunes or only interspersed shifting sand. Forage bases can be established on flat land in between sand dunes where water resources are relatively abundant.

Such measures are important especially as 11% of China's total land area, about one million square kilometres, is desert. Just over half of this is dune desert, the rest being gravel and other types. The Academia Sinica runs a desert research institute which studies desert distribution and type, the processes which form them, their natural conditions and ways of combating destructive sandstorms and using water and soil resources in sandy areas and in reclaimed land. It also publishes a quarterly journal *Desert Research*. In addition, the state has set up experimental forest farms and twenty demonstration stations for desert control. Many species of plants, which will survive in the desert, have been grown at the stations.

T. B. Tang

IN BRIEF

EEC report

ENERGY, uranium prospecting and the use of weedkillers containing dioxin were topics for questions last week at the European Parliament in Luxembourg. EEC Commissioner, Richard Burke, told the Parliament that the Commission is considering the possibility of including wind energy in the next research programme. The EEC already has plans for building a prototype plant to generate electricity from solar energy and there were suggestions for a comparable unit based on wind energy. However, it was unlikely, according to Burke, that wind energy could be more than peripheral because of the few areas within the EEC where

it would be suitable. Initial results for uranium prospecting in the EEC were reported as encouraging and those for Greenland as good.

There was concern over the use of weedkiller 2,4,5-T, which may contain traces of dioxin, the chemical released around the North Italian town of Seveso following an explosion at a chemical factory. The weedkiller is presently allowed in all EEC countries except Italy, Antonio Giolitti told the Parliament. Limiting its use should be considered following each item of information, he said. The Commission's proposed directive on harmonising safety regulations on dangerous manufacturing industry is to be discussed—

for the third time—by EEC government experts on 13 and 14 October.

Leakey in Europe

To mark the inauguration of its European chapter, the L. S. B. Leakey Foundation has endowed two post-graduate students at St John's College, Cambridge. Named after Dr Louis S. B. Leakey and based in the United States since 1968, the foundation has given more than \$1,000,000 to support the study of man's origins, behaviour and survival. The first task of the new chapter, Dr Bernard Campbell its vice-chairman said last week, will be to raise its own funds mainly to support anthropological research.

A FEW months ago (19 May, page 201) I voiced pessimism about the laetrile movement, but now I am in a more contemplative mood. Eleven State Legislatures have passed measures authorising the sale of laetrile. A most interesting day of reckoning is ahead, because no one, least of all the lawmakers, seems to know what laetrile is. Vials containing the solution are sometimes microbially contaminated. The 'potency' of various samples ranges from 14–87% of claims, and one batch of 'laetrile' capsules was found to consist of dehydrated iced tea, which was probably an improvement, because iced tea, unlike amygdalin ('street laetrile'), does not liberate cyanide in the digestive tract. Dr Gerald Lewis fed experimental animals with laetrile and sweet almonds; the almonds contain beta-glucosidase, which hydrolyzes amygdalin, and the animals died of cyanide poisoning. A child died similarly when she ate her father's laetrile tablets. I pointed this out to a pro-laetrile California state senator, and he told me that aspirin kills, too.

I am somewhat encouraged by the fact that the 'establishment' is finally bestirring itself. The Director of the National Cancer Institute, who opined that Ernst Krebs' theory of laetrile action was a great idea, and he wished it would work, has now departed; the US Surgeon General, Julius Richmond, now says that laetrile is useless and may be dangerous.

The best recent news stories were those concocted by the laetrile proponents at hearings before Senator Edward Kennedy on 12 July. Dr John Richardson, whose licence to practise medicine has been revoked, said that he, Ernst Krebs Jr and Robert Bradford were the victims of a conspiracy

by "the major oil and drug companies, the Food and Drug Administration (FDA), the American Medical Association, the National Cancer Institute, the American Cancer Institute"; he also suggested that the Rockefeller family was involved. The witnesses, including Bradford, were

Iced tea or laetrile?



THOMAS H. JUKES

unable to write the formula for laetrile "on a piece of paper", but Bradford, an engineering technician, said that "orthodox medicine was not qualified" to evaluate clinical tests of laetrile. Krebs, who is appealing a six-month jail sentence for violating his probation by selling laetrile, was reported as seeking to leave the impression that he was promoting laetrile for "purely humanitarian reasons". Carol Hehmyer, who prosecuted him, testified that he and his associates in San Francisco took in more than \$500,000 in two years by "selling laetrile and other outlawed medicines".

The 'victimisation' of Richardson, according to newspaper articles, seems to be rendered tolerable by his income (he grossed \$783,000 in 1974) and his opulent lifestyle. Bantam Books have printed 200,000 copies of his new book, *Laetrile Case Histories*. The FDA caustically pointed out in 'The Federal Register', 5 August, that Richardson's book states that he threw out the cases with "the weakest medical histories" and described only 62 out of 4,000 patients! In his book he also tells how he got the idea of laetrile promotion from Ernst Krebs Jr, whose biochemical theories, exposed as fantasies by Professor David Greenberg in 1974, served to convince Andrew McNaughton Jr, a former Canadian test pilot who is the leading entrepreneur of laetrile in Tijuana.

The scientific exposition by Krebs took place, according to McNaughton, in conversation at a chance meeting in a drugstore in Miami some years ago. Such erudite technicalities were not needed to persuade the legislators of 11 states. They responded to chanting crowds who wore T-shirts emblazoned with the words "Laetrile Works, You Bet Your Life". However, a slim majority of the California State Assembly Health and Welfare Committee was unconvinced. They killed the laetrile-legalising bill by a vote of six to five. The bill had passed the corresponding State Senate Committee unanimously. The author of the bill, Mr William Campbell, who for some reason is a member of the senate committee, says he will try again in January. Perhaps a more useful form of legislation would be a constitutional amendment to inhibit state governments from legalising quack remedies.

correspondence

Detecting dead bodies in 1718 and 1976

SIR,—I reported (*Nature* 262, 816 (1976)) that dead bodies of a dog and a cat were detected by the presence of fruit-bodies of an agaric species (*Hebeloma vinosophyllum*) appearing on the ground above the buried bones, and suggested the possibility that a victim of homicide buried in soil could be located by this or similar fungi. In response to a 'Science report' in *The Times* (2 September 1976) summarising my article, Dr Denys W. Tucker, formerly of British Museum (Natural History), informed me of a passage published in 1718, (Aubrey, J. *The Natural History and Antiquities of the County of Surrey* 111, (E. Curll, London 1918)), which contains the following lines (pages 225–26) written by John Aubrey of his visit to Woking in Surrey.

"The Grave-Digger here told me, that he had a Rule from his Father, to know when not to dig a Grave upon a Corps not rotted; which was, when he found a certain Plant about the Bigness of the Middle of a Tobacco-Pipe, which came near the Surface of the Earth, but never appear'd above it. It is very tough, and about a Yard long; the Rind of it is almost black, and tender, so that, when you pluck it, it slips off, and underneath is red; it hath a small Button at Top, not much unlike the Top of an *Asparagus*: Of these sometimes he finds two or three in a Grave. He is sure it is not a *Fern* Root.

He hath with Diligence trac'd to its Root, and finds it to spring from the Putrefaction of the dead Body. . ."

The phenomenon described here is close to what I suggested, but is not exactly the same as that expected of ammonia fungi including the above-mentioned species (Sagara, N. *Contr. biol. Lab. Kyoto Univ.* 24, 205–276 (1975)). "It" reminds us of the rhizomorph, pseudorhiza, or aborted fruit-body produced by certain fungi, but does not fit those of any known ammonia fungi. Further, if we interpret "the Putrefaction of the dead Body" as putrefying corpse, the ammonia fungi would not appear at such an early stage of decomposition.

I have been discussing the matter with some noted mycologists and botanists from the UK and Japan, but so far we have not succeeded in pointing out any possible organism for the structure described by J. Aubrey. I would appreciate receiving suggestions as to what it might be and any similar information from any part of the world.

I thank Dr Tucker for the information, and Drs Roy Watling, Geoffrey C. Ainsworth, Rokuya Imazeki, Siro Kitamura, Osamu Sinoda, and Minoru Hamada for the discussion.

Yours faithfully,

NAOHIKO SAGARA

Biological Laboratory,
Yoshida College,
Kyoto University, Japan

The twin paradox

SIR,—Tom Wilkie's remark (28 July, page 295) that the resolution of the twin paradox lies in the fact that the equivalence of the twins is destroyed by the necessity for the 'moving' one to reverse his motion continues to ignore the fact, pointed out in my letter in *Nature* of 31 August, 1973, that, if that were so, the equivalence would be maintained during the first half of the journey, before reversal occurs. Hence, in the example there cited, Paul, the 'moving' twin, would reach the distant planet a teenage boy, and the reversal and return would restore him to babyhood. It is not customary in scientific research to ignore such obviously remarkable and important points. Will Mr Wilkie therefore kindly say whether he accepts this necessary consequence of his suggested resolution of the paradox, or agrees with me that it makes such a resolution impossible?

Yours faithfully,

HERBERT DINGLE

Hull, UK

Tom Wilkie replies: I stand by every word of my article and do not accept that the above is a necessary consequence. I am writing to Professor Dingle privately on this matter.



Competition 14 asked for a boring or meaningless paragraph. £10 to E. R. A. Beck of Vienna for these introductory remarks:

The subject to be considered in this session of the symposium is one on which much research has been done, much grey matter has been activated and much fruitful discussion has already taken place. Its importance is well known to all of us here today, whether we face it at the laboratory bench or at an adminis-

trative, policy-making or governmental level. Indeed, the fact that an international organisation has chosen to devote an afternoon to an exchange of information on this topic is sufficient evidence that interest has transcended national boundaries. In some form or other, it affects the developed and the developing countries, the technologically advanced and the less-advanced peoples. There is, as yet, no consensus on a solution, nor indeed on a programme for the coming period. In my talk, therefore, I shall attempt to focus attention on some of the more significant points, and express some humble thoughts of my own as to how we might, together, approach the task of seeking a methodology for tackling this absorbing question, ensuring as we do so that our endeavours will also serve those facing this problem in the nations less well endowed with trained manpower and sophisticated equipment. (Aside, speaker to chairman: "I say, which symposium

is this; 'Advanced techniques in control of the screw-worm fly', or 'Special constraints in seeding methods for MHD generators?')

Honorable mention to J. R. Richards (Tadcaster, North Yorkshire) for a genuine paragraph from *The Mind of Light* by Sri Aurobindo.

Competition 15. The front cover of *Nature* used to carry a quotation from Wordsworth:

'To the solid ground
Of nature trusts the Mind that builds
for aye'.

£10 for the best quotation or verse, serious or humorous, suitable for these days. Entries to Competition 15, *Nature*, 4 Little Essex Street, London WC2 by October 20.

news and views

Intercalation complexes of DNA

from Donald Voet

It has now been sixteen years since Lerman proposed that aminoacridine dyes bind to DNA by intercalating between its stacked bases. Since then, numerous other planar aromatic molecules, many of which are potent drugs or mutagens, have been found to bind to DNA in a similar manner. Yet the detailed geometries of these intercalation complexes have hitherto been only poorly defined because of the low quality of the available fibres of nucleic acids complexed with intercalation agents. The situation has been greatly remedied recently by the elucidation of several X-ray crystal structures of self-complementary dinucleoside phosphates in complex with various intercalating agents.

X-ray studies have shown that self-complementary dinucleoside phosphates such as cytidyl-(3',5')-guanosine [CpG] often crystallise as miniature double helices. By this it is meant that the dinucleoside phosphate forms two symmetry-related Watson-Crick base pairs with a neighbouring molecule and that their sugar-phosphate backbones have the conformational parameters found in a double helix. In an intriguing extension of this work Sobell and his coworkers have recently reported the X-ray structures of several intercalation complexes of self-complementary dinucleoside phosphates. These are the 2:2 complex of ethidium with 5-iodouridylyl-(3',5')-adenosine [iodo-UpA] (Tsai *et al.* *J. molec. Biol.* **114**, 301; 1977); the 2:2 complex of ethidium with 5-iodocytidylyl-(3',5')-guanosine [iodoCpG] (Jain *et al.* *J. molec. Biol.* **114**, 317; 1977); the 2:2 complex of 9-aminoacridine with iodoCpG (Sakore *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 188; 1977) and the complex of proflavine with iodoCpG (Seshadri *et al.* *Abstracts Amer. Crystallogr. Assoc. Meeting, Michigan State Univ.* 1977). These complexes, which are all heavily hydrated, have quite different crystal structures from one another. Yet their molecular structures show striking

similarities. In all these structures the dinucleoside phosphates form miniature double helices that intercalate an ethidium or an acridine molecule. Thus the base pairs in these structures are separated by about 6.8 Å which is twice the usual base stacking distance found in the B form of DNA (the usual native form).

The intercalation agents are accommodated in the complex by the unwinding of the miniature double helices from the conformations found in nucleic acid double helices. In doing so all of these dinucleoside phosphates have adopted a rather distinctive sugar puckering pattern in which the ribose sugar of the pyrimidine nucleoside is in the C3' *endo* conformation and that of the purine nucleoside is in the C2' *endo* conformation. The same C3' *endo*-(3', 5')-C2' *endo* puckering alternation has been observed in the pseudo intercalation complex of 9-aminoacridine with ApU (Seeman *et al.* *Nature* **253**, 324; 1975). (In this structure the bases pair in a Hoogsteen arrangement without double helix formation. However the base pairs alternate with 9-aminoacridine molecules as in the foregoing intercalation complexes.) Furthermore, computerised model building studies by Alden and Arnott (*Nucleic Acids Res.* **2**, 1701; 1975) have suggested that the most satisfactory conformation for an intercalation complex of B-DNA incorporates the above puckering alternation.

It has long been known that, at saturating concentrations of intercalating agents, DNA will intercalate one molecule for every two base pairs. This observation has led to the 'neighbour exclusion' model which states that the binding of intercalation agents to DNA is subject to the restriction that adjacent binding sites be unoccupied. This model is neatly rationalised by the requirement of a C3' *endo*-(3',5')-C2' *endo* pucker alternation at the intercalation site. Such an alternation can maximally occur between every second base pair.

The foregoing crystal structures account for solution studies which indicate that ethidium preferentially

binds to sequences of the type pyrimidine-(3',5')-purine. In the above structures there are extensive stacking interactions between the nucleic acid bases and the intercalating molecules. However, model building studies show that in complexes which have these base sequences reversed the degree of ring overlap is much reduced. Presumably these latter complexes are less stable than the former. This result also explains why no intercalation complexes of ApU or GpC have been crystallised.

The various structural features common to the foregoing intercalation complexes, which include an 8° relative tilt of the base pairs towards the wide groove, have prompted Sobell *et al.* (*J. molec. Biol.* **114**, 333; 1977) to propose a detailed model for intercalation complexes of B-DNA. In this model, which is substantially based on computer-generated model building, the helix axis is kinked about the intercalation site by 8° and is unwound by 10°. This latter figure is in reasonable agreement with the results of solution studies of intercalation complexes. In addition the sugars about the intercalation site have the pucker alternation described above.

The structural features of this model have led Sobell *et al.* to extend their model building studies to generate a general model for the kinking of B-DNA. In this model the helix axis is bent by 40° into the wide groove through a conformational change that is made possible by a C3' *endo*-(3',5')-C2' *endo* pucker alternation about the kinking site. Then, depending on the interval along the helix axis of these kinks, various families of supercoiled DNA can be generated and Sobell *et al.* present several striking computer generated drawings of these superhelical DNAs. Perhaps the most interesting of these is the one in which a kink occurs every 10 base pairs. This gives rise to a left-handed superhelical structure that contains 1½ turns per 140 base pairs and has an axial length of 80 Å. These and other structural features make it an attractive model for the DNA in nucleosomes.

The foregoing models result from the great similarities among the four crystalline intercalation complexes reported by Sobell and coworkers. But into many ointments a fly will fall. In this case the fly is in the guise of the crystal structure of a 3:2 complex of proflavine with CpG that is reported by Neidle *et al.* in this issue of *Nature* (page 304). This structure, which results from a collaboration between researchers at Kings College, London, and The Institute of Cancer Research, Philadelphia, consists, in part, of miniature double helices into which proflavine molecules are intercalated. The spacing between adjacent C:G base pairs is the expected 6.8 Å. But what is unexpected and potentially damaging to the plausibility of Sobell's models is the fact that all the sugars are in the C3' *endo* conformation rather than having the C3' *endo*-(3',5')-C2' *endo* pucker alternation. Furthermore the miniature double helix is not unwound relative to the helical conformation of uncom-

plexed RNA-11. Rather, the miniature double helix accommodates the intercalated proflavine molecule by a stretching distortion involving small changes in the backbone and glycosidic torsion angles.

It may be that the structure reported by Neidle *et al.* is simply a red herring. After all, a dinucleoside phosphate could hardly be expected to behave as rigidly as a polynucleotide, especially in its interactions with other molecules. But the same criticism can be levelled at the structures reported by Sobell and coworkers. Furthermore, all of the dinucleoside phosphates discussed here are composed of ribonucleotides and therefore cannot be considered to be ideal models for DNA. (The fact that acridine fluoresces red when complexed with RNA and green with DNA indicates that the acridine molecule senses a difference between RNA and DNA). What is clearly necessary to settle the question of the conformation of an intercalation complex of DNA is the

crystal structure of an intercalation complex of a DNA fragment that is at least four base pairs in length. Rumour has it that there are several groups now attempting to crystallise such a complex.

In a wider sense these studies have shown that 'small molecule' crystallography continues to be a valuable tool in elucidating biological structures. The structures discussed here all contain several molecules in their asymmetric unit and hence are very large according to present day standards for 'small molecules'. Indeed, the asymmetric unit of the 9-aminoacridine: iodoCpG complex, which contains 248 non-hydrogen atoms, approaches the size of some of the smaller proteins whose structures were solved using 'macromolecular' crystallographic techniques (rubredoxin for example has 401 non-hydrogen atoms). Thus it seems that the differences between 'small molecule' and 'macromolecular' crystallographic techniques may soon grow indistinct. □

Elementary particles—a rich harvest

from David J. Miller

A symposium on Lepton and Photon Interactions at High Energies was held in Hamburg on 25–31 August, 1977.

A SCIENTIFIC journalist recently tried to persuade me that high-energy physics was one of the fields of science, and there might be others such as molecular biology, where enough progress has been made for the moment. He could not have maintained such an attitude if he had been at this year's conference. The momentum of progress in the field is unabated, with new and exciting experimental data being matched by a significant growth in theoretical understanding.

Evidence for a fifth quark?

The most exciting result of all came from L. Lederman, whose Columbia Fermilab group have now not only confirmed the Υ (upsilon) bump which they see in muon pairs from proton-proton collisions, but have shown that it is composed of at least two clear resonances, one produced three times more strongly than the other. It is almost certainly another pair of objects like the J/ψ and the ψ' ; but this time the theorists were ready for it. There is even a name for this whole class

of particles—'Onium'. Positronium—the pseudo atom made up from an electron and its antiparticle the positron—has been with us for many years. The ψ and ψ' have been called 'charmonium' because they behave as if they are made of a charmed quark and antiquark. A considerable amount of new evidence was reported on the level-structure of charmonium—not only the familiar J/ψ and ψ' 3S states which couple to e^+e^- by way of a photon, but the 3P states which lie between, and the rather less well established 1S states. A surprise has come as the theories have been refined. The non-relativistic theory of heavy quarks in a potential well had sufficient input from the 1^3S (J/ψ), 2^3S (ψ') and 1^3P that it was possible to predict a 3^3S state above the ψ' , at 3,770 MeV/ c^2 —just above the threshold for pair-producing the charmed D mesons. Experimenters at the e^+e^- storage rings SPEAR in California and DORIS in Hamburg had not investigated this region very carefully because of interest in the complex structure just beyond it from 4,000 to 4,450 MeV/ c^2 . They went back to look for the 3,770 only a few months ago and it was found as a clear signal exactly where predicted. If the upsilon is the next onium then it must be made from a pair of even heavier quarks than the charmed quark, so non-relativistic

potential theory should work even better in this case. As soon as Lederman's talk ended the theorists got out their pencils and paper, and in a talk a few hours later K. Gottfried was able to show that all of the known properties of the upsilon could be accommodated in an onium picture. There is therefore almost certainly a new quark flavour in addition to the everyday 'up' and 'down' quarks, the strange quark and the charmed quark.

More on charm

It is only about a year since it became clear that we had indeed seen charmed particles—the D mesons produced in e^+e^- annihilations. Many new properties of the Ds were reported at Hamburg and also evidence for their strange and charmed relative the F. The pseudoscalar Ds had been hard to separate from the vector D* mesons which decay directly into a D and a pion, but the discovery of the 3,770 has brought an unexpected bonus. It decays strongly into DD pairs, but it is not massive enough to give D*. One can therefore set the energy of the e^+e^- rings at 3,770 and study the rather complicated weak decays of the Ds without any extraneous pions. The other approaches to producing charm have been making slower progress. Neutrino experiments at Fermilab and at CERN are now accumulating large

numbers of events with two muons of opposite sign in the final state, or with a muon and an electron. All properties of these events seem to be consistent with charm's being produced in about 5% of neutrino interactions. So far no wholly satisfactory result has been reported on the production of charmed baryons. The Columbia-Fermilab experiment by Wonyong Lee's group has a clear signal with antilambda and three pions. They should also see something with lambda and three pions but they do not, so the significance of the antilambda signal must be questioned. It seems likely that the bubble chamber neutrino experiments will be the first to establish the existence of charmed baryons, but the results from several groups on strange particles from charmed particle decay are fluctuating wildly due to small statistics and no clear conclusions can yet be drawn.

More signs of τ

For two years now there have been signs from SPEAR and from DORIS that the increase of e^+e^- cross sections at about 4 GeV/ c^2 is due to two thresholds, not just to the charm threshold. M. Perl, using SPEAR, was the first to identify a class of events which have a muon and an electron with opposite signs and in which a great deal of the energy escapes as unseen neutral particles. New detectors at SPEAR and new data from DORIS have confirmed the existence of these events and it looks increasingly convincing that they are caused by a new heavy lepton, the tau (τ), a big brother to the electron and the muon. In particular, the cross section for producing them seems to rise smoothly and steadily from threshold towards the correct asymptotic value for the pair production of spin- $\frac{1}{2}$ particles, with mass about 1,850 to 1,900 MeV/ c^2 , coupled to the photon in the normal way. There was some excitement early in the conference when it seemed that one of the most easily predicted decays of the τ (to a pion and a neutrino) was not seen, but the experimenters looked more carefully at their data and were eventually convinced that there was no anomaly.

Neutrino physics

In neutrino physics the new results from the CERN SPS have come at the same time as the second generation results from Fermilab, with the consequence that some of the odder effects suggested by single experiments in the past can now be seen to have gone away. In particular the Harvard - Pennsylvania - Wisconsin - Fermilab, 'HPWF', suggestion of a threshold effect at neutrino energy of about 40 GeV has not been confirmed by any

Has the *src* gene product been found?

from Robin Weiss

AVIAN sarcoma viruses carry a gene, *src*, which is required for the transformation of fibroblasts and the induction of sarcomas, but which is superfluous for virus replication. Because mutants of *src* have been isolated that are temperature sensitive for the maintenance of the transformed state of the cell, it is widely assumed that *src* codes for a transforming protein. On page 346 in this issue of *Nature*, Brugge and Erikson report the detection of a transformation-specific antigen which appears to be a good candidate for the *src* protein. Sera obtained from rabbits bearing tumours induced by avian sarcoma virus (ASV) react with a protein of 60,000 daltons present in transformed cells, which is unrelated to virion structural proteins. The same protein is found in ASV-transformed avian and mammalian cells, strongly indicating that it is a virus-coded protein. Cells infected with transformation-defective, *src* deletion mutants of ASV do not express this protein; neither could it be immunoprecipitated from cells infected with temperature-sensitive *src* mutants and incubated at the non-permissive temperature.

This is not the first time a transformation-specific antigen has been identified in ASV-transformed cells. There is an extensive literature (reviewed by Kurth *Biomembranes* 8, 167; 1976) on a tumour-specific cell surface antigen (TSSA) to which Brugge and Erikson make no reference. It will be important to see whether the 60,000 dalton protein is related to TSSA.

Several attempts have been made to

identify the *src* gene product by *in vitro* translation of ASV RNA. Pawson, Martin and Smith (*J. Virol.* 19, 950; 1976) were not able to identify any polypeptide present in translation products of the ASV genome that was absent from those of a transformation-defective (*td*) mutant. Two recent reports, however, each claim that two polypeptides of approximately 18,000 and 25,000 daltons can be detected after translation of ASV RNA but not of *td* ASV RNA in the reticulocyte system (Kamine & Buchanan *Proc. natn. Acad. Sci. U.S.A.* 74, 2011; 1977; Beemon & Hunter *Proc. natn. Acad. Sci. U.S.A.* 74, 3302; 1977). The work of Beemon and Hunter is particularly interesting because they have demonstrated by analysis of tryptic peptides that the two polypeptides absent in *td* ASV translation products are unrelated to the precursor polypeptide of the virion core proteins. Since *src* is located near the 3' end of the ASV genome it is not easily translated from undegraded genomic RNA preparations, but selection of smaller fragments containing the poly(A) at the 3' end of the molecule proved effective for the translation of these presumed *src* products.

The antiserum that reacts with the 60,000 dalton polypeptide should be useful for selective precipitation of *in vitro* translation products. In their report, Brugge and Erikson already quote a further paper in press on that subject.

Robin Weiss is a member of staff at the ICRF Laboratories, London.

other group. But there are new effects on a more subtle level which are beginning to be believed because they are seen in so many experiments using very different techniques. Until now it has been usual to interpret neutrino data in terms of the simplest quark-parton picture. This will clearly not work any longer. For some years it had been clear that electron-proton and muon-proton scattering data required important corrections to the simple quark model. Now the same corrections have been shown to apply to the charged current neutrino data. Theorists are very pleased about this, because the kind of correction required is just what would be expected from the more formal theory of quarks—quantum chromodynamics 'QCD'.

Neutral current neutrino data has also been improving rapidly, and it is clear that neutral current interactions producing strongly interacting particles (hadrons) are very well described by the Salam-Weinberg GIM model (see *News and Views* 264, 398; 1976) otherwise known as $SU(2) \times U(1)$. P. Sandars (University of Oxford) gave a status report on the other end of the neutral current field—the search for the atomic effects of interference between the weak neutral current and the electromagnetic interaction. Here there are problems. Sandars's group and a group at Washington State University have performed optical rotation experiments with laser light passing through bismuth vapour. If the Weinberg-Salam model is true, and

has the parameters determined from the hadronic neutral-current experiments, then they should have seen large effects due to these parity-violating interferences. But neither group sees any effect, and they have spent a great deal of time checking both their experimental sensitivity and the theory in order to be sure (*News and Views* 264, 505; 1976).

S. Weinberg and others discussed the meaning of these results. It seems that $SU(2) \times U(1)$ is to the weak interaction what the naive quark-parton model has been to QCD; a first approximation which has fitted a surprisingly large part of the data. Now it will be necessary to enlarge the model to accommodate the new quarks and leptons, the absence of atomic neutral currents and perhaps also whatever it is that is causing trimuon events. The HPWF group still sees these events, though the big new CERN neutrino experiment is not yet claiming a definite effect. There is particular interest in two of the HPWF events in which all three of the muons have been given very large energies. This suggests that they have all come from the 'neutrino end' of a neutrino-nucleus interaction—something which no one was able to explain satisfactorily in the framework of current weak-interaction theory.

Plans for the future

The last morning was devoted to the future ('and its alternatives', a phrase quoted by W. Panofsky of SLAC from a US Government memorandum). Panofsky, and H. Schopper of the host laboratory, reviewed the plans for their laboratories and for their e^+e^- storage rings PEP and PETRA which will start work at about 30 GeV in the centre of mass (15+15 GeV beams) in 1979 and 1978 respectively. There was a fascinating talk from J. W. de Wire of Cornell University on CESR, the 8+8 GeV e^+e^- storage ring, which is being built on a minute budget by the standards of the big laboratories. Cornell is one of the last universities in the world to be able to build its own high energy physics facilities. The record of achievement on their 10 GeV electron synchrotron has been excellent and they are now especially confident of doing a good job with CESR because the upsilon falls right in the middle of their useful range of energies. Schopper described how the energy of DORIS will be extended to reach the upsilon, but the commitment to build PETRA on a very rapid schedule makes it impossible to do this immediately. The usefulness of having more than one laboratory doing e^+e^- physics at the same energy has been illustrated repeatedly not only by the results from SPEAR and DORIS on charm and τ production but also by

the latest results from the lower energy French and Italian machines, DCI and ADONE, which are still a long way from having cleared up the problems which remain in the energy region below 3.1 GeV. This region was left almost untouched in the rush to discover the 'new physics' and the Onium-structure of the old quarks—up, down and strange—is still not understood. These two machines will have to collect much more data before we can hope to sort it out.

Although our ideas of QCD and the weak interaction still leave many questions unanswered, J. D. Bjorken of Stanford drew attention in the final talk to the major discoveries which can be confidently predicted for the next 10 or 20 years. As energies rise, the old fashioned strong interaction and the electromagnetic interaction will have a decreasingly important role compared with the quark-gluon coupling of QCD and the charged and neutral currents of weak interactions. As well as discussing the zoo of new objects which might be produced as this regime is reached, he compared the merits of different types of machine which have been proposed to get there. The clear leader is the large e^+e^- machine LEP, as requested by the European Committee on Future Accelerators (*see News and Views* 267, 12; 1977). It would provide data on almost every major question in particle physics, and would also be able to sit at the mass of the neutral current intermediate boson and become a Z^0 factory—mass producing the Z^0 boson in a resonant mode so that the Z^0 couplings to all the quarks and leptons can be investigated simultaneously. Next in usefulness come high energy pp storage rings. These will give us an idea of effects beyond the range of extrapolation of our theories and at energies which other kinds of machine cannot reach. Cosmic rays have given hints of odd behaviour at tens of thousands of GeV (tens of TeV), but it will be storage rings such as ISABELLE at Brookhaven New York, rumoured to be about to be funded in the next ERDA budget, which will make it possible to take such effects seriously. Fixed target proton machines such as the SPS and the Fermilab machine will always be needed to provide neutrino, muon and hadron beams. The Russians are planning a 3 TeV machine and Fermilab's 1 TeV machine is still going ahead—now named the 'Tevatron'.

In the light of Bjorken's discussion there was some disappointment among many physicists at the current plans for the CERN laboratories near Geneva. The only new project which is being asked for by the directorate is an anti-proton on proton colliding beam facility to be added to the SPS. Yet it

is now well known that the antiquark content of the proton is sufficiently large that a high flux pp machine like ISABELLE should produce much more quark-antiquark physics than any conceivable $\bar{p}p$ machine. The other suggested development for the SPS, an electron ring called CHEEP (though it will cost a little more than the anti-proton facility), has a great deal more theoretical backing. The quark-parton model was evolved in response to the first deep-inelastic electron-proton scattering results. If CERN builds CHEEP it will have a unique tool to investigate many anticipated high energy effects by way of deep inelastic processes which are much easier to understand than quark-antiquark scattering. If CERN does not build CHEEP then it is possible that a proton ring could be added to PETRA instead, but it seems improbable that Germany would maintain its present level of subscription to CERN if it had such an advanced home-based programme.

Despite the inevitable arguments about tactics and despite the knowledge that funds will be hard to find if economic crises continue, there is confidence that the machines after PEP and PETRA will be built within a reasonable period. Bjorken mentioned something which particle physicists feel very strongly; the case for building the next generation of accelerators can be made more precisely and with more confidence now than has been possible for any previous generation of accelerators. Here is a field where progress must continue. □

Recognition of lysosomal enzymes

R. Colin Hughes

IN the late 1960s, Elizabeth Neufeld and her colleagues showed that 'corrective factors' secreted by skin fibroblasts established from normal individuals could eliminate intracellular deposits of mucopolysaccharides that build up in mutant cells established from the skin of mucopolysaccharidoses patients. Such patients lack the particular lysosomal enzymes to catabolise these substances in the normal way (see Neufeld *et al. A. Rev. Biochem.* 44, 357; 1975; Dawson & Lenn in *Handbook of Clinical Neurology* 27, 143; 1976). Later the 'corrective factors' were identified as precisely those lysosomal enzymes missing in each of the mutant cell lines (Bach

R. C. Hughes is a research scientist at the Medical Research Council, National Institute for Medical Research, Mill Hill, London.

et al. Proc. natn. Acad. Sci. U.S.A. **69**, 2048; 1972; Cantz *et al. J. biol. Chem.* **247**, 5456; 1972; von Figura & Kresse *Biochem. biophys. Res. Commun.* **48**, 262; 1972). More recently, Sly *et al. (J. Pediatr.* **82**, 249; 1973) reported a child with a mucopolysaccharide storage disease due to a deficiency of β -glucuronidase whose fibroblasts were corrected by purified bovine or human enzyme (Hall *et al. Archs Biochem. Biophys.* **155**, 32; 1973; Brot *et al. Biochem. biophys. Res. Commun.* **57**, 1; 1974). Since lysosomal enzyme deficiencies produce severe problems for the patients the encouraging idea of an enzyme replacement therapy prompted by these experiments has been pursued vigorously. Such efforts are likely to be aided by the impressive progress being made in showing how lysosomal enzymes added externally to cells find their way to the abnormal intracellular storage materials.

This process involves specific pinocytosis and apparently utilises part of the normal life cycle of these enzymes, which according to Hickman and Neufeld (*Biochem. biophys. Res. Commun.* **49**, 992; 1972) are first secreted to appear on the outside of cells and are then quickly taken up again by the same or adjacent cells to perform their enzymic function. Just why the lysosomal enzymes go through this complicated scheme is completely unknown. The important point is that one step in the proposed scheme involves recognition by fibroblast cell surface receptors. Some of the molecular features of this recognition are becoming clearer and there are several surprises.

The removal of carbohydrate from human β -hexosaminidase, which like most lysosomal hydrolases is a glycoprotein, was found to abolish its uptake by fibroblasts and led to the idea that recognition of the enzyme by fibroblasts involves a carbohydrate moiety (Hickman *et al. Biochem. biophys. Res. Commun.* **57**, 55; 1974). The classic work of Ashwell and Morell had already shown that carbohydrate specificity was critically involved in recognition of glycoproteins by mammalian liver, a reaction that explained the rapid clearance of these glycoproteins from the circulation. The properties of the liver cell receptor are such that only glycoproteins with terminal galactose residues are recognised and taken up by the hepatocyte (see Ashwell & Morell *Trends in Biochemical Sciences* **2**, 76; 1977 for recent discussion). Although the concept is clearly similar this receptor is sharply distinguishable from the receptor on fibroblasts which mediates the uptake of lysosomal hydrolases. The clearest evidence on the molecular

detail of this interaction so far has been obtained by Sly and his colleagues using human β -glucuronidase.

Previous studies (Glaser *et al. Archs Biochem. Biophys.* **166**, 536; 1975; Brot *et al. Biochem. biophys. Res. Commun.* **57**, 1; 1974) had shown that the enzyme appeared in two forms separable by a charge difference. Only the more acidic form, concentrated particularly in platelets but relatively scarce in placenta, was taken up by skin fibroblasts established from the patient with β -glucuronidase deficiency. At the recent ICN-UCLA meeting on Cell Surface Carbohydrates and Biological Recognition in Keystone, Colorado, Sly reported (*J. supramol. Structure Supplement* (1977) in the press) the competitive inhibition of β -glucuronidase uptake by certain yeast mannans containing phosphate and by simple hexose phosphates, in particular D-mannose-6-phosphate. Furthermore, the high uptake form of the enzyme was converted to a less acidic low uptake form without loss of enzymic activity by treatment with highly purified alkaline phosphatase. More detailed reports (Kaplan, Archord & Sly *Proc. natn. Acad. Sci. U.S.A.* **74**, 2026; 1977; Kaplan, Archord & Sly *J. clin. Invest.* (1977), in the press) substantiate these suggestions for a novel receptor on fibroblasts that recognises hexose phosphate on the lysosomal glycoproteins β -glucuronidase, β -hexosaminidase and β -galactosidase. The same kind of recognition is involved in the uptake of acidic forms of β -glucuronidase from liver, urine, spleen and placenta and the hexosaminidase secreted by normal human fibroblasts also seems to contain the phosphohexosyl group for the fibroblast receptor.

Phosphorylated sugars are very rarely found in mammalian glycoproteins. The sensitivity to alkaline phosphatase suggests a phosphomonoester, similar perhaps to some forms of haemoglobin which contain terminal D-glucose-6-phosphate residues (Haney & Bunn *Proc. natn. Acad. Sci. U.S.A.* **73**, 3534; 1976). Davis *et al. (FEBS Lett.* **65**, 3035; 1976) recently reported the isolation of phosphoglycoproteins from rat brain and demonstrated phosphomannosyl residues. Although phosphomannosyl is a well known constituent of yeast glycoproteins, this is the first example found in mammalian glycoproteins.

Other workers (Hieber *et al. Biochem. biophys. Res. Commun.* **73**, 710; 1976; Fedn *Proc.* **36**, 653; 1977) have been interested mainly in β -galactosidase and believe mannose also regulates uptake of this enzyme by fibroblasts. Whether the mannose residues are phosphorylated is not yet clear. Hieber *et al.*, concluded that this

was a distinct uptake system different from that which mediates β -glucuronidase uptake. Hieber *et al.* have, however, shown that the glycopeptides (carbohydrate chains containing just one or two amino acid residues) inhibit assimilation of β -galactosidase by fibroblasts. The exciting implications of these findings are that such glycopeptides could serve as recognition markers to get proteins rather specifically and efficiently into cells and perhaps opens up the possibility of enzyme replacement therapy in diseases in which a 'high uptake' human enzyme is not readily available.

Several questions concerning the role of the fibroblast surface receptor in lysosomal organisation are raised by these findings. It has been known for many years that β -glucuronidase for example is bound to the inner surface of the lysosomal membrane. Is the same recognition marker used for this interaction as that involved in pinocytosis? This is a distinct possibility since the inner surface of the lysosomal membrane (presumably carrying the specific receptor protein) appears on the outer surface of the cell during lysosomal discharge in the model of Hickman and Neufeld (*op. cit.*). Second, does the fibroblast surface receptor play a part in controlling the levels of extracellular and potentially lethal lysosomal enzymes? This may be so but collateral evidence has revealed yet another recognition system which, like that already discussed, involves mannose-rich carbohydrate chains of glycoproteins but does not require these residues to be phosphorylated.

When rat lysosomal enzymes are injected intravenously in rats they are cleared from the circulation within a few minutes (Stahl *et al. Nature* **264**, 86; 1977; *Proc. natn. Acad. Sci. U.S.A.* **73**, 4045; 1976) and end up in the liver. The system is clearly different from the clearance of galactose terminal glycoproteins, desialysed orosomucoid, by the Ashwell-Morell system which resides in hepatocytes, since there is no inhibition of lysosomal enzyme clearance by orosomucoid unless both terminal sialic acid and galactose residues are removed to expose N-acetylglucosamine terminals. According to Sly *et al. (Fedn Proc.* **36**, 653; 1977; *Biochem. biophys. Res. Commun.* **77**, 409; 1977) clearance of human placenta β -glucuronidase in the rat was similar to that reported by Stahl *et al. (op. cit.)* for rat enzymes, and the liver clearance system was shown to involve Kupffer cells rather than hepatocytes. This clearance system was inhibited even better by mannose terminal glycoproteins than by N-acetylglucosamine terminal glycoproteins. Clearance of N-acetylglucosamine terminal glycopro-

teins was antagonised by simultaneous injection of mannose terminal glycoproteins and *vice versa*, suggesting that there are unlikely to be independent Kupffer cell receptors responsible for clearance of mannose or N-acetylglucosamine-terminal glycoproteins respectively. Presumably the Kupffer cell receptor has relaxed specificity and binds to mannose residues present in both glycoprotein types. It has recently been suggested that the hepatocyte receptor also does not discriminate between galactose and glucose terminals in some circumstances (Stowell *et al.* *Fedn Proc.* **36**, 653; 1977). In this property the mammalian receptors resemble the plant lectins which in general also bind to several different sugars. Since both forms of β -glucuronidase are efficiently cleared from the circulation, the Kupffer recognition system does not require a phosphorylated site on the lysosomal enzyme, raising fascinating speculation on the potential role of phosphorylation in

deciding the circulatory fate and target cell of glycoproteins.

In summary, therefore, the original findings of Ashwell and Morell have opened up to reveal a remarkable collection of cellular receptors for glycoproteins. These receptors differ in specificity according to cell type, for example the preference of the mammalian hepatocyte receptor for galactosyl terminals, the Kupffer and fibroblast cell receptors for mannosyl residues and phosphomannosyl residues respectively while the identification of the avian hepatic receptor as a binding activity for N-acetylglucosaminyl terminated glycoproteins (Lunney & Ashwell *Proc. natn. Acad. Sci. U.S.A.* **73**, 341; 1976) warns against extrapolation across species. The list almost certainly will continue to grow and underlines the feeling that carbohydrate structure is involved in a variety of recognition systems, and defects in such recognition may correlate with some human disease states. \square

Diffusion in polymers

from Paul Calvert

SEMICRYSTALLINE polymers behave as if they consisted of two phases, crystal and amorphous, arranged in alternate layers. Most simple properties are a function of the fractional crystallinity but are insensitive to the structure or arrangement of the phases. Some recent measurements on the diffusion of chain molecules in polyethylene serve as a reminder that diffusion can tell us about structural details.

According to the two-phase model a semicrystalline polymer consists of plate-like crystals of about 10 nm thickness separated by amorphous regions of the same size. The crystals have the same properties as hypothetical large crystals and the amorphous material should be the same as completely amorphous polymer. This idea works well for bulk properties such as density, modulus, heat capacity and expansion coefficient (see Wunderlich *Macromolecular Physics I*, Academic Press, 1973). There are probably crystal defects and special crystal surface structure but they do not affect bulk properties.

It seems reasonable that motion in the amorphous phase will be limited by the crystals, but in polymers such as polystyrene and polyethylene terephthalate I am not aware of any evidence for a change in glass transition temperature or mobility between com-

pletely amorphous and partly crystalline polymers. Boyer has suggested that this happens in polyethylene (*Macromolecules* **6**, 288; 1976) but the situation is still confused. Intercrystalline links are chains running from one crystal to another stitching the structure together (Keith & Padden *J. appl. Phys.* **42**, 4585; 1971). These should be more frequent in quickly cooled samples where the crystals are small and have proved useful in explaining effects of molecular weight and cooling rate on mechanical properties. Unfortunately, no very convincing way of detecting them in normal samples has been found (although see Wunderlich *Makromol. Chem.* **175**, 977; 1974). So, although strength and deformation behaviour do depend on structural factors other than crystallinity, and although likely factors have been discussed, there is not much supporting evidence. Diffusion measurements could be very informative about this.

Many measurements of diffusion of gases in polymers were made in the 1950s and early 1960s when plastic packaging was becoming important (see Crank & Park *Diffusion in Polymers* Academic Press, 1968; Rogers in *Physics and Chemistry of the Organic Solid State* **2**, Interscience 1965). It is probably these workers whom we should thank for the disappearance of the soggy potato crisp. In semicrystalline polymers it was found that gas solubilities were proportional to the

amorphous fraction, implying that gases are excluded from the crystals. Michaels and coworkers (*J. Polymer Sci.*, **41**, 53; 1959; **50**, 393, 413; 1961) described diffusion by $D = D_a / \tau \beta$ where D_a is the diffusion coefficient in pure amorphous material, τ is a 'tortuosity factor' dependent on crystallinity and β is an immobilisation factor which is dependent on the size of the diffusing molecule. They initially found that in branched and Ziegler polyethylenes τ was proportional to amorphous content to a power between -1 and -2 rather than the expected inverse proportionality. In later measurements on linear polyethylene (*J. appl. Phys.* **35**, 3165; 1964) they did find diffusion to be simply proportional to amorphous content in samples crystallised by cooling. By annealing, however, they could produce samples of the same crystallinity but with different diffusion rates. This topic has essentially remained in this state since.

Klein and Briscoe made measurements of the diffusion of long chain amides in polyethylene (*Nature* **257**, 386; 1975; *Polymer* **17**, 481; 1976), and have recently extended this to study morphological effects on the diffusion of chain molecules (*Nature* **266**, 43; 1977; *J. Polymer Sci. (Phys.)* (in the press)). They envisage the diffusion of a chain molecule in the interlamellar regions as the motion of a large snake across the floor of a forest, winding amongst the intercrystalline links. This is based on the ideas of de Gennes (*J. Chem. Phys.* **55**, 572; 1971; *J. de Physique* **36**, 1199; 1975), and monte carlo calculations for two dimensional snake-like diffusion ('reptation') around obstacles by Doi (*J. Phys. A* **8**, 417; 1975). With some rather sweeping assumptions about the frequency of intercrystalline links they are able to calculate relative diffusion rates for two compounds containing 45 and 25 carbon atoms in rapidly and slowly cooled samples. The calculated ratios are in respectable agreement with experiment. The calculated and observed diffusion rates are faster in the more crystalline, slowly cooled, sample in contradiction to the results of Michaels for gases, and to simple crystallinity theories.

At the moment this is a lot of theory based on little data, and there are many unanswered questions including why annealed samples are different from continuously cooled ones, why Eby found more rapid diffusion in film surfaces (*J. appl. Phys.* **35**, 2720; 1964) and whether diffusion is homogeneous throughout the spherulites. Nonetheless, a quantitative theory is much better than a qualitative model and we should learn a great deal about morphology in proving it right or wrong. \square

review article

Mathematical models in applied ecology

Gordon R. Conway*

Since the 1950s there has been a growing interest in mathematical models as guides to harvesting fish and game or controlling pests and vector borne disease. So far there are few indications that we can use such models in day-to-day ecological management, but experience confirms that they have a powerful role to play in developing management strategies and in the formulation of policy.

CONTEMPORARY research in applied ecology can be criticised on several counts: it is predominantly empirical, the effort is fragmented into different fields and there is little attempt to link economic and social factors with ecological factors. These criticisms are by no means new. That they have to be repeated is a reflection of the considerable complexity of most problems in applied ecology and the very real intellectual and practical difficulties of integrating principles derived from pure ecology with principles arising out of the social sciences.

In the 1960s Watt laid the foundations for a coherent theory of applied ecology¹. His starting point was the perception that all natural resource management fields are related to one another by common dependence on the science of ecology, and by a common concern with the problem of optimisation. He also recognised that they are universally characterised by processes such as dispersal, predation, competition and weather effects whose mathematical properties are typically non-linear and complex. The development of a theory thus also required the elaboration of a common set of analytical tools and mathematical models.

Watt's pioneer efforts stimulated the development of a wide range of applied ecological models²⁻⁶. For the most part these, and earlier models, have been aimed at answering questions of strategy. Built on knowledge derived from the study of undisturbed organisms in their natural environment, they have been directed at the strictly ecological problems of maximising harvests of domesticated or wild animals and plants and minimising populations of pests and diseases. Underlying their development there has often been a conscious search for those natural thresholds and non-linearities in the dynamics of populations which can be successfully exploited for harvesting or control. At their best these models have become powerful didactic tools, guiding research and determining policy.

Strategic models, however, are purely ecological in orientation with no economic and social component. True policy models, which explicitly include these components, have so far been developed largely for fisheries, although in recent years they have begun to appear in the field of pest control. They are, of course, considerably more complex than strategic models but the few successful examples graphically demonstrate the critical importance of understanding the consequences of the interaction between the biological non-linearities of a system and those introduced by economic factors.

More recently, a third kind of model has appeared bearing on the day-to-day decisions involved in the harvesting or control of a specific population. Such tactical models have the formidable task of coping in some detail not only with temporal and spatial heterogeneity, but also with the weather variables which tend to be dominant at the tactical level. As a consequence most are

formulated as computer simulations. So far, very few, if any, have shown practical success.

Together, strategic and policy models may be able to correct the deficiencies of much of today's applied ecological research, by providing a stronger theoretical base, a sense of coherence between the different fields and an understanding of the nature of the policy questions and their answers. That may in turn lead to tactical models for use in everyday management.

This review begins with a brief historical note, which is followed by a discussion of the relative merits of small and large scale modelling in applied ecology. The main body of the review examines the relative progress of policy, strategic and tactical models in the harvesting of fish and game and the control of pests and vector borne disease.

Early models

The origins of contemporary modelling lie in nineteenth-century epidemiology⁷ and demography⁸ models. From the former there is a direct link to the model of malaria transmission developed by Ross at the beginning of this century⁹⁻¹². His original work was of enormous significance not only in epidemiology but also in the broader field discussed here because he clearly established the critical implications of the non-linearities in the system for control strategies (D. J. Bradley, inaugural lecture at the London School of Hygiene and Tropical Medicine). The model demonstrated that malaria could theoretically be eradicated without the need to eradicate the mosquito population as had previously been supposed: there was a critical threshold below which transmission of the malaria parasite ceased.

The nineteenth-century demographic models provided the basis for early theoretical studies of harvesting strategies. In the 1930s Graham¹³ and Hjort *et al.*¹⁴ independently drew attention to the management implications of the logistic equation as a model of population growth:

$$dx/dt = F(x) = rx(1 - x/K) \quad (1)$$

where r is proportional growth rate and K is the asymptotic stable equilibrium, traditionally referred to as the carrying capacity of the population environment. For this model the maximum sustainable yield (MSY) of, say, a fish population is obtainable by harvesting at a level $x = K/2$.

In subsequent decades few useful extensions were made to this basic work; it was not until the 1950s that progress was made with the development of a new generation of malaria and fish harvesting models and the first attempts to model agricultural and forest pests.

Large scale modelling

At the same time many ecologists became interested in the ability of modern computers and computer languages to mimic in detail

*Centre for Environmental Technology and Department of Zoology and Applied Entomology, Imperial College of Science and Technology, London SW7, UK.

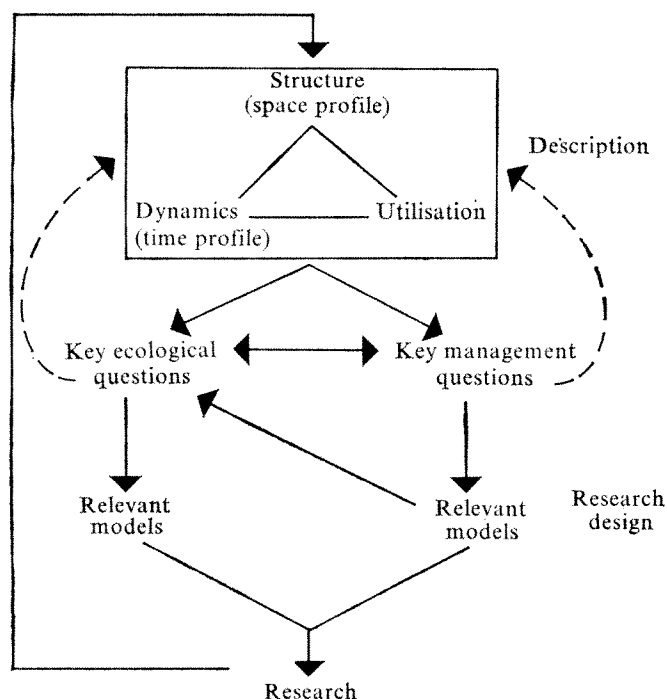


Fig. 1 A suggested procedure for applied ecosystem analysis, designed for a multidisciplinary team working on a continuing seminar basis. The procedure although flexible, is intended to ensure that ecological and management issues are raised simultaneously³⁶.

the dynamic and structural complexity of ecological systems¹⁵⁻¹⁹. This interest played an important part in the evolution of the International Biological Programme²⁰⁻²³ and in several of the IBP biome studies a central role was given to large scale ecosystem models.

A common approach to ecosystem modelling, both in the IBP and elsewhere has been to define ecosystems in terms of series of compartments—producers, consumers and decomposers, and an abiotic compartment—linked by equations describing energy (carbon) flows and materials cycling. The resulting models are essentially mechanistic, the hope being that the sum effect of the modelled interactions between and within compartments is sufficient to provide predictions of changes in the ecosystem, particularly the biomass of its constituent species, over relatively short periods.

A final assessment of the biome models would be premature now, since with some exceptions²⁴⁻²⁷ the relevant documentation is unpublished. There is no doubt that the models have conferred a better understanding of the complexity of ecosystem structure and dynamics, particularly on those engaged in the construction of the models^{21,28}. But so far there is little evidence that they have been useful in answering specific management questions²⁹. More successful in this respect have been aquatic ecosystem models which have taken both biotic and water quality into consideration, using mass balance equations^{30,31}. These can be used, for example, to assess the effects of the siting and quality of waste water discharges on rivers, lakes and other bodies of water. In general, though, while energy and material budgeting provides a convenient basis for linking ecosystem components the resulting models cannot easily be used by policy makers and managers whose prime concerns are the monetary and social outcomes of manipulating ecosystems. In practice, management issues are best served by purpose-built models³²⁻³⁴.

The alternative approach is not to attempt, at least at first, to match complex ecosystems with complex models³⁵, but to assume that their essential structure and behaviour can be understood and predicted through a limited number of key processes and that management problems can similarly be solved by altering a few key decisions³⁶. The task of the applied ecologist then becomes that of identifying the key questions and of choosing such appropriate mathematical models as will provide the answers (Fig. 1).

This approach, with its explicit avoidance of large-scale models, carries no guarantee that important features of the ecosystem will not be missed. However, in the absence of holistic models which are both sufficiently realistic and tractable it is probably more fruitful to concentrate on smaller-scale models designed to answer specific chosen questions, relying on multi-disciplinary interaction and the use of various graphic and flow diagram techniques to provide a holistic framework for the question posing. There is a greater likelihood of building models which are both practical and likely to contribute significantly to the emerging body of theory.

The remainder of this review is devoted, with some exceptions, to models which are based either implicitly or explicitly on these assumptions.

Vector borne disease

The new malaria model^{37,38} produced by Macdonald in the 1950s was based on that of Ross but was more biologically realistic, for example incorporating the possibility of superinfection. Central to the model is a function for the rate of malaria reproduction, for stable populations and static epidemiological conditions, of the form

$$Z_0 = bC/r \quad (2)$$

where Z_0 is the basic reproduction rate of malaria, or the number of infections distributed in a community as a direct result of the presence of a single, primary non-immune case, b is a measure of the probability of a human becoming infected if he is bitten by a mosquito with sporozoites in its glands, r is the recovery rate in man and C , the vectorial capacity

$$C = \frac{ma^2p^n}{\ln(1/p)} \quad (3)$$

where m is a measure of the density, a the man biting rate and p the daily survival rate of the mosquito population, and n the length of the parasite cycle in the mosquito.

Formulated like that the model is an elegant and powerful didactic tool with important implications for the broad strategies of malaria control. Simple inspection of the model reveals that strategies of killing larval mosquitoes, or administering prophylactic or curative drugs have a linear effect on the malaria reproduction rate, through their respective impacts on the terms m , b and r . However the strategy of insecticide spraying against the adult mosquitoes not only lowers m but also reduces p , and since this term enters the function non-linearly, small reductions in p result in disproportionately large reductions in the reproduction rate. This insight has been important in justifying the reliance on adulticide spraying as a principal malaria control strategy since World War II.

More recently, however, an attempt to use Macdonald's model to guide a local field control project has met with failure³⁹. Simulation studies⁴⁰, were used to prescribe the specific tactics for a strategy of chemotherapy combined with DDT spraying, aimed at interruption of malaria transmission in the Kankiya district of North-Central Nigeria, where earlier campaigns had failed. Part of the subsequent failure of the project was due to operational problems; the insecticide spraying was seriously delayed and proved ineffective against the particular vector population, *Anopheles gambiae*. It also became clear, however, that there were fundamental problems in the formulation and application of the model. The problems, moreover, seem similar to those encountered when attempts have been made to use models for tactical decisions in other fields such as fish harvesting or agricultural and forest pest control.

First, Macdonald's model is too aggregated and thus ignores both spatial heterogeneity and the important population subdivisions of age and state; the parameter b , for example, subsumes a complex of different biological processes including the host immune response which results in different immunological classes in the human population. Second, even when parameters seem

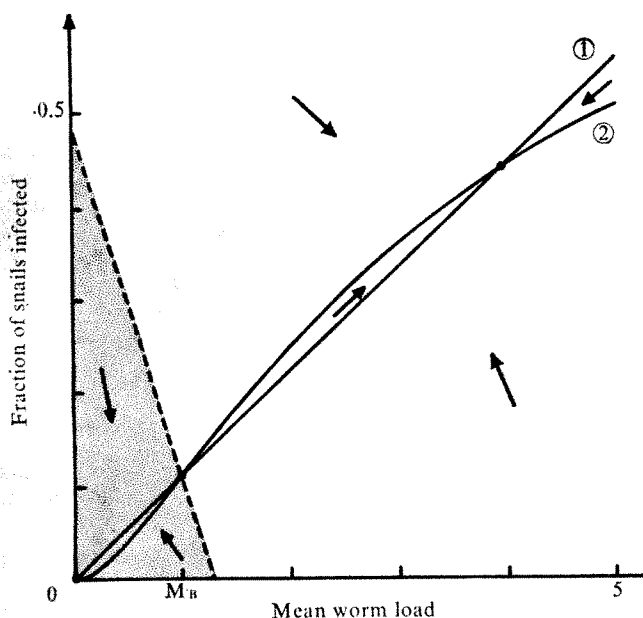


Fig. 2 Dynamics of Schistosome transmission based on elaboration of Macdonald's model. The two isoclines for stationary mean worm load (1) and fraction of snails infected (2) intersect at two stable points and at an unstable 'breakpoint' M_B below which the disease goes to extinction⁴⁷.

valid and well defined, the survival rate p for example, they are often difficult to measure with accuracy in the field. Third, being essentially deterministic and designed for stable populations, the model is not appropriate for the specific local conditions in Nigeria which are characterised by a seasonal and explosive epidemics.

Reviewing this episode Najera³⁹ acknowledges the value of Macdonald's model in its time but argues for the development of a third generation of malaria models, reflecting greater biological realism and the change from the objective of malaria eradication to malaria control. A deterministic model developed by Dietz *et al.*⁴¹ which recognises seven distinct epidemiological classes in the human populations is a step in this direction. As with earlier models a threshold vectorial capacity is specified below which malaria cannot maintain itself, but unlike Macdonald's model, where 100% endemicity is rapidly reached once the threshold is passed, the immune mechanism now realistically regulates the endemic level, and the model thus provides a truer measure of control in terms of the reduction in endemicity. Partial field tests have been applied to the model and it is currently being used to evaluate control strategies.

Schistosomiasis has recently received increasing attention from mathematical modellers⁴²⁻⁵². The disease is considerably more complex and much of the modelling effort has been focused on specific components of the disease cycle. There have, however, also been significant attempts to uncover the non-linearities in the overall life cycle of the disease which involves the transmission of the schistosome worms between the human and snail host. The classic model is again due to Macdonald⁵¹ who demonstrated the existence of a theoretical threshold or 'breakpoint' in the mean worm load in man, below which the disease goes to extinction (Fig. 2). The model further revealed that the threshold condition is more sensitive to changes in the probability of transmission from snail to man than man to snail and led him to suggest that the strategic priority should go to ensuring safe water supplies rather than improved sanitation. Macdonald's results have been modified by several subsequent workers: Bradley and May^{47, 52}, for example, have shown that the assumption of a non-random distribution of worms in man significantly alters the threshold criteria.

May also observes that further non-linearities are likely to be introduced in the schistosome model once the economic costs of control are considered. So far no-one has attempted to combine economic and ecological features in a vector borne disease model,

although cost-benefit estimates have been derived for a number of situations⁵³⁻⁵⁶. Such estimates tend to be unsatisfactory because of the difficulty of obtaining realistic measures of benefits which in many cases are intrinsically unquantifiable. Loss of earning capacity as a result of morbidity or mortality can be calculated but there remains the problem of translating this loss into actual benefits following control, given the prevailing pattern of high unemployment and underemployment in the less developed countries. For the present it is more important to incorporate costs alone into epidemiological models, in particular so as to illuminate the changing cost pattern of a control or eradication campaign as it nears its objectives. The recent collapse of several malaria control campaigns suggests insufficient understanding of how maintenance costs and operations are determined by the epidemiological features of low endemicity. A related issue is the interaction of control campaigns for different diseases and the question of when it is 'safe' to switch budget priorities from one control campaign to another.

Harvesting

Policy models which integrate ecological and economic factors have been most successfully developed for fishery harvesting⁵⁷. This is partly due to the ease with which the basic logistic model used to describe fish population growth can be manipulated and extended⁵⁸⁻⁶⁰. Despite, or perhaps because of, its simple assumptions it has proved a powerful tool for illuminating fishery policy issues. The success of the 1946 Overfishing Convention has been attributed in part to Graham's forceful arguments based on the model⁶¹.

In the 1950s Schaefer⁶² incorporated fishing effort into the basic equation

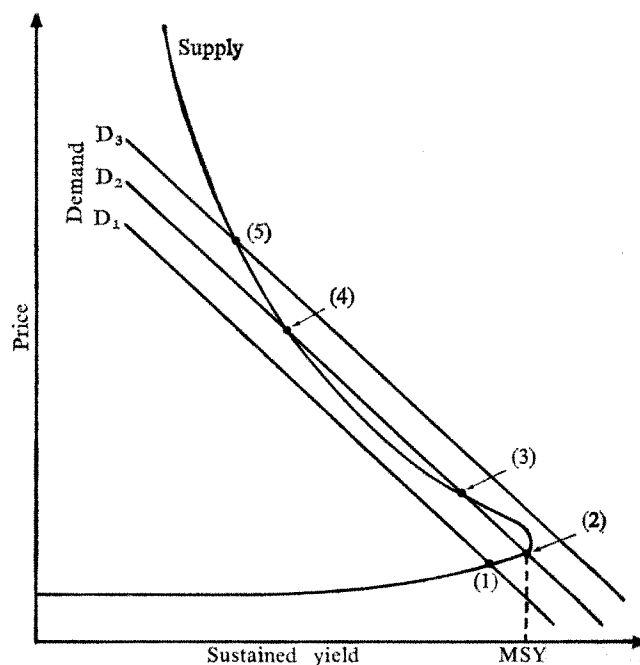
$$dx/dt = rx(1 - x/K) - qEx \quad (4)$$

where E is effort (for example vessel-days per unit time) and q the catchability coefficient. Gordon⁶³ then completed the economic formulation

$$\pi(x, E) = pqEx - cE \quad (5)$$

where $\pi(x, E)$ is net economic revenue, p and c are unit price and

Fig. 3 Instability of supply and demand in the open access fishery as revealed by the Schaefer model. For demand curve D_1 , the equilibrium is at (2) or (4) depending on the starting population. However, a slight demand shift to D_3 results in inevitable overfishing (5)⁵⁷.



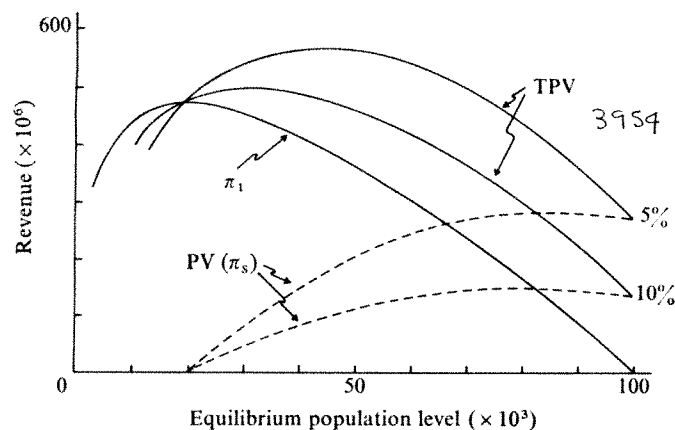


Fig. 4 Present value curves for blue whale harvesting at 5% and 10% discount rates. The total present value (TPV) is the sum of the net depletion revenue (π_1) obtained from depleting the initial stock (100,000) to the sustained yield level, and the present value of that sustained yield ($PV(\pi_s)$). Using a simple Schaefer model the MSY would be at 75,000 whales equilibrium population⁷¹.

cost, and used the model to show that in the open access fishery uncontrolled effort leads to an equilibrium at which total revenue equals total cost, so that the economic rent is completely dissipated. More recently several workers have extended his analysis to incorporate questions of supply and demand. For the open access fishery the supply curve for sustained yield is of the form

$$Y = rc(1 - c/pK)/p \quad (6)$$

High prices thus lead to biological overfishing. If the elasticity of demand is also taken into account the instability of the system becomes even more apparent (Fig. 3)^{57,64,65}.

The Schaefer model has played an important part in attempts to introduce some degree of regulation in otherwise open access fisheries. It provided, for example, one argument which led to the 1966 ban on blue whale harvesting^{66,67} and, in modified form, is currently used in calculating maximum sustainable yields (MSYs) and catch limits for all currently exploited whale stocks.

In the last two years, however, a number of criticisms have been levelled at fishery policy based on setting MSYs and catch quotas in this fashion¹²². One criticism stems from the deterministic nature of the models used. Beddington and May⁶⁹ demonstrate that in a randomly fluctuating environment the relative variability in population magnitude and hence yield increases systematically as harvesting effort increases. Moreover this effect is more pronounced when management is based on constant catch quotas rather than on a direct regulation of effort.

A more fundamental criticism is that the economic analysis ignores the important capital component in fishery management. As Clark and Munro^{57,70} forcefully point out a fishery is a capital asset and by definition its value is equal to the present value (PV) of the net future revenues it is expected to yield. In practice the commercial fishery aims not for an MSY but to maximise

$$PV = \int_0^{\infty} e^{-\delta t} - \pi(x, E) dt \quad (7)$$

where δ is the continuous discount rate per annum.

Clark has illustrated the practical consequences of this assumption in terms of blue whale harvesting⁷¹, where the maximum total present value is obtained at a stock level well below the MSY derived from a simple Schaefer model (Fig. 4). This result arises because the intrinsic rate of increase, r , of whale populations is of the order of 5–10%, which is similar to or often less than prevailing commercial discount rates. In many commercial fisheries the bionomic growth rate δ/r is less than unity and the MSY is a sensible first approximation to a management goal but where δ

exceeds r , conservation can only be achieved by decisions which, in effect, replace the high commercial discount rate by a politically derived social rate.

As a practical management tool the Schaefer model is also too dependent on the availability of a long run of catch data. It may be the only approach where age grading of fish is impossible but for the majority of commercial fish an age class model is feasible and indeed essential if the detailed population dynamics, and in particular any non-linear stock-recruitment phenomena, are allowed for adequately. The first significant advance in this direction was the Beverton and Holt model⁷² which gives the annual biomass yield Y in equilibrium for a population consisting of cohorts of all ages

$$Y = R \exp(F t_{\mu}) \int_{t_{\mu}}^{\infty} \exp(-(M + F)t) w(t) dt \quad (8)$$

where R is annual recruitment, F and M the fishing and natural mortality coefficients, $w(t)$ the weight which is usually computed by the von Bertalanffy function and μ is the mesh size, so that t_{μ} is the age when fish are first caught. The model can thus be used to prescribe management strategies in terms of fishing mortality and mesh size. Its main application has been for North Sea demersal fish stocks. Beverton and Holt recommended a mesh size of the order 110 mm for plaice and haddock with a 80–90 mm mesh to ensure survival of sole and whiting, and by the early 1960s minimum mesh sizes of that order were in operation in the North Sea⁶⁶.

More recently, the Leslie matrix formulation^{73–76} has been used to represent the age structure of fish and game populations subject to harvesting

$$\mathbf{x}_{i+1} = \mathbf{A} \mathbf{D} \mathbf{x}_i \quad (9)$$

the matrix \mathbf{A} having elements

$$a_{ij} = \begin{cases} f_j & \text{for } i = 1 \\ p_j & \text{for } i = 1 \\ 0 & \text{otherwise} \end{cases}$$

where f_j and p_j are the age specific fecundity and natural survival. The diagonal matrix \mathbf{D} with elements θ_i is the proportion of the i th age class surviving exploitation. The model has the merit of being conceptually simple and intelligible to non-mathematical biologists, although accurate field measurement of the necessary age specific parameters may present difficulties. Beddington⁷⁸ has, however, obtained reasonable estimates for red deer populations in Scotland. The harvest at equilibrium is given by

$$q = \sum_i (1 - \theta_i) n_i \quad (10)$$

and this is inserted in a Cobb–Douglas production function for the yearly crop

$$P = q X_3 = \beta X_1^{r_1} X_2^{r_2} X_3^{r_3} \quad (11)$$

where the X s are the number of stalkers, the area of the estate and the deer population respectively. Given the pattern of deer forest ownership in Scotland the estate size is not easily adjusted but the model can be used to provide a guide to the most profitable labour input.

Models such as the Beverton and Holt and Leslie matrix together with a variety of simulation models developed in the last few years provide the beginnings of tactical harvesting models, but there are many formidable problems to be overcome. In particular there are practical and theoretical difficulties in obtaining dynamic optimisation solutions to age structure models. These problems will be compounded when attempts are made to produce tactical versions from the few existing policy orientated models for multispecies harvesting and when, eventually, harvesting models encompass the whole ecosystem^{78,79}.

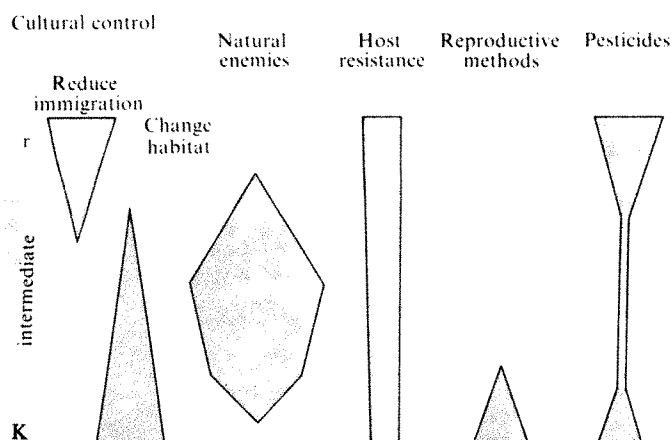


Fig. 5 Appropriate control strategies for pests categorised in terms of the r - K continuum⁸⁸.

Pest control

A further critical problem facing fishery ecologists is that of estimating parameters, in particular for density dependent processes^{61,80}. The theoretical consequences of different functional forms of these processes have been understood for some time but there are very considerable difficulties in obtaining accurate data. Insect populations, by contrast, can be more easily observed and experimented upon, so that considerably more is known about their population dynamics. This is particularly true of the relationships between insects and their predators and parasites, for which there is a range of mathematical models that have been well tested against laboratory and, less well tested against field data⁸¹⁻⁸⁵.

Surprisingly, however, there has been little effort to use these models to formulate strategies for pest control. A notable exception is Hassell and May's⁸⁶ analyses of the characteristics of insect parasitoids as agents of biological control. In a model which explicitly deals with spatial heterogeneity, population change for the host pests (X) and the parasitoids (P) is given by

$$X_{t+1} = \lambda X_t \sum_{i=1}^n [\alpha_i \exp(-a(c\alpha_i^\mu P_t)^{1-m})] \quad (12)$$

and

$$P_{t+1} = X_t - X_{t+1}/\lambda \quad (13)$$

where λ is the net rate of host increase, the $\{\alpha_i\}$ set defines the host distribution and μ , a and m are measures of aggregation, searching efficiency and mutual interference in the parasitoids. In general, high levels of a lead to low equilibria and high levels of m and μ to stability in the host population. Since highly specific parasitoids tend to show these features more markedly than polyphagous ones, it is argued that specific natural enemies are preferable as biological control agents where, for example in perennial orchards, equilibrium populations can be more readily maintained.

Recently, Conway⁸⁷ and Southwood⁸⁸ have attempted to provide a stronger link between theoretical ecological models and practical pest control, by applying the r - K selection continuum to the choice of control strategies. They distinguish at one extreme, r -pests such as the desert locust, characterised by high rates of dispersal and highly damaging population explosions, and at the other extreme K -pests such as the codling moth, with lower rates of increase, regulated by competition and whose status as pests is achieved more because of the character of the damage they cause. In between lies a large category of pests normally regulated at various levels by parasites and predators. Simple models^{89,90} have been produced for the different categories and used to prescribe general rules for control (Fig. 5). Although these notions are still somewhat crude and oversimplified they provide the beginnings of a fairly rigorous strategy analysis for pest control.

A good example of the intelligent application of simple models of this order is in pesticide resistance. Again, surprisingly, there have been no attempts until very recently⁹¹⁻⁹³ to model resistance in spite of its critical importance in modern pest control practice. Comins^{94,95} has, however, developed a deterministic Mendelian model which demonstrates, for example, the existence of a hysteresis effect in the presence of migration from untreated populations, the population jumping from high susceptibility to high resistance as migration drops below a critical level. Density dependence is included in the model in the form

$$X_{t+1} = \lambda X_t^{1-b} \quad (14)$$

where b is the density dependence coefficient. For perfect density dependence ($b = 1$) resistance time decreases with increasing kill but where the relationship is undercompensating ($0 < b < 1$) intermediate kills result in the fastest resistance. In general, for reasonable levels of density dependence it seems that resistance can be suppressed by using highly effective non-residual insecticides, strictly confined to the target population.

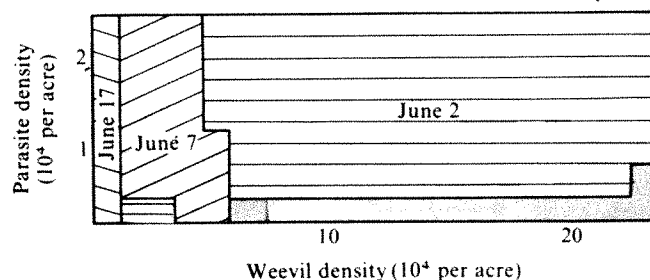
Simple density dependence models have also been used to explore the interrelationships between ecological and economic factors in pest control. In a study of the sugar cane froghopper, *Aeneolamia varia saccharina*, Conway *et al.*⁹⁶ show how the optimal spraying strategy is a function of the degree of density dependence between the different broods that the froghopper passes through in a season. In the absence of density dependence ($b = 0$) heavy spraying in the first brood is optimal but as the relationship goes from undercompensating to overcompensating ($1 < b < 2$) the spraying becomes progressively heavier in later broods. Models of this kind are not prescriptive but play an important part in determining which of a multitude of ecological factors merit further research before control decisions are made.

Dynamic programming⁹⁷ was used to arrive at the froghopper result and this technique has been used to derive optimal strategies for a number of pests^{91,92,98-101}. Its usefulness is restricted by the number of variables which can be included, but if this problem is resolved dynamic programming has a considerable potential as a tool for day-to-day management. As Shoemaker has shown⁹⁹ the results can be presented as simple decision rules in graphic form (Fig. 6), which allow an optimal strategy to be pursued even when the outcome of earlier decisions has not been as expected.

The problem of modelling age structure has also been tackled, as in harvesting models, by use of the Leslie matrix formulation^{96,102}. Recently Guttierrez, Wang *et al.*¹⁰³⁻¹⁰⁵ have used a continuous form, which is time and age dependent, to model both plant growth and the pest population on cotton and alfalfa. They seem to have been fairly successful in obtaining appropriate data but in general age class parameters are difficult to measure in many insect populations.

To date, most attempts at tactical models have taken the form of complex computer simulations. At worst they have suffered from the defects of the large-scale ecosystem models but there have been some notable exceptions, kept functional and useful because they have concentrated on the fairly circumscribed question of the optimal timing of pesticide application. Regev *et al.*⁹⁸ have developed such a model for the alfalfa weevil, *Hypera brun-*

Fig. 6 Optimal control strategies for the alfalfa weevil in terms of a standard insecticide spray (shaded area) and date of alfalfa harvesting¹⁰⁰.



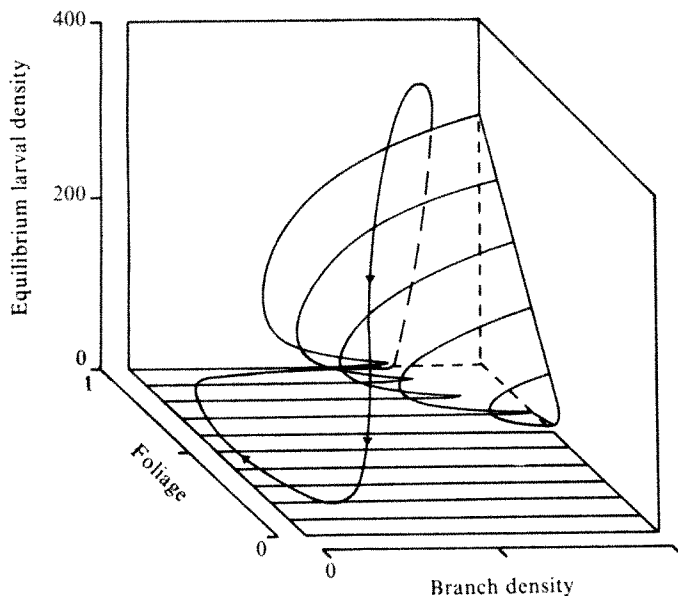


Fig. 7 Path of a single outbreak cycle of the spruce budworm in terms of living foliage per branch and different densities of branches per acre¹¹⁰.

neipennis, and demonstrated that higher profit would accrue from earlier spraying in the season. Kiritani and his colleagues^{106–109} have similarly used a simulation model of the dynamics of the green leafhopper *Nephotettix cincticeps*, and the *Lycosa* spiders which are its principal enemies to demonstrate the detrimental effects of BHC spraying which leads to destruction of the spiders and resurgence of the leafhopper. As a consequence the insecticide has been phased out from control campaigns in Japan.

Perhaps the most successful simulation model has been developed by Holling and his co-workers¹¹⁰ for the spruce budworm (*Choristoneura fumiferana*) in New Brunswick. At first a very complex model, it has been progressively bounded so that the essential dynamics of the system (Fig. 7) can be represented in terms of a limited number of variables relating to the pest, its natural enemies, the three main tree species, weather and, significantly, space. The final model reproduces the budworm dynamics for a 17,000 square mile region in terms of 265 discrete subdivisions. The model has been used to derive strategies of control under a variety of objectives, one set of outputs being in the form of graphical decision rules (compare Fig. 6) for spraying and logging obtained by a Markovian optimisation procedure.

Decision making

Underlying most policy models is the assumption that the farmer or trawler owner is a profit maximiser and hence is risk neutral; that is he values each successive increment in income in the same way. In practice, however, resource managers, whether at the individual, corporate or national level, exhibit a wide range of attitudes toward the outcomes of their decisions. Norton^{111–113} has recently illustrated the problem in the context of pest control and demonstrated the value of Bayesian techniques in the relatively common situation where the farmer is risk averse; that is, he values initial increases in income far higher than later increases. For example Bayesian analysis of potato blight control in England with the objective of a long run average profit maximisation leads to schedule spraying in the Southwest and Fens and no spraying in the Southern and Northern Regions. Because of the high variance associated with no spraying, however, the risk averse farmer in the latter regions may decide to spray anyway. Bayesian analysis can be further usefully extended to assessments of the value of pest forecasting. It is often naively assumed that a forecast is always useful but in practice the value depends on critical threshold levels in the accuracy of the forecast.

Such decision making has eventually to be placed in the broader context of ecosystem and regional management, which is likely to have to resolve conflicting and often incommensurate objectives. The most promising of the multivariate decision techniques is multi-attribute utility analysis^{114,115}, which has the advantage of dealing with uncertainty in a rigorous manner and of directly involving the decision maker in the definition and weighting of objectives. In common with other techniques, however, it is essentially static in that it seeks to identify an optimal plan which is adhered to for the foreseeable future. But, experience shows that objectives change or hidden objectives become manifest and unsuspected and unforeseeable events arise to disturb the management plan. Walters¹¹⁶ argues that this problem is compounded by a tendency for a wrong policy or design decision to beget further wrong decisions when dramatic problems arise so that, in practice, there is a progressive foreclosure of options. These are issues which have been addressed in the spruce budworm¹¹⁰ and a related salmon study^{117–119}. A general conclusion is that the main goal of such studies and the models they generate is not to solve an economic maximisation function but to create some kind of robust system which is resilient to all conceivable ecological, economic, social and political change^{120,121}.

No unified theory

It is very clear from this review that mathematical modelling in the fields of vector borne disease, harvesting and pest control has proceeded at different rates and in largely different directions, despite Watt's vision of a unified science of resource management. Yet, it is also clear that each field could well profit from the experience of the others. Policy models, of the kind developed for fishery management are urgently required in both pest and vector borne disease control. Equally, the notions and models of density dependence that are being elaborated for pest control could provide a more powerful basis for determining strategies for harvesting and disease vector control. There is also a growing, albeit halting experience, in each field of attempts to incorporate the discrete variables of age, state and space in tactical models and to develop appropriately dynamic optimisation techniques, which needs to be shared. From this kind of cross fertilisation a common theory and practice of applied ecology could begin to emerge.

I thank D. J. Bradley, C. W. Clark, R. M. May and colleagues at Imperial College for helpful comments and criticism. Financial support from the Office of Resources and Environment in the Ford Foundation and the Natural Environment Research Council is gratefully acknowledged.

- ¹ Watt, K. E. F. *Ecology and Resource Management* (McGraw-Hill, New York, 1968).
- ² Conway, G. R. in *Insects: Studies in Population Management* (eds Geier, P. W., Clark, L. R., Anderson, D. J. & Nix, H. A.) 103–120 (Ecological Society of Australia, Canberra, 1973).
- ³ Jeffers, J. N. R. (ed.) *Mathematical Models in Ecology* (Blackwell Scientific, Oxford, 1972).
- ⁴ Patten, B. C. (ed.) *Systems Analysis and Simulation in Ecology* 1 & 2 (Academic, New York, 1971 & 1972).
- ⁵ Ruesink, W. G. *A. Rev. Ent.* **21**, 27–44 (1976).
- ⁶ Van Dyne, G. M. & Abramsky, Z. in *Study of Agricultural Systems* (ed. Dalton, G. E.) 23–106 (Applied Science, London, 1975).
- ⁷ Farr, W. J. *Soc. Sci.* 233–236 (1866).
- ⁸ Verhulst, P. F. *Correspondance Mathématique et Physique* **10**, 113–121 (1838).
- ⁹ Ross, R. *Report on the prevention of Malaria in Mauritius*, London (1908).
- ¹⁰ Ross, R. *The Prevention of Malaria*, 2nd ed. London (1911).
- ¹¹ Ross, R. *Nature* **87**, 466–467 (1911).
- ¹² Ross, R. *Proc. R. Soc. A* **92**, 204–230 (1916).
- ¹³ Graham, G. M. J. *Cons. perm. int. Explor. Mer.* **10**, 264–274 (1935).
- ¹⁴ Hjort, J., Jahn, G. & Ottestad, P. *Hvalrad. Skr.* **7**, 92–127 (1933).
- ¹⁵ Garfinkel, D. in *Computers in Biomedical Research* 2 (eds Stacey, R. W. & Waxman, B.) 205–216 (Academic, New York, 1965).
- ¹⁶ Holling, C. S. *Mem. Entomol. Soc. Can.* **48**, 86 (1966).
- ¹⁷ Jones, J. G. W. in *Rep. Grassld. Res. Inst.* 1965, 51–52 (1966).
- ¹⁸ Van Dyne, G. M. *Oak Ridge Natl. Lab. Tech. Memo.* 3957, 40 (1966).
- ¹⁹ Watt, K. E. F. (ed.) *Systems Analysis in Ecology* (Academic, New York, 1966).
- ²⁰ Neuhold, J. M. in *Systems Analysis and Simulation in Ecology* 3 (ed. Patten, B. C.) 7–12 (Academic, New York, 1975).
- ²¹ O'Neill, R. V. in *Ecological Modelling in a Resource Management Framework* (ed. Russell, C. S.) 251–282 (Resources for the Future, Washington, 1975).
- ²² Van Dyne, G. M. in *Mathematical Models in Ecology* (ed. Jeffers, J. N. R.) 111–172 (Blackwell Scientific, Oxford, 1972).
- ²³ Worthington, E. B. (ed.) *The Evolution of IBP* (Cambridge University, Cambridge, 1975).
- ²⁴ Park, R. A., Scavia, D. & Clesceri, N. L. in *Ecological Modeling in a Resource Management Framework* (ed. Russell, C. S.) 13–48 (Resources for the Future, Washington, 1975).
- ²⁵ Park, R. A. *et al.* *Simulation* **23**, 33 (1974).
- ²⁶ Patten, B. C. (ed.) *Systems Analysis and Simulation in Ecology* 3 & 4 (Academic, New York, 1975 & 1976).
- ²⁷ Shugart, H. H., Goldstein, R. A., O'Neill, R. V. & Munkin, J. B. *Oecol. Plant.* **9**, 231 (1974).
- ²⁸ Woodmansee, R. G. *Critique and analysis of the grassland ecosystem model ELM 93* (US IBP Grassland Biome, Natural Resource Ecological Laboratory, Colorado State University, 1975).
- ²⁹ Watt, K. E. F. in *Systems Analysis and Simulation in Ecology* 3 (ed. Patten, B. C.) 139–152 (Academic, New York, 1975).

- ³⁰ Chen, C. W. & Orlob, G. T. in *Systems Analysis and Simulation in Ecology* 3 (ed. Patten, B. C.) 476-588 (Academic, New York, 1975).
- ³¹ Kelly, R. A. in *Systems Analysis and Simulation in Ecology* 4 (ed. Patten, B. C.) 3-45 (Academic, New York, 1976).
- ³² Biswas, A. K. *Ecological Modelling* **1**, 31-48 (1975).
- ³³ Holcomb Research Institute. *Environmental Modeling and Decision Making* (Praeger, New York, 1976).
- ³⁴ Mitchell, R., Mayer, R. A. & Downhower, J. *Science* **192**, 859-865 (1976).
- ³⁵ Watt, K. E. F. *Simulation* **28**, 1-3 (1977).
- ³⁶ Walker, B. H., Norton, G. A., Conway, G. R., Comins, H. N. & Birley, M. J. *appl. Ecol.* (in the press).
- ³⁷ Macdonald, G. *The Epidemiology and Control of Malaria* (Oxford University, London, 1957).
- ³⁸ Macdonald, G. *Publ. Hlth Rep., Wash.* **76**, 753-764 (1961).
- ³⁹ Najera, J. A., *Bull. Wild Hlth Org.* **50**, 449-457 (1974).
- ⁴⁰ Macdonald, G., Cuellar, C. B. & Foll, C. V. *Bull. Wild Hlth Org.* **38**, 743-755 (1968).
- ⁴¹ Dietz, K., Molineux, L. & Thomas, A. *Bull. Wild Hlth Org.* **50**, 347-357 (1974).
- ⁴² Cohen, J. E. *Ann. Rev. Ecol. Syst.* (in the press).
- ⁴³ Cohen, J. E. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 237-256 (Blackwell Scientific, Oxford, 1976).
- ⁴⁴ Fine, P. E. M. (ed.) *Mathematical Models of Schistosomiasis* (Edna McConnell Clark Foundation, New York, 1976).
- ⁴⁵ Hairston, N. G. in *Epidemiology and Control of Schistosomiasis (Bilharzia)* (ed. Ansari, N.) (Basel, University Park, Baltimore, 1973).
- ⁴⁶ Lee, K. L. & Lewis, E. R. *IEEE Trans. Biomed. Eng.* **BME-23**, 225-233 (1976).
- ⁴⁷ May, R. M. *Math. Biosci.* (in the press).
- ⁴⁸ Naselli, I. *Theor. Pop. Biol.* **10**, 47-69 (1976).
- ⁴⁹ Naselli, I. *Theor. Pop. Biol.* **10**, 133-144 (1976).
- ⁵⁰ Naselli, I. & Hirsch, W. M. *Commun. pure appl. Math.* **26**, 395-453 (1973).
- ⁵¹ Macdonald, G. *Trans. R. Soc. trop. Med. Hyg.* **59**, 489-506 (1965).
- ⁵² Bradley, D. J. & May, R. M. *Proc. R. Soc. trop. Med. Hyg.* (in the press).
- ⁵³ Fenwick, A. *Bull. Wild Hlth Org.* **47**, 573-578 (1972).
- ⁵⁴ Fenwick, A. & Figenschou, B. H. *Bull. Wild Hlth Org.* **47**, 567-572 (1972).
- ⁵⁵ Meurs, L. A. *Economic Project Evaluation with Philippine Cases* (University of the Philippines, Manila, 1969).
- ⁵⁶ Wright, W. H. *Bull. Wild Hlth Org.* **47**, 559-566 (1972).
- ⁵⁷ Clark, C. W. *Mathematical Bioeconomics. The Optimal Management of Renewable Resources* (Wiley-Interscience, New York, 1976).
- ⁵⁸ Christy, F. T. *Alternative Arrangements for Marine Fisheries: An Overview* (Resources for the Future, Washington DC, 1973).
- ⁵⁹ Graham, M. *Cont. int. L'Explor. Mer. Rapp. P.-v. Reun.* **132**, 72-78 (1952).
- ⁶⁰ Gulland, J. A. *The Management of Marine Fisheries* (University of Washington, Seattle, 1974).
- ⁶¹ Cushing, D. *Marine Ecology and Fisheries* (Cambridge University, London, 1975).
- ⁶² Schaefer, M. B. J. *Fish. Res. Bd Can.* **14**, 669-681 (1957).
- ⁶³ Gordon, R. L. *J. Polit. Econ.* **62**, 124-142 (1954).
- ⁶⁴ Anderson, L. G. *J. Fish. Res. Bd Can.* **30**, 509-518 (1973).
- ⁶⁵ Copes, P. *Manchester School of Social and Economic Studies* **40**, 145-163 (1972).
- ⁶⁶ Cushing, D. *Fisheries Resources of the Sea and their Management* (Oxford University, Oxford, 1975).
- ⁶⁷ Chapman, D. G., Allen, K. R. & Holt, S. J. *Rep. int. Comm. Whal.* **14**, 32-106 (1964).
- ⁶⁸ Gambell, R. *Appl. Biol.* **1**, 247-343 (1976).
- ⁶⁹ Beddington, J. R. & May, R. M. *Science* (in the press).
- ⁷⁰ Clark, C. W. & Munro, G. R. *J. Envir. Econ. Management* **2**, 92-106 (1975).
- ⁷¹ Clark, C. W. *Advisory Committee on Marine Resources Research ACMRR MM SC 65* (F.A.O., London, 1976).
- ⁷² Beverton, R. J. H. & Holt, S. J. *On the Dynamics of Exploited Fish Populations* (Min. Ag. Fish & Food Fisheries, Investigation Series 219), London, 1957).
- ⁷³ Beddington, J. R. & Taylor, D. B. *Biometrics* **29**, 801-809 (1973).
- ⁷⁴ Leslie, P. H. *Biometrics* **35**, 213-245 (1948).
- ⁷⁵ Reed, W. J. *Math. Biosci.* **22**, 313-337 (1974).
- ⁷⁶ Usher, M. B. in *Mathematical Models in Ecology* (ed. Jeffers, J. N. R.) 29-60 (Blackwell Scientific, Oxford, 1972).
- ⁷⁷ Beddington, J. R. *J. Envir. Management* **3**, 91-103 (1975).
- ⁷⁸ Walsh, J. J. in *The Ecology of the Seas* (eds Cushing, D. H. & Walsh, J. J.) 388-407 (Blackwell Scientific, Oxford, 1976).
- ⁷⁹ Steele, J. H. *The Structure of Marine Ecosystems* (Harvard University, Cambridge, 1974).
- ⁸⁰ Parrish, B. B. (ed.) *Fish Stocks and Recruitment Rapp. P.-v. Reun. Cons. int. Explor. Mer* **164** (1973).
- ⁸¹ Beddington, J. R., Free, C. A. & Lawton, J. H. *J. anim. Ecol.* **45**, 791-816 (1976).
- ⁸² Gilbert, N., Gutierrez, A. P., Frazer, B. D. & Jones, R. E. *Ecological Relationships* (Freeman, San Francisco, 1976).
- ⁸³ Hassell, M. P. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 71-93 (Blackwell Scientific, Oxford, 1976).
- ⁸⁴ Murdoch, W. W. & Oaten, A. *Adv. Ecol. Res.* **9**, 2-131 (1975).
- ⁸⁵ Royama, T. *Res. Popul. Ecol. Suppl.* **1**, 1-91 (1971).
- ⁸⁶ Hassell, M. P. & May, R. M. *J. anim. Ecol.* **42**, 693-726 (1973).
- ⁸⁷ Conway, G. R. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 257-281 (Blackwell Scientific, Oxford, 1976).
- ⁸⁸ Southwood, T. R. E. in *Origins of Pest, Parasite, Disease and Weed Problems* (eds Cherrett, J. M. & Sagar, G. R.) 35-54 (Blackwell Scientific, Oxford, 1977).
- ⁸⁹ Southwood, T. R. E., May, R. M., Hassell, M. P. & Conway, G. R. *Am. Nat.* **108**, 791-804 (1970).
- ⁹⁰ Southwood, T. R. E. & Comins, H. N. *J. anim. Ecol.* **45**, 949-965 (1976).
- ⁹¹ Hueth, D. & Regev, U. *Am. J. agr. Econ.* **56**, 543-552 (1974).
- ⁹² Regev, U., Shahit, H. & Gutierrez, A. P. in *Proc. IASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IASA, Laxenburg, in the press).
- ⁹³ Taylor, C. R. & Headley, C. S. *Can. Ent.* **107**, 237-242 (1975).
- ⁹⁴ Comins, H. N. *J. theor. Biol.* **64**, 177-197 (1977).
- ⁹⁵ Comins, H. N. *J. theor. Biol.* **65**, 399-420 (1977).
- ⁹⁶ Conway, G. R., Norton, G. A., Small, N. J. & King, A. B. S. in *Study of Agricultural Systems* (ed. Dalton, G. E.) 193-229 (Applied Science, London, 1975).
- ⁹⁷ Bellman, R. *Dynamic Programming* (Princeton Univ., Princeton, 1957).
- ⁹⁸ Regev, U., Gutierrez, A. P. & Feder, G. *Am. J. agr. Econ.* **58**, 188-195 (1976).
- ⁹⁹ Shoemaker, C. A. *Math. Biosci.* **18**, 1-22 (1973).
- ¹⁰⁰ Shoemaker, C. A. in *Proc. IASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IASA, Laxenburg, in the press).
- ¹⁰¹ Watt, K. E. F. *Can. Ent.* **95**, 525-636 (1963).
- ¹⁰² Conway, G. R. in *Proc. XV International Congress of Entomology* 541-552 (Washington, DC, 1976).
- ¹⁰³ Gutierrez, A. P., DeMichele, D. W. & Wang, Y. in *Proc. XV International Congress of Entomology* 553-559 (Washington, DC, 1976).
- ¹⁰⁴ Wang, Y., Gutierrez, A. P. & Oster, G. *Can. Ent.* (in the press).
- ¹⁰⁵ Gutierrez, A. P. in *Proc. IASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IASA, Laxenburg, in the press).
- ¹⁰⁶ Kiritani, K. in *Proc. XV International Congress of Entomology* 591-598 (Washington, DC, 1976).
- ¹⁰⁷ Kiritani, K. in *Proc. IASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IASA, Laxenburg, in the press).
- ¹⁰⁸ Sasaba, T. & Kiritani, K. *Res. Popul. Ecol.* **16**, 231-244 (1975).
- ¹⁰⁹ Sasaba, T., Kiritani, K. & Urabe, T. *Res. Popul. Ecol.* **15**, 9-22 (1973).
- ¹¹⁰ Holling, C. S., Jones, D. D. & Clark, W. C. in *Proc. IASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IASA, Laxenburg, in the press).
- ¹¹¹ Norton, G. A. *Am. appl. Biol.* **84**, 444-447 (1976).
- ¹¹² Norton, G. A. *Agroecosystems* **3**, 27-44 (1976).
- ¹¹³ Norton, G. A. & Conway, G. R. in *Origins of Pest, Parasite, Disease and Weed Problems* (eds Cherrett, J. M. & Sagar, G. R.) 205-226 (Blackwell Scientific, Oxford, 1977).
- ¹¹⁴ Keeney, R. L. *IASA Research Memorandum* 75-43 (1975).
- ¹¹⁵ Keeney, R. L., Wood, E. F., David, L. & Csontos, K. *IASA Collaborative Publication* 76-3 (1976).
- ¹¹⁶ Walters, C. J. *IASA Research Report* 75-12 (1975).
- ¹¹⁷ Hilborn, R. & Peterman, R. M. in *Management for People* (ed. Ellis, D. V.) 68-98 (University of Victoria, British Columbia, 1977).
- ¹¹⁸ Hilborn, R. & Walters, C. J. *J. Fish Res. Bd Can.* **34**, 64-72 (1977).
- ¹¹⁹ Keeney, R. L. *J. Fish Res. Bd Can.* **34**, 49-63 (1977).
- ¹²⁰ Holling, C. S. *Am. Rev. Ecol. Syst.* **4**, 1-24 (1973).
- ¹²¹ Holling, C. S. *Proc. Conf. Future Energy Strategies* (Oak Ridge Associated Universities, in the press).
- ¹²² Larkin, P. A. *Trans. Am. Fish. Soc.* **106**, 1-11 (1977).

articles

Ocean-floor magnetotelluric sounding over North Central Pacific

Jean H. Filloux

Scripps Institution of Oceanography, La Jolla, California, 92093

Observations on the sea floor of naturally occurring electromagnetic signals reveal information on the deep structure of the Earth. Recent magnetotelluric soundings on the North Central Pacific have produced conductivity measurements which may relate to the partially molten zone or asthenosphere on which the well lubricated oceanic plates slide.

OBSERVATIONS over a wide range of frequencies of slowly fluctuating natural electric and magnetic fields at the Earth's surface can be used to infer the distribution of electrical conductivity in the Earth's crust and mantle^{1,2}. Because of the

strong dependence of the electrical conductivity of terrestrial materials on temperature and composition³ the magnetotelluric (MT) technique is now accepted as a valid and attractive method of geophysical investigation on land⁴⁻⁸. Providing that electric and magnetic fluctuating fields can be recorded on the sea floor, the MT method is also applicable to indirect exploration of the oceanic basement although high frequency shielding by the ocean tends to mask the influence of crustal layers⁹. A recent MT sounding on the North Central Pacific suggests a rapid initial conductivity increase downward through lithosphere and upper mantle, weakening sharply and probably reversing trend around 180±40 km. The resulting high conductivity layer may relate to the partially molten zone

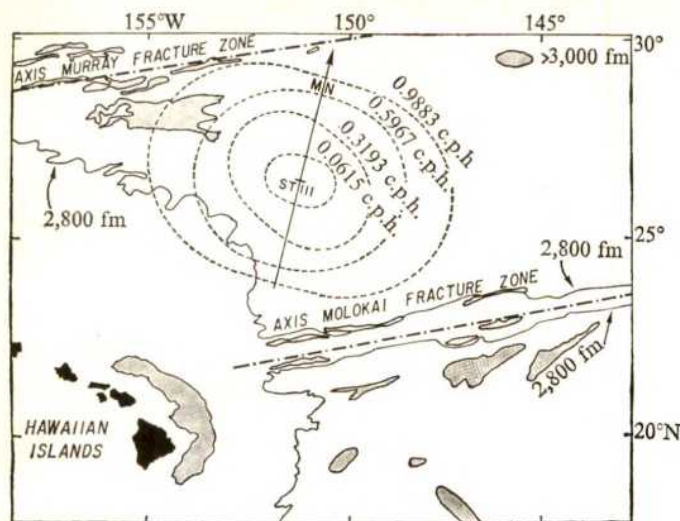


Fig. 1 Location of the observation station ($26^{\circ}35.9'N$, $151^{\circ}09.8'W$). The four elongated figures represent the ratio of the electric field to the magnetic field at right angle for four frequencies and with respect to the azimuth of the electric field. Important bathymetric features are also shown.

or asthenosphere on which the well-lubricated oceanic plate is allowed to slide.

The first suggestion of MT exploration at sea is in Cagniard's original paper², together with a symbolic diagram and the assertion that performing MT soundings on the ocean floor presents no special difficulty. In spite of Cagniard's optimism, as well as the actual simplicity inherent in the principles of the MT method, its application to submarine geophysics has progressed extremely slowly, probably because of the natural hostility of the oceanic environment and the remoteness of the deep sea floor.

The first MT success at sea was scored^{10,11} with a unidirectional MT sounding 600 km off Central California in 1965. In spite of many limitations, this contributed important confirmatory evidence of the considerable difference between continental and oceanic lithospheres, thus enhancing interest in the MT method in offshore geophysics. Newer data¹² collected in 1973 in the West Central Atlantic are being analysed. Nevertheless, the anticipated contribution of sea floor MT to submarine geology and geophysics has remained unproven until very recently.

In autumn, 1976, a new MT sounding was performed in the North Central Pacific with redundant equipment much superior to previously available instrumentation, see Fig. 1. As a result, the electric to magnetic field coherence and concurrently the resolution in characterisation of conductivity structure, improved enough to produce a meaningful structural picture of the local sea floor basement which seems compatible with other accepted knowledge.

Fundamental to this recent advance was the development of instruments with extremely low drift and noise, yet with extremely high resolution (10^{-7} V m⁻¹ and 0.2 nT). Electrode switching by means of a salt bridge chopper^{9,13} permitted essentially total rejection of electrode noise. (The natural signal of the optimum silver-silver chloride electrodes¹³ is typically 10^8 times larger than the signal to be recorded.) Conceptually, the magnetic sensors borrow from high grade fibre and magnet technology already optimised in the time of classical physics and from the most recent advances in modern optometrics.

Recording MT fluctuations

The first 2 weeks of a 3 week redundant recording cross coverage by two electric field recorders and two magnetometers are plotted on Fig. 2. **H** is the magnetic variation to the magnetic North, **D** to the magnetic East, and **Z** downward; **E_x** and **E_y**,

are the horizontal electric field components to the magnetic North and East, respectively.

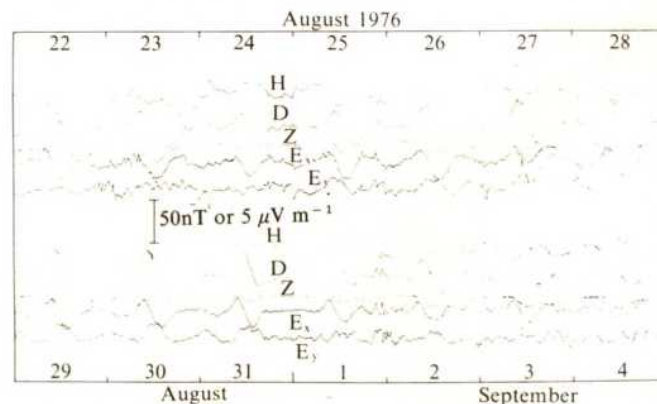
If Fig. 2 were compared with similar records covering a long period of time the following would be noticed. The daily modulation of the various fields is smooth and regular, particularly with respect to component **D**, although high frequency signals are often present. This relatively quiet behaviour is consistent with the time of collection of data with respect to the 11-yr sunspot and solar storm cycle which had reached its low point. The time reference in the above plot is Hawaii Standard Time (HST). Local noon at our station is therefore slightly early; the maximum descending slope of **D** at midday is slightly ahead with respect to noon, and the difference is consistent with the station longitude.

Although magnetic field disturbances (originated in the ionosphere and beyond, and controlled by fluctuations in the solar wind) as well as their electric field counterpart are numerous on the record, their amplitude is moderate to low, particularly with respect to the higher frequencies: in addition to the inherent low activity characteristic of the present solar cycle state, the shielding of the ocean greatly reduces such fluctuations above a few c.p.h. and essentially suppress them above 15 to 20 c.p.h. Note that most intense electric fields occur when the rate of change of the magnetic field is highest, a fact well exemplified by the rapid excursion of **H** early on 24 and on 27 August and the corresponding large electric spikes in **E_y**, at right angle to **H**.

The power spectra of the four components, **H**, **D**, **E_x**, **E_y**, have been calculated using a fast Fourier transform and band averaging as shown in Fig. 3; note that both spectra and frequency scales are logarithmic. The number of bands resulting in individual frequency estimates is not regular and is roughly five times larger towards the high frequency end. This choice is in part due to our effort to separate subtidal and intertidal bands which must be appreciably contaminated by the signals from strong oceanic tidal motions. (Electric fields of the motional type are generated by the displacement of oceanic water across the field lines of the Earth's main magnetic field.) The tidal bands as well as the relevant spectral estimates are indicated: clearly their energy density is substantially higher than in the adjacent intertidal bands. This excess is split between ionospheric signals from the daily variation and oceanic signals, the latter contributing mostly in **E_x** and **E_y**. Because of the mixing of these processes these bands are not used in the analysis that follows.

The reduced smoothness towards lower frequency is not simply due to the existence of the tidal peaks: in this area the number of Fourier bands forming each estimate is reduced, being limited by the duration of the record available. But, the existence of another source of noise below 0.3 c.p.h. is strongly

Fig. 2 Sea floor electric and magnetic variations for two periods of one week: **H** horizontal magnetic field to the magnetic north, **D** to the east, **Z** downward; **E_x** horizontal electric field to the magnetic north, **E_y** to the east. Time and scales indicated.



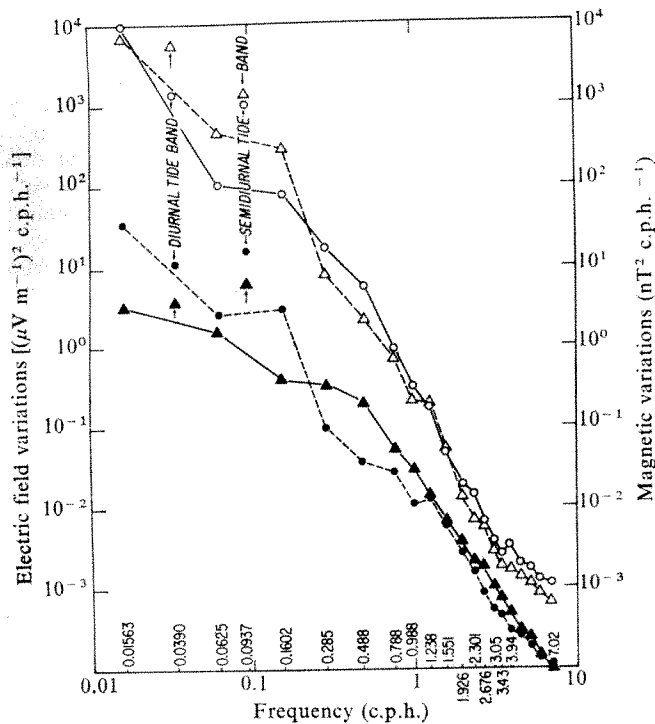


Fig. 3 Spectra of electric and magnetic variations. Notice special estimates corresponding to diurnal and semidiurnal tide bands, characterised by the higher energy density resulting from oceanic tidal motions. Δ , D ; \circ , H ; \bullet , E_x ; \blacktriangle , E_y .

suspected, with internal waves and internal tides as prime candidates.

The spectra of H , D and E_x , E_y show very similar behaviour, characterised by a very steep slope toward high frequencies. Also, features in the otherwise rather straight spectral curves, such as a jog around 1 c.p.h. track well, D with respect to E_x , and H with respect to E_y , as expected.

To the extreme right there is a sudden break toward a lesser slope in both H and D , occurring earlier in H than in D . This feature represents the instrumental noise contamination generated by the tape recorder stepping motor. Its greater effect on H compared to D simply relates to the closer proximity of suspension H to the recorder. (Shielding and spacing has suppressed this problem.)

In the following, the individual Fourier spectra are used to calculate cross-spectra and coherencies between all pairs of electric and magnetic components. From these estimates both impedances $Z(f)$ and admittances $A(f)$ are independently estimated according to

$$\begin{vmatrix} E_x \\ E_y \end{vmatrix} = \begin{vmatrix} Z_{xH} & Z_{xD} \\ Z_{yH} & Z_{yD} \end{vmatrix} \begin{vmatrix} H \\ D \end{vmatrix} \quad \text{or} \quad E(f) = Z(f) B(f)$$

and

$$\begin{vmatrix} H \\ D \end{vmatrix} = \begin{vmatrix} A_{Hx} & A_{Hy} \\ A_{Dx} & A_{Dy} \end{vmatrix} \begin{vmatrix} E_x \\ E_y \end{vmatrix} \quad \text{or} \quad B(f) = A(f) E(f)$$

where Z_{xH} etc., A_{Hx} etc., are the elements of the impedance and admittance tensors $Z(f)$ and $A(f)$, with f the frequency, $B(f)$ the vector magnetic field (H , D) and $E(f)$ the vector electric field (E_x , E_y).

If the assumption fundamental to the originally described MT method of an isotropic horizontally layered basement were to be satisfied, in the absence of ionospheric contamination¹⁴, (1) the diagonal elements of $Z(f)$ and $A(f)$ would be zero (the induced electric field is at right angle with the inducing field), (2) the absolute value of the antidiagonal elements would be equal and independent of the reference frame orientation and

(3) $Z(f)$ would be the exact inverse of $A(f)$.

In the presence of a contamination of electric and magnetic signals, equations (1) and (2) relate to the alternate assumptions that all noise affects only the electric, equation (1) or only the magnetic field, equation (2).

The hypothesis of isotropy can be easily tested visually by plotting the ratio of E to B at right angles to E as a function of the azimuth of E for various frequencies, see the result in Fig. 1. The moderate degree of elongation of the expected ideal circular patterns is consistent with a relatively minimal anisotropy. This situation greatly simplifies interpretation of the observed electromagnetic signals.

In the absence of precise knowledge concerning the distribution of noise in E and B we have assumed at first that both E and B are equally affected. We have in addition retained the combination of Z_{xD} and Z_{yH} which, according to Berdichevskiy¹⁵ minimises the effect of possible local heterogeneity of the conductivity distribution. The impedance thus arrived at and used in the interpretation is

$$Z'(f) = (Z_{\text{eff}}/A_{\text{eff}})^{\dagger} \quad (3)$$

where Berdichevskiy's Z_{eff} effective¹⁵ is defined by

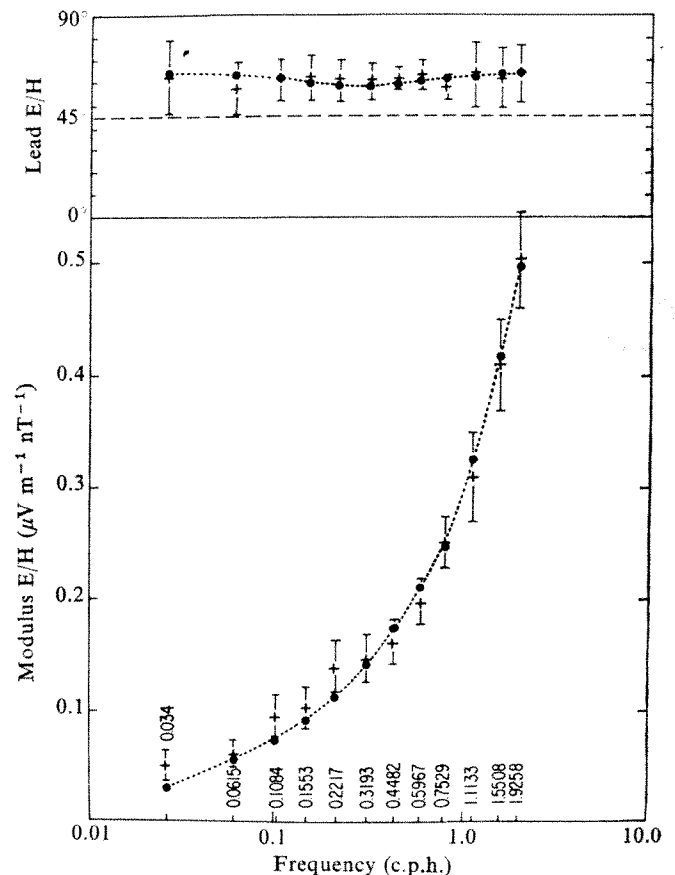
$$Z_{\text{eff}} = \frac{1}{2} (Z_{xD} - Z_{yH}) \quad (4)$$

and with

$$A_{\text{eff}} = \frac{1}{2} (A_{Hy} - A_{Dx}) \quad (5)$$

The resulting set of 12 impedances (sometimes called MT ratios, E/B) are plotted in amplitude and phase in Fig. 4 together with error bars at 95% confidence level (the frequency scale is logarithmic). The dotted line represents the impedance function characteristic of an isotropic horizontally layered model optimum with respect to the observed impedances.

Fig. 4 Impedances, or magnetotelluric ratios, derived from crossspectra between electric and magnetic signals, in amplitude (lower box) and phase (upper box). Error bars are indicated at the 95% confidence level. --- \bullet ---, the optimum model; +, observations.



Conductivity distribution and depth

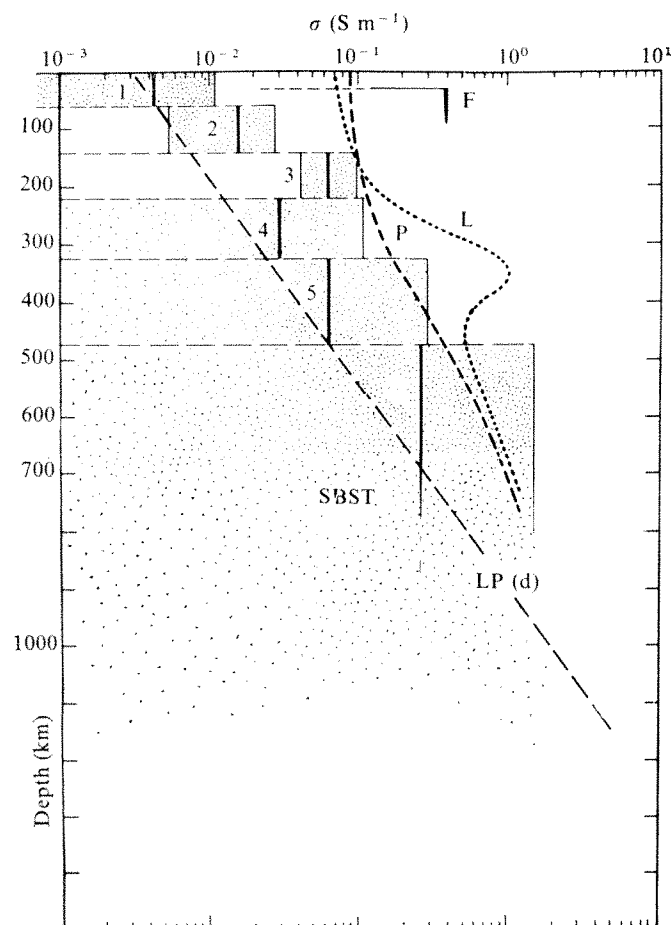
The method used to select the conductivity distribution with depth that best fits the observations will now be outlined briefly; 12 frequency estimates are available providing 12 amplitudes and 12 phases, thus a total of 24 constraints in a least square fit of the model predictions. The allowable number of model parameters thus must be substantially less than 24. With each horizontal layer involving thickness and conductivity—thus two parameters—a realistic model should include appreciably less than 12 layers. Using n layers with pre-determined thicknesses and conductivities the resulting impedances are calculated. The mismatch model to observation is estimated in terms of the variance between observed and predicted impedance functions.

The effect of changing the conductivity on the variance is then studied for each layer and improved conductivity values are updated accordingly. Convergence is rapid for the layers well characterised by the available cross-spectral estimates. From this rate of convergence, or equally well from the sensitivity of the variance to small conductivity changes, important implications result about the validity of the optimum model and the range of conductivity values acceptable in the various layers.

Following these guide lines we propose for the conductivity dependence with depth and for its range of uncertainty the five layer model with infinite half space substratum shown on Fig. 5. The layers are horizontal and isotropic. Their thicknesses are, from top downwards 60, 80, 80, 100 and 150 km respectively.

The layers best defined by the set of available impedances and

Fig. 5 Optimum six layer model of an isotropic, horizontally layered sea floor structure. Shaded area indicates range of uncertainty. Best resolved layer is layer 3, with a significantly high conductivity probably related to the asthenosphere. Estimates from other authors also shown.



in the order of decreasing resolution are 3, 2 and 4; the increased uncertainty in layer 1 adjacent to the ocean results from the high frequency cut off due to oceanic shielding, while decreasing resolution below layer 4 is a consequence of the low frequency impedance unreliability, itself related to the finite record length and increased oceanic motional noise. The influence of this signal is strongly supported by the much higher interpretability of the impedance estimates when noise contamination is assumed to be affecting mainly the electric field.

Earlier estimates of the Earth's electrical conductivity with depth are also shown in Fig. 5 as interpretative background, namely global averages proposed by Lahiri and Price¹⁶ (LP) and Parker¹⁷ (P), Larsen's profile below Hawaii¹⁸ (L) and Filloux's profile¹⁹ (F) 600 km offshore from Central California. The implied conductivity at the observation site is intermediate between that proposed by Lahiri and Price on the low side and that of Parker on the high side. A conductivity maximum is strongly suggested by the high conductivity average over an 80 km thickness in layer 3, with the value of 0.065 S m^{-1} . The actual conductivity maximum may, of course, be much higher, with a correspondingly smaller thickness. Its most probable depth of occurrence ranges within $180 \pm 40 \text{ km}$.

Although not very well resolved, the crustal conductivity is moderate to low, which is consistent with a sediment layer only a few hundred-metres thick and with the relatively old age of the local lithosphere: the matching seafloor spreading magnetic anomaly¹⁹ corresponds to reversal number 31 and to a plate age of 72 Myr (ref. 20).

The probability of a high conductivity tongue within layer 3 should be tested against processes implied by our present understanding of plate tectonics and ocean floor spreading, and against profiles of other physical properties of terrestrial materials over the same depth and, if possible, the same area.

A most attractive and immediate implication is that layer 3 contains the shear and partly molten layer or asthenosphere between mantle and lithosphere which permits plate motion. This view is in rough agreement with the range of occurrence of the oceanic low shear velocity layer²¹, namely 130–270 km. It is also compatible with Odegard's interpretation of the Cannikin/airborne experiment²² which suggests for an area broadly inclusive of our station, a low P-wave velocity layer extending from 210 to 320 km. Indirect estimates, however, of the lithospheric thickness^{23,24} suggest only 75 km for the local plate age. The discrepancy may be more apparent than real, relating to the still little known thickness of the asthenosphere in general and to the presently limited information concerning electrical conductivity of mantle materials, itself closely dependant on temperature and small changes in composition³.

The other MT profiles available from not-too-distant areas, that of Larsen (L), Fig. 5, and that of Filloux (F), like the present one predict or imply high conductivity layers and probable inversions. It is logical to attempt to relate these features and to tentatively predict that their significance may have something in common. Certainly the very high conductivity at a very shallow depth in the F profile is compatible with a much younger lithospheric age, about 32 Myr. The extremely high conductivity maximum in Larsen's model occurs at a considerably greater depth and may relate to the origin of the Hawaiian island chain.

In conclusion, and to give the foregoing analysis a realistic perspective, future MT sea floor work can be carried out with a considerably improved efficiency. Specifically, the following advantages can be readily attained: (1) longer records, up to one year, can be achieved with existing technology; (2) the frequency range can be readily extended upward by a factor of 4 and perhaps occasionally by a factor of 8 to 16; (3) broad coverage with instrumental arrays shall permit study of multidimensional structures such as spreading centres, subduction zones where old crust is consumed and trenches, island chains and arcs; and (4) magnetic activity, at a low during the present experiment shall progressively rise during the next five years, providing more energetic ionospheric fluctuations, thus

allowing improved resolution in general, and particularly in shallower crustal structures.

I thank Drs Larsen and Winter for constructive criticisms and programming advice, L. Green and H. Moeller for technical assistance. This work was supported by the USNSF.

Received 31 May; accepted 19 July 1977.

- ¹ Tikhonov, A. N. *Doklady Akad. Nauk S.S.S.R.* 73, 275–295 (1950).
- ² Cagniard, L. *Annls Geophys.* 9, 95–125 (1953).
- ³ Shankland, T. J. S. *Phys. Earth planet Int.* 10, 209–219 (1975).
- ⁴ Cantwell, T. & Madden, T. R. *J. geophys. Res.* 65, 4202–4205 (1960).
- ⁵ Swift, C. M. thesis, M.I.T., (1967).
- ⁶ Hermance, J. F. *Trans. Am. Geophys. Union* 52, IUGG (1971).
- ⁷ Vozoff, K. & Swift, C. M. *Intl. Upper Mantle Proj. Contrb.* 152 (1966).

- ⁸ Vozoff, K. *Geophysics* 37, 98–141 (1972).
- ⁹ Filloux, J. H. *Phys. Earth Planet Int.* 7, 323–338, (1973).
- ¹⁰ Filloux, J. H. thesis, Univ. California, La Jolla, California (1967).
- ¹¹ Cox, C. S., Filloux, J. H. & Larsen, J. C. *The Sea* 4, Part I, 637–693 (Wiley, New York, 1971).
- ¹² Bennett, D. J. & Filloux, J. H. *Rev. geophys. Space Phys.* 13, 197–239 (1975).
- ¹³ Filloux, J. H. *J. Geomag. Geoelectr.* 26, 269–279 (1974).
- ¹⁴ Swift, D. W. & Westcott, E. M. *J. geophys. Res.* 69, 4149–4154 (1964).
- ¹⁵ Berdichevskiy, M. N. & Demitriev, V. I. in *Geol. Geoth. St. KAPG* (Adam, A. ed.) (Akademiai Kiado, Budapest, 1976).
- ¹⁶ Lahiri, B. N. & Price, A. T. *Phil. Trans. R. Soc.* A237, 509–540 (1939).
- ¹⁷ Parker, R. L. *Geophys. J. R. astr. Soc.* 22, 121–138 (1970).
- ¹⁸ Larsen, J. C. *Geophys. J. R. astr. Soc.* 43, 17–46 (1975).
- ¹⁹ Atwater, T. & Memard, H. W. *Earth planet. Sci. Lett.* 7, 445–450 (1970).
- ²⁰ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman III, W. C. & Le Pichon, X. *J. geophys. Res.* 73, 2119–2136 (1968).
- ²¹ Leeds, A., Knopoff, L. & Kausel, E. G. *Science* 186, 141–143 (1974).
- ²² Odegard, M. E. thesis, Univ. Hawaii (1975).
- ²³ Press, F. J. *geophys. Res.* 75, 6575–6581 (1970).
- ²⁴ Parker, R. L. & Oldenburg, D. W. *Nature* 242, 137–139 (1973).

Deep-sea carbonate and the deglaciation preservation spike in pteropods and foraminifera

W. H. Berger

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

The aragonite compensation depth fluctuated considerably during the last 20,000 yr. This fluctuation culminated in a preservation spike at about 14,000 yr BP. The aragonite preservation stratigraphy parallels that in calcitic foraminifera, and constitutes part of a world-wide phenomenon signalling considerable changes in ocean chemistry and providing a useful tool for correlation.

THERE is abundant evidence that the effects of carbonate dissolution on the deep sea floor changed considerably as a result of Pleistocene climatic fluctuations. Within the last 20,000 yr, there has been a drastic worldwide drop of the aragonite compensation depth and of foraminiferal preservation levels during deglaciation, and a subsequent rapid rise to the present position. This fascinating phenomenon, which was a geologically almost instantaneous preservation pulse of 'spike', is the subject of this article.

The deglacial preservation spike was reported by the marine geology group at Kiel, working from the research vessel Meteor on the continental slopes off Portugal and Morocco^{1–4}. It expresses itself most commonly as a pteropod-rich layer, at the very end of the last glacial. The interpretation of this event is complicated by evidence for downslope redeposition. However, in many cases the pteropod layer does not seem to be associated with redeposited material, and a change in dissolution effects seems indicated as pointed out by Diester-Haass⁵. More recently, she proposed mass mortality of pteropods to explain the phenomenon⁶.

Recent evidence from the western equatorial Pacific^{7–8} indicates that the deglacial preservation spike is a world-wide event. Shackleton⁹ has drawn attention to this possibility and has commented on some geochemical implications of such an event.

Before turning to such implications, of which there are many, let us review briefly the evidence for the preservation stratigraphy from the last glacial to the present, that is, the last 20,000 yr.

Evidence for preservation stratigraphy

A drastic rise in the calcite compensation level at the glacial-Holocene boundary was recognised in the North Pacific¹⁰ on the basis of published foraminiferal data^{11,12}. Quite generally, there are fewer calcareous foraminifera in

Holocene sections of cores from the North Pacific than in the underlying glacial ones^{13,14}; the change has been dated at 12,500 yr BP¹⁵. Analogous observations were made elsewhere (East Pacific Rise¹⁶; Equatorial Pacific¹⁷; Andaman Sea¹⁸). The recognition of a Holocene dissolution pulse led to a reinterpretation of Arrhenius's carbonate cycles in the equatorial Pacific¹⁹ in terms of dissolution cycles^{20–23} with increased dissolution during interglacials, as suggested earlier by Olausson²⁴. Subsequently it was discovered that the dissolution cycles appear shifted with respect to the glacial-interglacial cycles^{25–27}. One type of shift can be produced by centering a carbonate preservation maximum on deglaciation, some 4,000 yr after the glacial maximum.

On examining the literature, it becomes evident that such a preservation spike exists not only in the eastern North Atlantic^{1–4} and in the west equatorial Pacific^{7,8}, but also in the Caribbean and in the Gulf of Mexico²⁸, on the western slope of India^{29,30}, and in many places in the deep sea, although here slow sedimentation rates and wide spacing of sampling intervals make recognition subject to doubt in each individual case (Atlantic: refs 31–33; Pacific refs 22, 23, 25).

The magnitude and the timing of the preservation pulse can be assessed. The pteropod data from the slope off NW Africa^{3,4} indicate that the euglacial ACD (lower depth limit of pteropods) stood at 1.5 km, the deglacial one at 3.5 km, and the postglacial one at 0.5 km (Fig. 1a). The corresponding ACD levels in the western equatorial Pacific are 1 km (?), 1.7 km, and 0.5 km (Fig. 1b). Similar fluctuations can be documented elsewhere and in shells other than pteropods (Table 1).

It will be noted that preservation during pre-deglacial

Table 1 Magnitude of preservation spike in calcareous shells

Type of level	Area	Depth (km) during			Source
		Post-G.	De-G.	Eu-G.	
ACD	Off NW Africa	0.5	3.5	1.5	Refs 3, 4
ACD	Off Portugal	1.1	3.6	2.8	Ref. 2
ACD	Off W India	0.3	2.5	1.2	Ref. 29
ACD	W. Eq. Pacific	0.5	1.7	1(?)	This article
RCD	W. Eq. Pacific	2.8	3.8	3.1	This article

ACD: lower depth limit of pteropods. RCD: lower depth limit of *Globigerina rubescens*, a planktonic foraminifer that is highly susceptible to dissolution. Post-G.: postglacial.

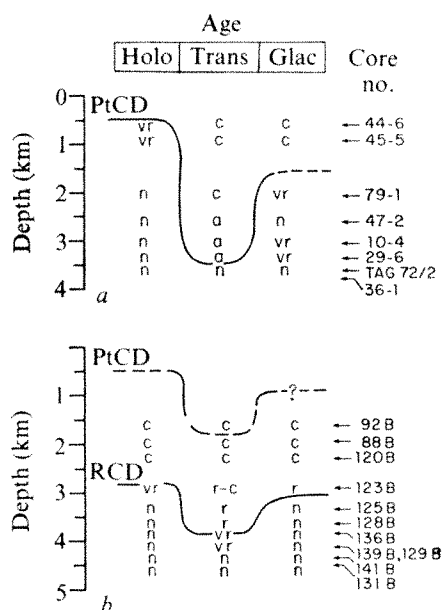


Fig. 1 Excursion of the compensation depth for pteropods (PtCD) and for *Globigerina rubescens* (RCD) during the transition period from glacial to Holocene time. *a*, Off NW Africa, based on data given by Diester-Haass (refs 3 and 4). *b*, Western equatorial Pacific, own data.

('euglacial') is indeed better than during postglacial time, in agreement with earlier assessments based on accumulation rate comparisons²⁰. However, the preservation story is clearly more complicated than a simple glacial-interglacial dichotomy would suggest.

The timing of the preservation spike is of crucial importance if the geochemical driving mechanisms are to be discovered. The data in Kudrass² suggest that the spike is centred in 13,500 yr BP. Our own data from the equatorial Pacific⁸ and those of Chen and of van Donk (in ref. 20) indicate maximum preservation during maximum change in the oxygen isotope signal. In the Gulf of Mexico, this period of maximum change is dated at about 15,000 yr to 12,500 yr BP^{34,35}, although the exact timing is in some dispute. In the west equatorial Pacific, the period of maximum change (and the preservation spike) seems to be close to 14,000 yr BP (ref. 8). The same period is centred between 13,500 and 14,000 yr BP in the dated isotope sequence of a core in the equatorial Atlantic²⁶. One may conclude that the spike has its maximum at about 14,000 yr BP.

The spike is narrow, perhaps no more than 1,000 yr wide. Considering the effects of bioturbation, the resolution of such an event in deep sea cores represents the limit for this type of record. A further constraint on the timing is given by the rapid increase of dissolution following this spike, starting at about 13,000 yr BP^{14,15}. In fact, the very recording of the rapid onset of the dissolution pulse in deep sea sediment, despite benthic mixing and the presence of good preservation immediately preceding it, strongly suggests a flip-flop situation, with a dissolution spike immediately following a preservation spike.

Geochemical and stratigraphical implications

What are some of the geochemical and stratigraphic implications of the deglacial preservation spike?

Fundamentally, we can enhance preservation of carbonate in deep waters by increasing saturation, by decreasing the speed of bottom currents, or by suddenly increasing sedimentation rate, for example, by redeposition. I suggest that a combination of these factors is at work, rather than any one process. First, let us examine saturation.

The thermodynamic equations on which saturation determination depends contain the following alterable terms³⁷: (1) partial pressure of CO₂, and (2) temperature and chlorinity. Availability of CaCO₃ (3) enabling reactions to proceed towards equilibrium, also is a variable in the real ocean. We see that the first item refers to a property of the atmosphere, the second to the water itself, and the third to conditions at the sea floor.

To enhance preservation then, the following possibilities arise:

- Lowering of the CO₂ pressure in the atmosphere. Shackleton⁹ has suggested that the rapid growth of the biosphere during deglaciation would lead to extraction of CO₂ from the atmosphere-ocean system, and hence result in improved preservation of carbonate. Both a change from glacial aridity to postglacial humidity in tropical latitudes³⁸ and the recession of tundra and ice would be responsible for this 'biosphere effect'. If one adds the dead carbon ('thanatosphere') that would build up together with the growing forests and below the rising sea level, this effect could indeed be substantial—of the order of 1% of the entire ocean-atmosphere CO₂ content. In areas of gentle depth gradients of saturation, such a change could result in marked excursions of the dissolution level. One problem with accepting Shackleton's biosphere effect as the main or sole factor is the timing of the major changes in floral cover. Apparently this major change is not centred on 14,000 yr BP, but rather occurs between 13,000 yr and 10,000 yr ago^{39,40}, that is, during the dissolution pulse rather than the preservation spike.

- Increase in temperature of deep ocean waters. Weyl⁴¹ speculated that abyssal waters would have warmed during glacials and Worthington⁴² pointed out that a low-salinity meltwater lid due to deglaciation would lead to stagnant bottom waters, affecting carbonate preservation. There is indeed evidence for such a glacial warming of deep water⁴³⁻⁴⁵, at least in the Atlantic Ocean. This 'glacial bottom water effect' does not seem to be focused enough to account for the preservation spike, although it must be taken into account in the overall glacial-postglacial preservation fluctuations.

- Decrease of the chlorinity of deep ocean waters. The addition of meltwater eventually decreases chlorinity by some 3%. This is most likely done with run-off water, which brings its own dissolved load of calcium carbonate. The exact effect on saturation of the ocean as a whole would seem to depend on the magnitude of this load, which would depend on the rate of recycling of the water, through continuous evaporation and precipitation, during the time of net addition to the ocean, and on the rate of concomitant chemical erosion.

- Increase of availability of CaCO₃ in abyssal depths. There is considerable evidence for large-scale redeposition of shallow water sediments in the Late Pleistocene, including pteropod-rich sediments^{2-4,15,28,35}. Such transport makes carbonate particles available at abyssal depths. Here bottom waters are greatly undersaturated and are able to dissolve such particles at a maximum rate. On rising, therefore, these waters will quickly reach saturation values which preclude rapid attack of calcareous shells. The timing of this 'neutralisation' of abyssal waters seems to be correct.

Two more possibilities can be added, considering that the ocean is not in equilibrium:

- Decrease in the rate of extraction of carbonate in surface waters, by calcareous organisms. A reduction of precipitation of shells in surface waters will increase the overall saturation of the ocean with the respective substance and therefore enhance preservation^{46,47}. (Thus, the relationship between overall fertility and preservation of biogenous sediments is exactly the reverse of that usually suggested.) It is possible that a rapid dumping of meltwater, combined with incomplete mixing, would temporarily reduce produc-

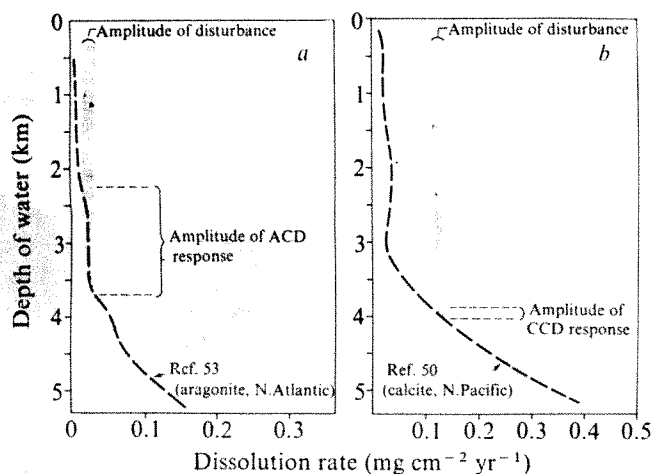


Fig. 2 Compensation depth fluctuations as a response to a disturbance (for example change in supply). The amplitude of the response is strongly governed by the depth gradient of dissolution, and its relationship to the disturbance. The reaction of the system to a given disturbance provides insights into the state of undersaturation.

tivity, although there is no evidence for such a 'fertility effect'.

● Increase in the rate of supply of alkalinity to the ocean as a whole. The redeposition of shelf carbonates (the fourth item, above) constitutes addition of alkalinity. Also, the alkalinity supply may be increased during deglaciation by an increase in the suspended and dissolved load of river input, through erosion of glacial weathering products (loess, calcareous desert crusts, morainal debris). If conditions fluctuated between arid and humid and before the plant cover could re-establish itself, this would provide for maximum erosion. Also, sea level was still considerably depressed, leaving the upper shelf areas exposed for erosion, and facilitating bypassing of the lower ones by the sediment introduced. Considering the evidence for transport of terrigenous and shelf deposits into abyssal waters, the 'neutralisation effect' could have operated earlier, on a reduced level, throughout glacial conditions, aided by additional supply whenever arid conditions changed to humid.

It should be noted that the type of stagnation visualised by Worthington⁴², due to a hypothetical meltwater lid, should lead to a decrease in bottom currents (enhancing preservation) but also to a build-up of CO_2 (enhancing dissolution). In this scenario, when ocean mixing finally catches up with the meltwater, CO_2 is released to the atmosphere, and a climatic optimum ensues. Increased CO_2 , together with rapid extraction of carbonate on the flooded shelves, probably accounts for the dissolution pulse following the preservation spike.

It is obviously tempting to go on speculating about the relationship between CO_2 fluctuations and preservation and dissolution pulses, especially in view of the man-made CO_2 input to the atmosphere and its presumed ultimate effects on carbonate deposition and climate⁸. Suffice it to say that the type of analysis presented here, which is an example of preservational stratigraphy, has considerable potential for palaeoclimatic studies in the deep sea as well as in shallow waters⁴⁴.

The most obvious use of the preservation spike is for correlation and dating of deep sea cores. Relative preservation aspects can be determined extremely rapidly by visual inspection, for example, by noting the degree of fragmentation of foraminifera in the fine sand fraction. Thus, the peak preservation is easily found. We have already made considerable use of this method in the deep-sea sediment

laboratory at the Scripps Institution of Oceanography. A word of caution is in order, however. Before determining sedimentation rates by this method, the displacement of a spike by benthic mixing must be considered. This displacement is downward, by up to one mixed layer thickness, depending on the ratio between the thickness of the layer and the thickness corresponding to the actual duration of the event. For example, a rate of 1.93 cm per 1,000 yr would be calculated for the pteropod-containing core Bx 92 (Fig. 1b), if the centre of the pteropod preservation spike at 27 cm is divided by 14,000 yr, the estimated age of the event. Allowing for a mixed layer of 6 cm (ref. 17), and a duration of the spike of 2,000 yr (3–4 cm), the offset is 4.5–4 cm (mixed layer thickness minus one-half the width of the event, see ref. 49). Thus, the actual sedimentation rate, under these assumptions, is closer to 1.6 cm per 1,000 yr than to 1.9 cm per 1,000 yr, a non-trivial correction. The problem is not restricted to the dating method presented here, but is of a general nature. It is negligible only in cores with high sedimentation rates.

One final note of caution in interpreting the significance of preservation fluctuations: a change in preservation is a result of both the condition of the system (sensitivity, that is, depth gradient of potential dissolution rate) and the strength of the agent of change (disturbance, resulting in a change in the record). Depth gradients of dissolution can change both in space^{30–33} and in time^{34–36}. Disturbances of similar magnitude, therefore, can produce greatly differing amplitudes in preservational signals (Figs 2a, b).

In fact, the response amplitudes of high frequency ACD and CCD fluctuations through time may be a useful tool for determining variations in the strength of the depth gradient of undersaturation in the deep ocean. For example, relatively small disturbances in Late Cretaceous carbonate sedimentation in the central Atlantic apparently were responsible for large CCD excursions³⁷ presumably due to high sensitivity of the system (gentle dissolution depth gradient). In comparing the record of marginal seas to that of the open ocean, it must be kept in mind that these seas may not play exactly the same tune as the open ocean. Thus the Late Pleistocene and Holocene preservation stratigraphies of the Mediterranean, the Red Sea, the Sea of Japan and the Arctic Ocean are entirely different from the one described here (see refs 28, 58, 59).

This research was supported by the National Science Foundation and by the Office of Naval Research. Parts of the manuscript were written at the Geologisches Institut in Kiel, Germany. I thank Professor E. Seibold and my other colleagues in Kiel for their hospitality.

Received 28 April; accepted 18 May 1977.

- 1 Thiede, J. *Meteor. Forsch. Ergebn. Reihe C* 7, 15–102, (1971).
- 2 Kudrass, H.-R. *Meteor. Forsch. Ergebn. Reihe C* 13, 1–63 (1973).
- 3 Diester-Haass, L., Schrader, H.-J. & Thiede, J. *Meteor. Forsch. Ergebn. Reihe C* 16, 19–66 (1973).
- 4 Diester-Haass, L. *Meteor. Forsch. Ergebn. Reihe C* 20, 1–32 (1975).
- 5 Diester-Haass, L. & van der Spoel, S. *Palaeogeogr. Palaeoclimat. Palaeoecol.* (in the press).
- 6 *Fate of Fossil Fuel CO_2 in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 7 Berger, W. H. in *Fate of Fossil Fuel CO_2 in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 8 Berger, W. H. & Killingley, J. S. *Science* 197, 563–566 (1977).
- 9 Shackleton, N. J. in *Fate of Fossil Fuel CO_2 in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 10 Berger, W. H. *Mar. Geol.* 8, 111–138 (1970).
- 11 Nayudu, Y. R. *Science* 146, 515–517 (1964).
- 12 Saidova, H. M. *Progr. Oceanography* 4, 143–152 (1965).
- 13 Bandy, O. L. *Progr. Oceanography* 4, 27–49 (1967).
- 14 Barnard, W. D. & McManus, D. A. *Geol. Soc. Am. Bull.* 84, 2097–2100 (1973).
- 15 Duncan, J. R., Fowler, G. A. & Kulm, L. D. *Geol. Soc. Am. Bull.* 81, 561–566 (1970).
- 16 Broecker, W. S. & Broecker, S. *Soc. Econ. Geol. Paleont. Spec. Publ.* 20, 44–57 (1974).
- 17 Berger, W. H. in *Benthic Processes and Geochemistry of Interstitial Waters of Marine Sediments* (ed. Fanning, K. A. & Manheim, F. T.) (Joint Oceanogr. Congress, Edinburgh, in the press).
- 18 Frerichs, W. E. *Science* 159, 1436–1458 (1968).
- 19 Arrhenius, G. *Rep. Swed. Deep Sea Exped.* 5, 1–228 (1952).
- 20 Broecker, W. S. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 239–265 (Yale University, 1971).
- 21 Berger, W. H. *J. Foraminif. Res.* 3, 187 (1973).
- 22 Thompson, P. R. & Saito, T. *Geology* 2, 333–335 (1974).

- ²³ Thompson, P. R. *J. Foram. Res.* **6**, 208–227 (1976).
²⁴ Olausson, E. *Progr. Oceanography* **3**, 221–252 (1965).
²⁵ Luz, B. & Shackleton, N. J. *Cushman Found. Foram. Res. Spec. Publ.* **13**, 142–150 (1975).
²⁶ Pisias, N. G., Heath, G. R. & Moore, T. C. *Nature* **256**, 716–717 (1975).
²⁷ Moore, T. C., Pisias, N. G. & Heath, G. R. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
²⁸ Chen, C. *Nature* **21**, 1145–1149 (1968).
²⁹ von Stackelberg, U. *Meteor. Forsch. Ergebn. Reihe C* **9**, 1–73 (1972).
³⁰ Zobel, B. *Meteor. Forsch. Ergebn. Reihe C* **12**, 9–73 (1973).
³¹ Ruddiman, W. F. *Geol. Soc. Am. Bull.* **82**, 282–302 (1971).
³² Thiede, J. *1973 Geol. Soc. Am. Bull.* **84**, 2749–2754 (1973).
³³ Damuth, J. E. *Deep-Sea Res.* **22**, 725–743 (1975).
³⁴ Kennett, J. P. & Shackleton, N. J. *Science* **188**, 147–150 (1975).
³⁵ Emiliani, C., Gartner, S., Lidz, B., Eldridge, K., Elvey, D. E., Huang, T. C., Stipp, J. J. & Swanson, M. F. *Science* **189**, 1083–1088 (1975).
³⁶ Wiseman, J. D. H. *Rep. Swed. Deep Sea Exped.* 288–354 (1965).
³⁷ Gieskes, J. M. in *The Sea* (ed. Goldberg, E. D.) **5**, 123–151 (1974).
³⁸ Damuth, J. E. & Fairbridge, R. W. *Geol. Soc. Am. Bull.* **81**, 189–206 (1970).
³⁹ van der Hammen, T., Wijmstra, T. A. & Zagwijn, W. H. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 391–424 (Yale University, 1971).
⁴⁰ Wright, H. E. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 425–464 (Yale University, 1971).
⁴¹ Weyl, P. K. *Meteorol. Monogr.* **9**, 37–62 (1968).
⁴² Worthington, L. V. *Meteorol. Monogr.* **8**, 63–67 (1968).
⁴³ Duplessy, J. C., Chenouard, L. & Vili, F. *Science* **188**, 1208–1209 (1975).
⁴⁴ Streeter, S. S. *Quat. Res.* **3**, 131–141 (1973).
⁴⁵ Schnitker, D. *Nature* **248**, 385–387 (1974).
⁴⁶ Berger, W. H. *Geol. Soc. Am. Bull.* **81**, 1385–1402 (1970).
⁴⁷ Berger, W. H. & Roth, P. H. *Rev. Geophys. Space Phys.* **13**, 561–585, 624–635 (1975).
⁴⁸ Alexandersson, E. T. *Science* **189**, 47–48 (1975).
⁴⁹ Berger, W. H. & Heath, G. R. *J. Mar. Res.* **26**, 134–143 (1968).
⁵⁰ Peterson, M. N. A. *Science* **154**, 1542–1544 (1966).
⁵¹ Ben-Yaakov, S., Ruth, E. & Kaplan, I. R. *Science* **184**, 982–984 (1974).
⁵² Ben-Yaakov, S., Ruth, E. & Kaplan, I. R. *Deep Sea Res.* **21**, 229–243 (1974).
⁵³ Milliman, J. D. *Geology* **3**, 461–462 (1975).
⁵⁴ Berger, W. H. *Nature* **236**, 392–395 (1975).
⁵⁵ van Andel, T. J. H., Heath, G. R. & Moore, T. C. *Geol. Soc. Am. Memoir* **143**, 1–134 (1975).
⁵⁶ Heath, G. R., Moore, T. C. & van Andel, T. J. H. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
⁵⁷ Berger, W. H. & von Rad, U. *Init. Rep. Deep Sea Drilling Project* **14**, 787–954 (1972).
⁵⁸ Ujiie, H. & Ichikura, M. *Trans. Proc. Paleont. Soc. Japan, N. S.* **91**, 137–150 (1973).
⁵⁹ Herman, Y. *Marine Geology and Oceanography of the Arctic Seas* 283 (Springer, Heidelberg, 1974).

Structure of a dinucleoside phosphate–drug complex as model for nucleic acid–drug interaction

S. Neidle, A. Achari & G. L. Taylor

Department of Biophysics, University of London King's College, 26–29 Drury Lane, London WC2, UK

Helen M. Berman, H. L. Carrell, J. P. Glusker & W. C. Stallings

The Institute for Cancer Research, 7701 Burholme Avenue, Fox Chase, Philadelphia, Pennsylvania 19111

The crystal structure of a 3 : 2 complex of the frameshift mutagen proflavine with the dinucleoside phosphate cytidylyl-3'-5'-guanosine has been determined. The complex has one drug molecule intercalated between Watson-Crick base pairs of the nucleotide duplex. The other two proflavine molecules are bound to the exterior of the miniature double helix. The orientation of the base pairs in this miniature double helix has aspects similar to that found in RNA II.

It is well established that many drugs and carcinogens act by binding to nucleic acids; both nuclear double-stranded DNA and RNA have been shown to be targets for such interactions^{1,2}. Many such drugs are characterised by a planar aromatic structure, and the acridines, in particular, have been the subject of much study¹. The concept of drug intercalation between adjacent base pairs of the nucleic acid, first introduced by Lerman³, accounts satisfactorily for many observed properties of drug–nucleic acid complexes, and is widely accepted. Intercalation is believed to be responsible for the activity of many of these drugs as frameshift mutagens^{4–6}. In addition, intercalation, and the subsequent unwinding of nucleic acids, has emerged as an important tool in the study of superhelicity^{7,8}.

Intercalation necessitates an alteration in the geometry of the double helix at (and, presumably, adjacent to) the actual binding site. In normal B DNA the turn angle of rotation between adjacent nucleotide residues is 36°. In the original model for intercalation³ the helix uncoils by 45° from this value. Subsequent studies^{9–12} using various drugs including proflavine provide support for the intercalation hypothesis although the degree of concomitant unwinding remains uncertain with values ranging from –45° to 12°. Linked atom refinement techniques¹³ have been used to define further both the degree of unwinding as well as the exact conformational features of DNA complexed with proflavine.

Proflavine (Fig. 1) is involved in more than one mode of

binding^{1,14}. In addition to the strong intercalative process which occurs at low drug levels, there is a weaker binding of the drug, presumably external to the helix. Temperature-jump relaxation kinetics data^{15,16} show, however, that the strong binding process is itself a complex one, involving as an initial step, externally-bound drug molecules. It is apparent that a realistic visualisation of proflavine–DNA binding should involve both factors.

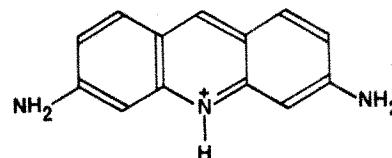


Fig. 1 A proflavine cation.

We have determined the crystal structure of a complex between proflavine and cytidylyl-3',5'-guanosine (CpG) (Fig. 2), in the hope that this would serve as a model for proflavine–nucleic acid interactions. We were encouraged in this belief by analyses of two other dinucleoside monophosphates^{17,18}, which showed the features of an RNA-like miniature double helix, and of several drug–dinucleoside phosphate complexes^{19,20} which demonstrated intercalative binding. The proflavine–CpG complex was deliberately produced without any heavy atoms so as to avoid the possibility that they might perturb the structure.

Crystallographic analysis

Large, well formed single crystals of the complex were produced from an aqueous solution of proflavine hemisulphate and free acid CpG. The crystals showed triclinic symmetry, space group *P*1, with cell dimensions *a* = 16.098 Å, *b* = 16.755 Å, *c* = 12.933 Å, α = 112.67°, β = 113.69° and γ = 99.36°. As has been observed with other dinucleoside phosphates²¹, however, the diffraction pattern displays pronounced pseudosymmetry such that it

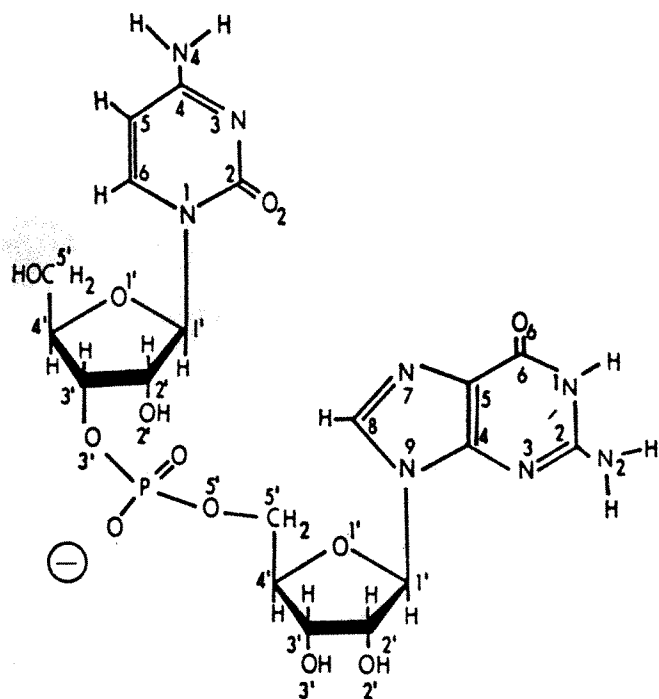


Fig. 2 The cytidyl 3',5'-guanosine anion.

was possible to index the reflections in a monoclinic cell, space group $C2$, with cell dimensions $a = 29.487$ Å, $b = 12.933$ Å, $c = 16.883$ Å and $\beta = 121.94^\circ$. Because of this ambiguity, the intensity data were measured on the basis of the space group $P1$. A crystal was sealed in a glass capillary in which mother liquor was included.

A total of 5,621 independent reflections was measured, to a limit of 1 Å resolution, on an automatic four-circle diffractometer using $\text{CuK}\alpha$ radiation. Of these, 4,073 reflections were considered to have intensity significantly greater than background. In addition, Friedel mates for 356 reflections were measured and the agreement, R_{sym} , for these 356 Friedel pairs was found to be 0.038. When the reflection data were transformed to space group $C2$, the agreement among the symmetry-related reflections was 0.139. The fact that the agreement for symmetry related reflections for space group $P1$ was significantly better than for space group $C2$ was taken as a strong indication that space group $P1$ is more probable. Accordingly, the structural analysis was carried out in the latter space group.

Numerous attempts at structure solution by direct and search methods were unsuccessful. The structure was eventually solved by location of the phosphorous atoms using the high resolution Patterson technique²², followed by a number of cycles of (E and F) Fourier syntheses to determine the rest of the structure. Early stages of the analysis of the crystal structure were greatly facilitated by the use of the interactive computer graphics system²³ at the Institute for Cancer Research. This permitted the rapid detailed evaluation of possible structural fragments at each stage of the analysis. Least squares refinements, as yet incomplete, have reached an R of 0.155 and the overall structure is well ordered; only a few atoms display high thermal motion.

Proflavine interacts with miniature double helix in several ways

A projection of the structure is illustrated in Fig. 3. It is a 3:2 proflavine-CpG complex and represents the first reported crystal structure of drug-dinucleotide complex which does not have 2:2 stoichiometry. The two dinucleotides are hydrogen bonded to form a miniature double

helix with Watson-Crick base pairs, separated by 6.8 Å. One of the proflavine molecules is symmetrically intercalated between the base pairs and protrudes into the wide groove. Each amino group of this proflavine forms a hydrogen bond to a phosphate oxygen on either side of the duplex. These interactions, therefore, probably serve to stabilise the observed symmetry of intercalation. The other two proflavine molecules stack asymmetrically above and below the miniature double helix. The asymmetry is such that these proflavines tend to stack more over the guanine residues thereby preserving the pseudo-twofold symmetry which may be described as an axis perpendicular to the helix axis and lying along the $\text{C}(9)\text{--N}(10)$ vector of the intercalated proflavine molecule.

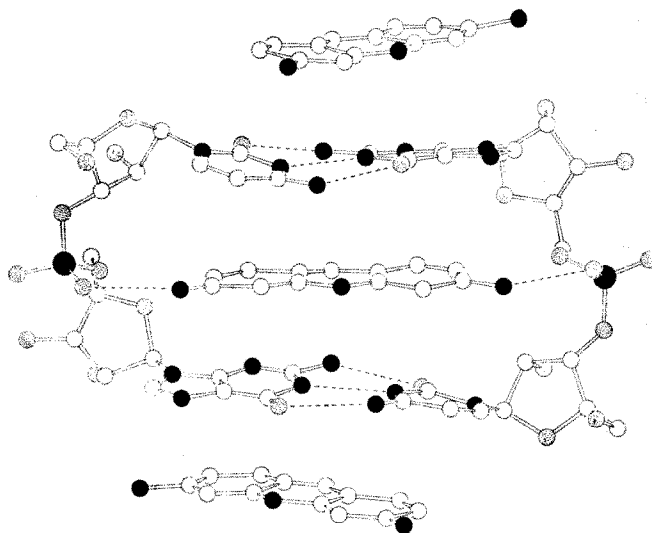
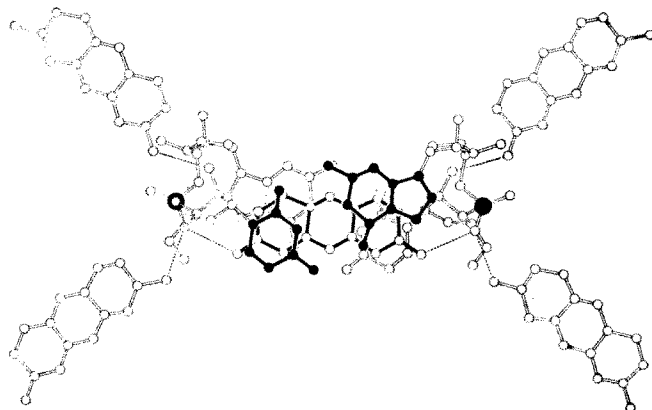


Fig. 3 A view of the structure of the complex, tilted slightly from the view parallel to the mean planes of the base pairs. Dotted lines represent hydrogen bonds. Nitrogen and phosphorus atoms are black and oxygen atoms are stippled.

Inspection of the non-intercalated proflavine molecules (translated to adjacent unit cells) shows that the molecules also bind externally to the duplex through a system of hydrogen bonding (Fig. 4). The external binding to the backbone of the duplex may be described by four unique hydrogen bonds—two from proflavine amino groups to phosphate oxygens and two more from proflavine amino groups to the $\text{O}(2')$ hydroxyl oxygens of the cytosine riboses. Note that the phosphate oxygens that are hydrogen bonded in this manner are also those that are hydrogen bonded to the amino groups of the intercalated proflavine

Fig. 4 A view of the structure, looking perpendicular to the planes of the base pairs. The two phosphorus atoms are shown in black, one as a heavy open circle and the other as a large solid circle. The atoms of the uppermost base pair are also in black and the intercalated proflavine has black bonds.



molecule. The packing in the crystal structure is such that the non-intercalated proflavines link adjacent base-paired duplexes by these external hydrogen bonds; when one amino group of an external proflavine molecule is hydrogen bonded to a phosphate oxygen, the other amino group of that proflavine molecule is hydrogen bonded to an O(2') hydroxyl oxygen of a neighbouring duplex (Fig. 5). The overall effect is a pattern of extensive cross-linking between adjacent miniature double helices.

The crystal structure of the complex is heavily hydrated, with 24 water molecules associated with each unit. This large degree of hydration lends credence to the relevance of this structure to nucleic acid-drug complexes. A full description of the many interactions involved will be published elsewhere.

Proflavine stacks with base pairs and also with other proflavines

THE sequence of stacked polycyclics is proflavine-base pair-intercalated proflavine-base pair-proflavine-proflavine-base pair-intercalated proflavine, and so on, so that proflavine-proflavine stacking also occurs (Fig. 5). In this case the long axes of the proflavine molecules lie at approximately 83° to each other. This is a different situation from that found in proflavine hydrochloride²⁴ where this angle is 180° and in proflavine hemisulphate where this angle is 150° (refs 2, 25). The skewness of stacking of proflavine molecules probably results from the fact that the terminal amino groups of proflavine are hydrogen bonded to phosphate oxygen atoms and to ribose O(2') hydroxyl groups. These hydrogen bonds position the molecule with respect to the base pairs and, to satisfy the crystallisation conditions, proflavine-proflavine stacking occurs as best it may.

The 3:2 nature of this complex, containing two mono-ionised anions of CpG (Fig. 2), suggests that one of the three proflavine molecules carries no charge. By symmetry one would expect this to be the intercalated proflavine molecule. Assignment of these charges must await further refinement and the location of hydrogen atoms.

The miniature double helix has unexpected features

Each base pair is twisted in a propeller-like fashion, much as has been found in other dinucleoside phosphate structures. The spacing between base pairs is 6.8 Å, as would be expected for a complex of this sort. What is not expected, however, is that the orientation of the two base pairs has aspects similar to that found in normal, uncomplexed RNA 11 (ref. 26). That is, the angle between the vectors connecting the glycosidic carbon atoms within each base pair is 33°, not the 8°–10° values found in the structures of iodo-CpG-ethidium bromide and iodo-CpG-9-amino-acridine.

Moreover, instead of having C(3') *endo* 3'-5' C(2') *endo* mixed sugar pucker²⁷, all the sugars are C(3') *endo*. The stretching (rather than unwinding) of this miniature helix is accomplished by systematic changes in the backbone and glycosidic angles. The implications of this surprising result with respect to the unwinding of polynucleotide structure are under investigation.

The results of this analysis allow us to explain various phenomena associated with proflavine complexation to nucleic acids. We have defined in geometric terms two modes of binding: (1) symmetrical intercalation with hydrogen bonding to the phosphate groups, and (2), hydrogen bonding to phosphate and ribose O(2') hydroxyl atom external to the helix. It is known that when acridines are used to stain cells, the RNA complex shows a red fluorescence whereas the DNA has a green fluorescence¹.

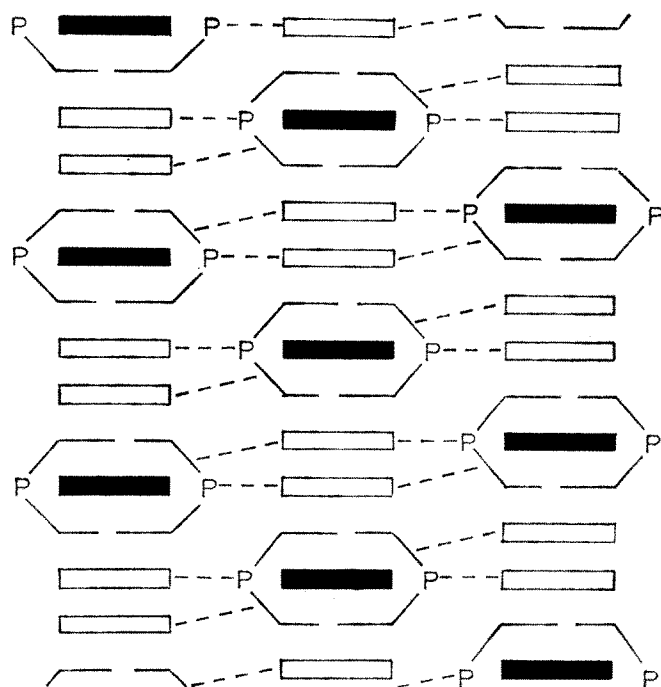


Fig. 5 A diagrammatic representation of the packing of the CpG and proflavine ions in the crystal lattice.

This difference may be explained, in part, by the hydrogen bonding between the O(2') hydroxyl of the ribose and the amino nitrogen of the proflavine since there is no O(2') hydroxyl group in DNA. The structure also shows the nature of the aggregation of the proflavine molecules themselves (see Fig. 5). Finally, the similarity of the orientation of the overlapping base pair planes to those in RNA 11 (as measured by the vectors between the C(1') glycosidic atoms), implies that proflavine does not unwind the helix.

This work was supported by grants CA-05322, CA-10925, GM-21589, CA-06927 and RR-05539 from the US NIH, an appropriation from the Commonwealth of Pennsylvania, and by grants from the Cancer Research Campaign, the SRC, and the Lawson Tait Trust. S.N. is grateful to the Royal Society and the Cancer Research Campaign for travel grants, and to the Institute for Cancer Research for their hospitality. We also thank G. M. Sheldrick, M. Spencer, L. Wakelin and M. Waring for helpful comments. *Note added in proof:* We have been informed by Alden and Arnott that they have developed a model for A DNA-proflavine binding which has many features similar to our structure. Sobell *et al.* have given a preliminary structural report of a 2:2 proflavine-iodo CpG complex (American Crystallographic Association meeting, August 1977).

Received 10 June; accepted 5 August 1977.

1. Peacocke, A. R. in *The Acridines* (ed. Acheson, R. M.) (Wiley, New York, 1973).
2. Neidle, S. & Jones, T. A. *Nature* **253**, 284–285 (1975).
3. Lerman, L. S. *J. molec. Biol.* **3**, 18–30 (1961).
4. Drake, J. W. & Baltz, R. H. *A. Rev. Biochem.* **20**, 11–37 (1976).
5. Roth, J. R. *A. Rev. Genet.* **8**, 319–346 (1974).
6. Crick, F. H. C., Barnett, L., Brenner, S. & Watts-Tobin, R. J. *Nature* **192**, 1227–1232 (1961).
7. Wang, J. C. *J. molec. Biol.* **89**, 783–801 (1974).
8. Waring, M. J. *J. molec. Biol.* **54**, 247–279 (1970).
9. Fuller, W. & Waring, M. J. *Ber. Bunsenges. Physik. Chem.* **68**, 805–808 (1964).
10. Lerman, L. S. *J. cell. comp. Physiol.* **64**, Suppl. 1, 1–18 (1964).
11. Neville, D. M. & Davies, D. R. *J. molec. Biol.* **17**, 57–74 (1966).
12. Bond, P. J., Langridge, R., Jennett, K. W. & Lippard, S. J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4825–4829 (1975).
13. Alden, C. & Arnott, S. *Nucleic Acid Res.* **2**, 1701–1717 (1975).
14. Peacocke, A. R. & Skerrett, N. J. H. *Trans. Faraday Soc.* **52**, 261–279 (1956).
15. Li, H. J. & Crothers, D. M. *J. molec. Biol.* **39**, 461–477 (1969).
16. Ramstein, J. & Leng, M. *Biophys. Chem.* **3**, 234–240 (1975).
17. Seeman, N. C., Rosenberg, J. M., Suddath, F. L., Kim, J. J. P. & Rich, A. *J. molec. Biol.* **104**, 109–144 (1976).

- ¹⁸ Rosenberg, J. M., Seeman, N. C., Day, R. O. & Rich, A. J. *molec. Biol.* 104, 145–167 (1976).
¹⁹ Tsai, C. C., Jain, S. C. & Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* 72, 628–632 (1975).
²⁰ Sakore, T. D., Jain, S. C., Tsai, C. C. & Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* 74, 188–192 (1977).
²¹ Hingerty, B. *et al.* *Biopolymers* 14, 227–236 (1975).
²² Sussman, J. L., Seeman, N. C., Kim, S. H. & Berman, H. M. *J. molec. Biol.* 66, 403–421 (1972).
²³ Bernstein, H. J. *et al.* *Crysmet—A Network of Intelligent Remote Graphics Terminals* Second Annual AEC Scientific Computer Information Exchange Meetings,

- Proceedings of the Technical Program* (1974) 148–158 BNL-18803 (Brookhaven National Laboratory, New York, 1974).
²⁴ Obendorf, S. K., Carrell, H. L. & Glusker, J. P. *Acta crystallogr.* B30, 1408–1411 (1974).
²⁵ Jones, A. & Neidle, S. *Acta crystallogr.* B31, 1324–1334 (1975).
²⁶ Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W. & Hodgson, A. R. J. *molec. Biol.* 81, 107–122 (1973).
²⁷ Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. & Sakore, T. D. *Proc. natn. Acad. Sci. U.S.A.* 73, 3068–3072 (1976).
²⁸ Schmechel, D. E. V. & Crothers, D. M. *Biopolymers* 10, 465–480 (1971).

Orientation of cell-surface antigens in the lipid bilayer of lymphocyte plasma membrane

Frank S. Walsh* & Michael J. Crumpton

National Institute for Medical Research, Mill Hill, London NW7, UK

Human HLA-A, B, C and Ia antigens were labelled by lactoperoxidase-catalysed iodination of the inner surface of lymphocyte plasma membrane and thus are transmembrane proteins. In contrast, membrane-bound human IgM and mouse IgM, IgD and Thy-1 antigen were not labelled on the inner membrane surface.

AN important role of the plasma membrane is to transmit biochemical signals generated by ligand–receptor interaction at the cell surface across the lipid bilayer and into the cell¹. The elucidation of the mechanisms of transmembrane informational transfer depends ultimately on the designation of the orientation of the membrane proteins in the bilayer. In this respect the transmembrane proteins are especially favoured candidates since they are exposed on both the outer and inner (cytoplasmic) membrane faces. Thus, this orientation has apparently been universally adopted by membrane transport proteins^{2,3}. It is, however, evident that transmembrane proteins are not a prerequisite for signal transmission and that other mechanisms also exist. For instance, the biological effects of certain outer cell surface hormone receptors are believed to be mediated by interaction with cytoplasmically disposed adenylate cyclases⁴.

In view of the apparent functional importance of transmembrane proteins, there are strong incentives to develop methods for their identification. One possible way is by comparing the patterns produced by covalent labelling of the outer and inner surfaces of cells or plasma membranes^{5–6}. Application of this approach to erythrocytes^{7–11} has demonstrated that both of the major erythrocyte glycoproteins, glycophorin and band 3, are transmembrane molecules. The results of these studies have led to the proposals that all membrane glycoproteins have a transmembrane orientation, that the non-glycosylated proteins are exposed on the inner surface only and that no protein is present exclusively on the outer surface unless it is associated with a glycoprotein which spans the lipid bilayer^{12,13}. The notable successes that have been achieved with erythrocytes are primarily due to two factors—the lack of intracellular membranes and the ready availability of sealed ‘inside-out’ membrane vesicles for labelling the inner membrane surface^{7,8}. Several proteins in various other systems have also been shown to be transmembrane—for example, (Na⁺, K⁺)-dependent ATPase¹⁴, intestinal brush border aminopeptidase¹⁵, cytochrome *c* oxidase¹⁶, Semliki forest virus coat protein¹⁷ and rhodopsin¹⁸. No generally applicable method for their detection has, however, emerged with the result that knowledge of the orientation of membrane proteins of nucleated cells has proceeded slowly.

Designation of transmembrane proteins

We describe here a method for defining the transmembrane proteins of lymphocyte plasma membrane. Lymphocytes were chosen for study because their surface structure regulates many

biological effects (for example, activation by antigen, ‘patching’ and ‘capping’ of surface receptors) and includes many antigens of known specificity (for example, Ia and Thy-1 antigens, IgM and IgD). Also, well-characterised preparations of the surface membrane are available¹⁹. The approach adopted for designating the orientation of membrane proteins relied on lactoperoxidase-catalysed iodination for covalent labelling, and the availability of inside-out membrane vesicles that were sealed to lactoperoxidase. Lactoperoxidase-catalysed iodination was selected in preference to other techniques because the conditions favouring vectorial labelling have been clearly defined²⁰. In particular, the free iodide concentration was adjusted to less than 10^{−6} M to promote iodination via an impermeable enzyme-activated iodine complex²⁰. The procedure suffers, however, from a fairly high degree of selectivity in that only proteins with exposed tyrosine and possibly histidine residues are labelled. A method for the preparation of inside-out vesicles of pig lymphocyte plasma membrane has been described previously²¹. Although the vesicles were permeable to trypsin and concanavalin A, a subpopulation of vesicles has been selected by dextran density gradient centrifugation that were sealed to dextran of 10,000 molecular weight and also, by implication, to lactoperoxidase (molecular weight 78,000). Lactoperoxidase-catalysed iodination of these vesicles followed by affinity chromatography on concanavalin A–Sephacrose gave a different pattern of labelled polypeptides than that obtained with viable pig lymphocytes²². At least eight labelled polypeptides of each sample comigrated on polyacrylamide gel electrophoresis in sodium dodecylsulphate and were, consequently, judged to have a transmembrane orientation. A disadvantage of this study was the inability to equate any defined surface component with a particular iodinated polypeptide. This problem has been circumvented by using antisera against defined surface components to precipitate the homologous antigen from ¹²⁵I-labelled, inside-out membrane vesicles after solubilisation in sodium deoxycholate. The orientation of the following cell surface antigens have been analysed using this approach: the HLA-A, B, C and Ia antigens, and IgM of cultured human lymphoblastoid B1 8 cells, as well as the Thy-1 antigen of mouse thymus cells, and IgM and IgD of mouse spleen cells.

Cell surface antigens

The choice of cell-surface antigens for study was determined by the availability of cells and specific antisera. The human B-lymphoblastoid cell line B1 8 was chosen because it represents a well-documented source of HLA-A, B, C and Ia antigens and membrane-bound IgM^{23,24}. Mouse thymus and spleen cells have the advantage of being a relatively rich source of Thy-1 antigen²⁵ and membrane-bound IgM and IgD²⁶ respectively. Goat anti-(human β_2 -microglobulin) serum raised by immunisation with purified urinary β_2 -microglobulin was used as a ligand for the human HLA-A, B and C antigens. This choice is based on the observation that β_2 -microglobulin is non-covalently associated with the products of the HLA-A, B and C genes on the cell surface²⁷. The antiserum was shown to be monospecific on the

* Present address: Laboratory of Biochemical Genetics, National Institutes of Health, Bethesda, MD 20014, USA

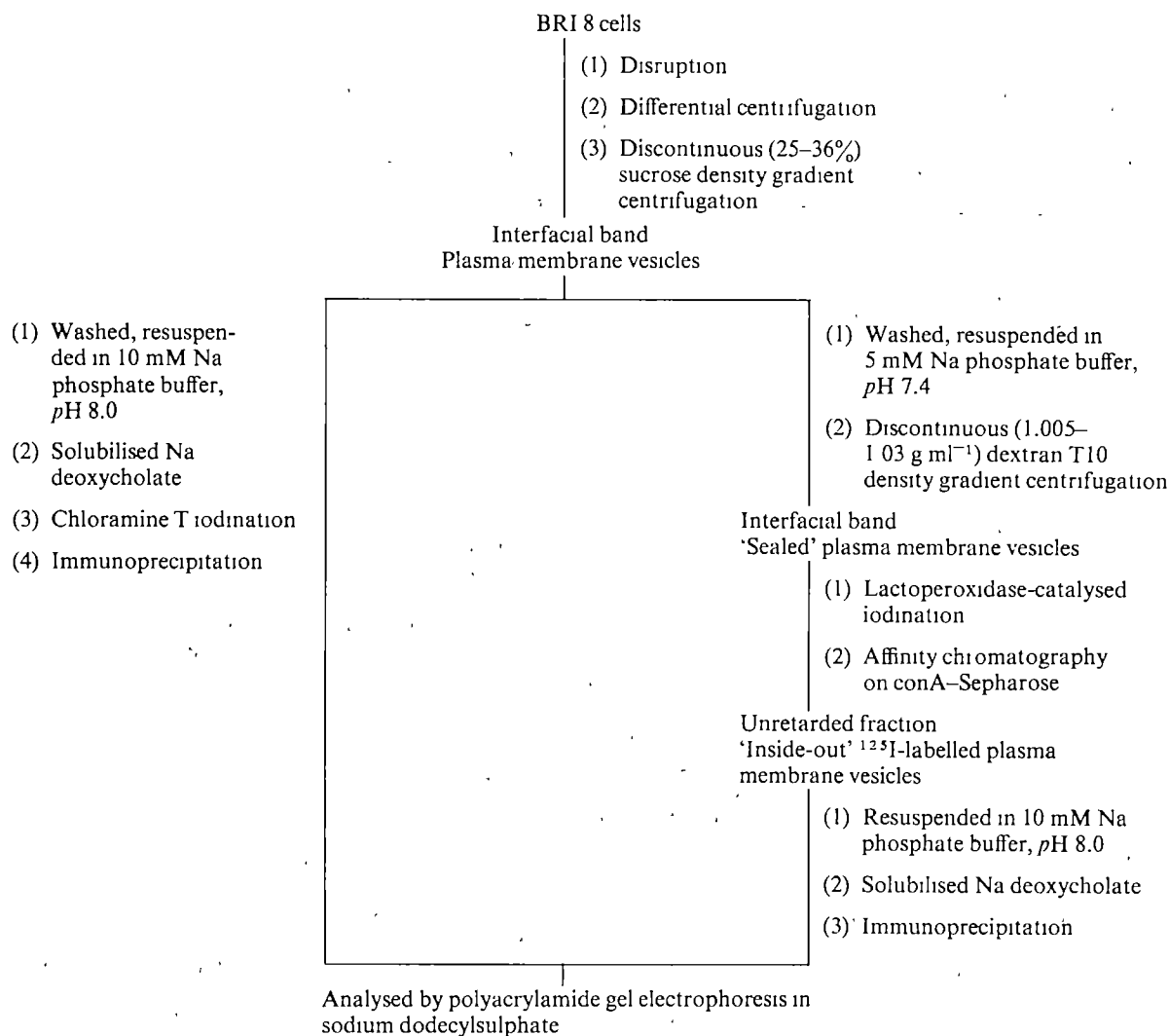


Fig. 1 Summary of experimental procedure for designating orientation of lymphocyte surface antigens. The procedure is shown for BRI 8 cells; an identical procedure was used for mouse spleen and thymus cells. The cells (about 80% viable; 5×10^7 cells ml^{-1}) were broken using the Stansted cell disruptor (model A0 612, disrupting valve 516, air pressure applied to disrupting valve was 55.2 kPa for BRI 8 and 250 kPa for mouse thymus and spleen cells). The plasma membrane fraction was separated as previously described^{19, 21, 23}, washed free of sucrose and resuspended in 5 mM Na phosphate buffer, pH 7.4. Sealed vesicles were separated by centrifugation on discontinuous gradients of Dextran T10 (Pharmacia) for 2 h at 40,000 r.p.m. in a Beckman SW41 rotor⁸. The vesicles that accumulated at the interface between 1.005 and 1.03 g ml^{-1} were impermeable to dextran of 10,000 molecular weight. This fraction represented at least 25% of BRI 8 plasma membrane but was no more than 10% of the membrane preparations from mouse spleen or thymus cells. The sealed vesicles (2–3 mg of membrane protein in 1 ml of 5 mM Na phosphate buffer, pH 7.4) were iodinated by adding 1 mCi Na^{125}I , 20 μg lactoperoxidase, 2.3 units glucose oxidase and glucose to a final concentration of 20 mM. After 15 min at 20 °C the reaction was terminated by adding 40 volumes of 5 mM Na phosphate buffer, pH 7.4, at 4 °C. The iodinated membrane was recovered by sedimentation at 75,000g for 30 min and inside-out vesicles were separated as described previously using concanavalin A–Sephrose 4B²¹. The ^{125}I -labelled inside-out membrane vesicles (about 1 mg of protein) were solubilised at 4 °C by addition of Na deoxycholate [10% (w/v) in 10 mM Tris-HCl buffer, pH 8.2] to a final concentration of 2% (w/v). After 30 min the solution was centrifuged at 100,000g for 1 h. Plasma membranes from BRI 8, mouse spleen and mouse thymus cells for control immunoprecipitations (about 1 mg of each) were solubilised in 1 ml of 2% (w/v) Na deoxycholate and non-vectorially labelled with Na^{125}I (1 mCi) by using Chloramine T (10 μg)³⁰.

basis of being inhibited completely by an authentic sample of β_2 -microglobulin, and having no detectable reactivity with erythrocytes and the lymphoblastoid cell line 'Daudi' which lack HLA-A, B and C antigens. A rabbit antiserum raised by hyperimmunisation with a glycoprotein fraction from BRI 8 plasma membrane was used to precipitate the HLA-Ia antigens—according to various criteria this antiserum recognised the human counterparts of the mouse Ia antigens^{24, 28}. Mouse thymocyte Thy-1 was precipitated by a xenogeneic cross-reacting antiserum raised against rat brain Thy-1^{25, 29} (from A. F. Williams). IgM from BRI 8 cells was precipitated by a commercial sample (Organon) of goat anti-(human IgM) serum, and mouse spleen membrane IgM and IgD were precipitated using an antiserum raised against mouse serum Ig (from M. C. Raff).

Numerous studies have shown that each of the above antigens is exposed on the cell surface. This disposition was confirmed for the cells chosen for study by using fluorescein-labelled anti-(Ig) sera to

reveal antigen-specific antibody bound to the cell surface in the fluorescence activated cell sorter. Thus, all BRI 8 cells were stained by anti-(β_2 -microglobulin), anti-(HLA-Ia) and anti-(IgM) sera relative to control cells which had not been incubated with specific antibody. Similar results were found with the anti-(Thy-1) serum on mouse thymus cells, whereas anti-(mouse Ig) serum stained about 50% of mouse spleen cells.

¹²⁵I-labelled inside-out plasma membrane vesicles

The preparation of iodinated inside-out plasma membrane vesicles from the various cell-types is summarised in Fig. 1. The initial purified plasma membrane preparation was not significantly contaminated by other subcellular fractions¹⁹. Previous results indicated that it represented a mixture of right-side-out and inside-out vesicles that were permeable to certain macromolecules²¹. Fractionation of the vesicles on a discontinuous density gradient

of dextran T10, in a manner similar to that used for preparing sealed erythrocyte vesicles⁸, yielded a population which by implication was impermeable to lactoperoxidase and which, thus, permitted vectorial labelling. The proportion of vesicles sealed to dextran T10 varied with the cell type and was not easily manipulated. Iodination of these vesicles was followed by fractionation on concanavalin A-Sepharose 4B (ref. 21) to separate the inside-out vesicles.

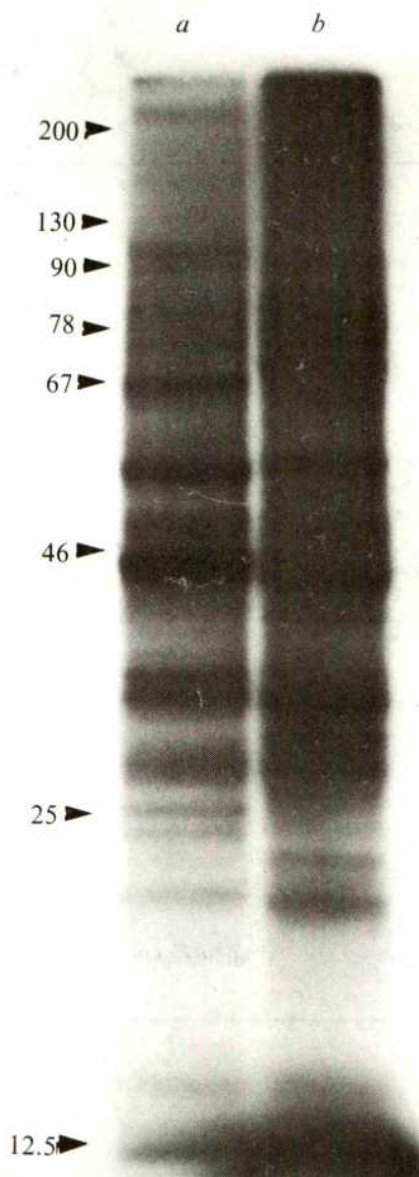


Fig. 2 Comparison of polypeptides labelled by lactoperoxidase-catalysed iodination of *a*, whole BRI 8 cells and *b*, inside-out plasma membrane vesicles. BRI 8 cells [10^7 in 100 μ l of phosphate buffered saline (PBS, 10 mM Na phosphate buffer 0.15 M NaCl, pH 7.4)], were iodinated by adding 300 μ Ci of Na 125 I, 20 μ g ml $^{-1}$ of lactoperoxidase, 2.3 units ml $^{-1}$ glucose oxidase and glucose to a final concentration of 20 mM. The reaction was allowed to proceed for 15 min at 20 $^{\circ}$ C and was terminated by the addition of 100 volumes of PBS. The cells were washed twice and finally resuspended in 1 ml of sodium dodecyl sulphate (SDS) sample buffer [2% (w/v) SDS, 10% (w/v) glycerol, 0.01 M dithiothreitol and 0.02% (w/v) bromophenol blue]. 125 I-labelled inside-out plasma membrane vesicles were prepared from BRI 8 cells as described in Fig. 1. They were sedimented by centrifugation at 75,000g for 30 min and resuspended in SDS sample buffer. Samples were boiled for 2 min and analysed by polyacrylamide gel electrophoresis in SDS using the Tris-glycine discontinuous buffer system of Laemmli³¹. Iodinated polypeptides were revealed by autoradiography. Molecular weights (given in thousands) were calculated by reference to the mobilities of standard proteins: myosin (200), β -galactosidase (130), phosphorylase (90), transferrin (78), bovine serum albumin (67), ovalbumin (46), Ig L-chain (25) and cytochrome C (12.5).

Figure 2 shows representative patterns of the iodinated polypeptides of 125 I-labelled whole BRI 8 cells (the outer surface) and of 125 I-labelled inside-out plasma membrane vesicles (the inner surface), as revealed by polyacrylamide gel electrophoresis in sodium dodecylsulphate. The clear differences between the patterns inspires confidence in the efficacy of the overall procedure. The samples contained more than 10 co-migrating polypeptides which on the basis of this criterion have a transmembrane orientation. Similar results have been obtained for pig lymphocytes²² as well as mouse thymus and spleen cells.

Immunoprecipitations

Immunoprecipitation analysis of the 125 I-labelled inside-out vesicles after solubilisation in sodium deoxycholate was undertaken to determine whether any of the inner-surface iodinated polypeptide chains could be equated with known cell surface antigens. A positive result would indicate that the antigen in question was exposed on both faces of the lipid bilayer and was, thus, a transmembrane protein. Each of the cell surface antigens chosen for study was also shown to be a component of the purified plasma membrane by immunoprecipitation from solubilised membrane that had been labelled non-vectorially by iodination using Chloramine T³⁰. The results for the various antigens are summarised in Fig. 3.

The HLA-A, B and C gene products are glycosylated polypeptides of 43,000 molecular weight²³. Immunoprecipitation of the 125 I-labelled inside-out membrane vesicles with the anti- β_2 -microglobulin serum gave one band only of molecular weight 43,000 (Fig. 3*b*), whose position was identical with that of the band of the control precipitate from the non-vectorially iodinated plasma membrane (Fig. 3*a*). If lactoperoxidase-catalysed iodination of the inside-out vesicles labels exposed tyrosine residues on the inner membrane surface only, then this result indicates that the HLA-A, B and C polypeptides span the lipid bilayer. The β_2 -microglobulin component of the HLA-A, B and C antigens was not detected because at the polyacrylamide gel concentration used (7.5%) β_2 -microglobulin was not resolved from the dye front and was run off the gel together with the radioactive iodide.

Similar results were obtained for the HLA-Ia antigens. These antigens comprise two non-covalently associated glycosylated polypeptides of 28,000 and 33,000 molecular weight²⁴. Figure 3*c* shows that the plasma membrane contained both polypeptides and Fig. 3*d* indicates that both are exposed on the inner membrane surface.

In contrast, neither the μ nor L chains of human IgM were labelled in the inside-out vesicles (Fig. 3*f*), although Fig. 3*e* indicates that the plasma membrane contained IgM and that both chains were iodinated when the Chloramine-T procedure was used. An identical result was obtained for mouse spleen cell Ig. Thus, the μ , δ and L chains were present in the plasma membrane (Fig. 3*g*), but were not labelled in the inside-out membrane vesicles (Fig. 3*h*). Similarly, Thy-1 antigen (molecular weight about 25,000³³) was a component of the thymus plasma membrane (Fig. 3*i*), but was not labelled in the inside-out vesicles (Fig. 3*j*).

Conclusions

The significance of the results depends on the subpopulation of plasma membrane vesicles used for the labelling studies being correctly designated as inside-out and sealed to lactoperoxidase. This designation is supported by two experiments. First, the failure to label certain known membrane components by lactoperoxidase-catalysed iodination (Fig. 3) argues strongly against the vesicles being leaky to lactoperoxidase. Second, the different labelling patterns given by intact cells and by the membrane vesicles (Fig. 2) argues that the outer surfaces of the cells and membrane vesicles possess different structures which is consistent with a different orientation. If these interpretations are correct then the prescribed method is applicable to the identification of lymphocyte transmembrane proteins as well as providing the means to probe the structure of the plasma membrane's inner surface. It seems likely that the approach will be generally applicable to a variety of cell types, especially since inversion of plasma orientation may be a general feature of cell breakage³⁴.

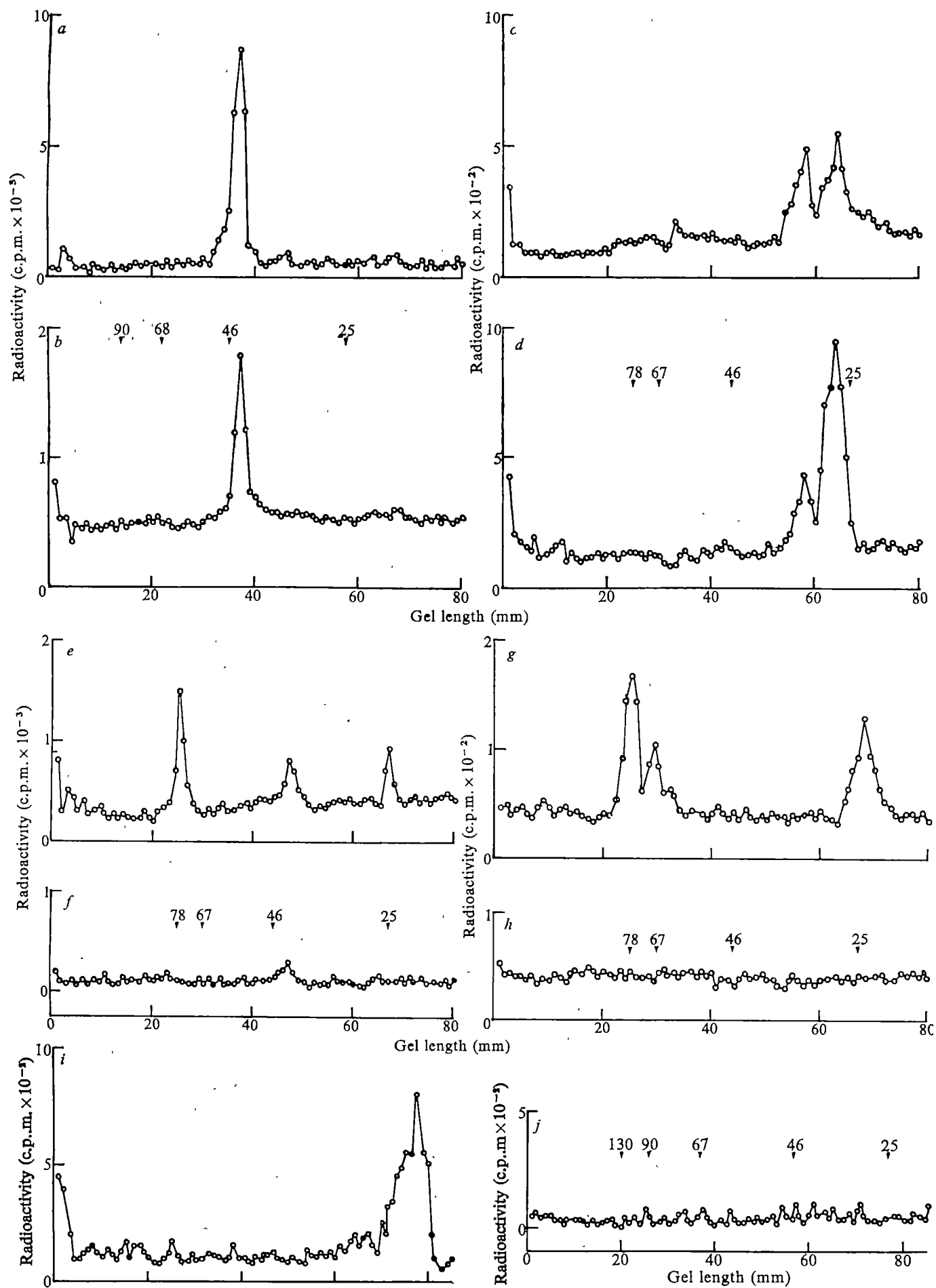


Fig. 3 For description see opposite

Fig. 3 Polyacrylamide gel electrophoresis patterns in SDS of immunoprecipitates prepared from ^{125}I -labelled inside-out membrane vesicles with *b*, anti-(human β_2 -microglobulin); *d*, anti-(HLA-Ia); *f*, anti-(human IgM); *h*, anti-(mouse Ig) and *j*, anti-(Thy-1) sera. These patterns should be compared with those obtained from the non-vectorially iodinated plasma membrane with *a*, anti-(β_2 -microglobulin); *c*, anti-(HLA-Ia); *e*, anti-(human IgM); *g*, anti-(mouse Ig) and *i*, anti-(Thy-1) sera. The preparation of ^{125}I -labelled inside-out membrane vesicles and ^{125}I -labelled plasma membrane is described in Fig. 1. Three methods of immunoprecipitation were used depending on the quantity of antiserum available. First, the Ig fractions of the anti-(β_2 -microglobulin), anti-(HLA-Ia), anti-(mouse Ig) and normal rabbit serum were coupled to cyanogen bromide activated Sepharose CL-4B at a concentration of 10 mg of protein per ml of gel sediment. Before interaction with specific antibody the deoxycholate-solubilised ^{125}I -labelled membranes (Fig. 1) were depleted of non-specifically bound protein by incubation for 30 min at 20°C on a 5 ml column of normal rabbit Ig-Sepharose CL-4B. Unadsorbed protein was eluted with 0.5% (w/v) Na deoxycholate. The eluate was incubated for 30 min at 20°C on 5 ml columns of immune Ig-Sepharose CL-4B that were subsequently washed with 0.5% (w/v) Na deoxycholate. The adsorbed material was eluted with 2% (w/v) SDS in 8 M urea, precipitated with ethanol (final concentration 66%) for 48 h at -20°C , solubilised in SDS sample buffer and analysed by polyacrylamide gel electrophoresis in SDS as described above. After electrophoresis and staining with Coomassie blue the gels were dried, divided into 1 mm slices and counted in a Packard gamma counter. Second, *Staphylococcus aureus* A protein attached to Sepharose CL-4B was used as the absorbent for the anti-(human IgM) serum. The protein prepared as previously described³² was coupled to Sepharose CL-4B (1 mg of protein per ml of gel sediment). The deoxycholate-solubilised ^{125}I -labelled BRI 8 membranes were pre-adsorbed with normal rabbit Ig-Sepharose CL-4B as described above. The eluate was incubated at 37°C with 100 μl of anti-(human IgM) serum for 1 h followed by 12 h at 4°C and then incubated for 30 min at 20°C with the *Staph* A-Sepharose CL-4B. Unadsorbed material was eluted with 0.5% (w/v) Na deoxycholate. The bound material was displaced with 2% (w/v) SDS in 8 M urea and treated as previously described. Third, a direct precipitation system was used for the mouse thymus Thy-1 antigen. Deoxycholate-solubilised ^{125}I -labelled membrane (300 μl) was incubated at 37°C for 30 min with 30 μl of anti-(Thy-1) serum, for a similar period with 50 μl of horse anti-(rabbit Ig) and then cooled to 4°C for 12 h. The resulting precipitate was washed four times with 0.5% (w/v) Na deoxycholate at 4°C , solubilised in 2% (w/v) SDS in 8 M urea and analysed as above.

The major limitation of the method concerns the selectivity of iodination which is restricted to exposed tyrosine residues. As a result, lack of labelling may reflect lack of an accessible tyrosine residue rather than absence of the polypeptide chain *per se*.

The plasma membrane of the human B-lymphoblastoid cell line BRI 8 contained at least 10 transmembrane proteins as assessed by the comigration of polypeptide chains which were iodinated on both the outer surface of intact cells and of inside-out membrane vesicles (Fig. 2). Similar results have been obtained with pig lymphocytes²². In this case the majority of the transmembrane proteins were further identified as glycoproteins on the basis of specific adsorption to *Lens culinaris* lectin-Sepharose²². A preferential disposition of glycoproteins to a transmembrane orientation is in accord with models of plasma membrane structure based on erythrocytes^{12,13}.

Direct evidence was obtained that the polymorphic, 43,000 molecular weight polypeptide chain of the HLA-A, B and C antigens spans the lipid bilayer. This conclusion is in accord with the orientation suggested previously on the basis of the amino acid composition of enzymatic cleavage products³⁵. It is also concluded that both of the polypeptides (28,000 and 33,000 molecular weight) which comprise the HLA-Ia antigens are transmembrane.

The significance of the lack of labelling of Thy-1 antigen and membrane-bound Ig in the inside-out vesicles is equivocal because of the selectivity of the labelling procedure. Other arguments may, however, be invoked in favour of the view that the lack of labelling of IgM is a true reflection of its orientation. Thus, membrane-bound IgM binds very much less detergent than typical integral membrane proteins including Thy-1 and HLA-A, B and C and Ia antigens^{24,33,36}. This difference in behaviour is consistent with the proposal that IgM dips much less extensively into the bilayer than known transmembrane proteins. Also, if the μ -chain of membrane-bound IgM spans the membrane it should be iodi-

nated, assuming its structure is identical with that of secreted IgM which has tyrosine as its C-terminal amino acid residue³⁷.

We thank Drs M. C. Raff and A. F. Williams for gifts of antisera, and Dr A. J. Edwards for examining the cell suspensions for surface antigens in the fluorescence activated cell sorter.

Received 25 April; accepted 18 July 1977.

- ¹ Crumpton, M. J., Allan, D., Auger, J., Green, N. M. & Maino, V. C. *Phil. Trans. R. Soc.* **B272**, 173-180 (1975).
- ² Singer, S. J. A. *Rev. Biochem.* **43**, 805-833 (1974).
- ³ Guidotti, G. *Trends biochem. Sci.* **1**, 11-13 (1976).
- ⁴ Greaves, M. F. *Nature* **265**, 681-683 (1977).
- ⁵ Bretscher, M. S. *J. molec. Biol.* **59**, 351-357 (1971).
- ⁶ Carraway, K. L. *Biochim. biophys. Acta* **415**, 379-410 (1975).
- ⁷ Steck, T. L. *J. Cell Biol.* **62**, 1-19 (1974).
- ⁸ Steck, T. L. *Meth. Membr. Biol.* **2**, 245-281 (1974).
- ⁹ Mueller, T. J. & Morrison, M. *Biochemistry* **14**, 5512-5516 (1975).
- ¹⁰ Reichstein, E. & Blostein, R. *J. biol. Chem.* **250**, 6256-6263 (1975).
- ¹¹ Boxer, D. H., Jenkins, R. E. & Tanner, M. J. A. *Biochem. J.* **137**, 531-534 (1974).
- ¹² Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43-49 (1975).
- ¹³ Rothman, J. E. & Lenard, J. *Science* **195**, 743-753 (1977).
- ¹⁴ Kyte, J. *J. biol. Chem.* **250**, 7443-7449 (1975).
- ¹⁵ Louvard, D., Semeriva, M. & Maroux, S. *J. molec. Biol.* **106**, 1023-1035 (1976).
- ¹⁶ Hackenbrock, C. R. & Hammon, K. M. *J. biol. Chem.* **250**, 9185-9197 (1975).
- ¹⁷ Garoff, H. & Simons, K. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3988-3992 (1974).
- ¹⁸ Blaurock, A. E. *J. molec. Biol.* **93**, 139-158 (1975).
- ¹⁹ Crumpton, M. J. & Snary, D. *Contemp. Top. molec. Immun.* **3**, 27-56 (1974).
- ²⁰ Hubbard, A. L. & Cohn, Z. A. in *Biochemical Analyses of Membranes* (ed. Maddy, A. H.) 427-501 (Chapman and Hall, London, 1976).
- ²¹ Walsh, F. S., Barber, B. H. & Crumpton, M. J. *Biochemistry* **15**, 3557-3563 (1976).
- ²² Walsh, F. S., Barber, B. H. & Crumpton, M. J. *Biochem. Soc. Trans.* (in the press).
- ²³ Bridgen, J. et al. *Nature* **261**, 200-205 (1976).
- ²⁴ Snary, D., Barnstable, C. J., Bodmer, W. F., Goodfellow, P. N. & Crumpton, M. J. *Scand. J. Immun.* **6**, 439-452 (1977).
- ²⁵ Trowbridge, I. S., Weissman, I. L. & Bevan, M. J. *Nature* **256**, 652-654 (1975).
- ²⁶ Goding, J. W. & Layton, J. E. *J. exp. Med.* **144**, 852-857 (1976).
- ²⁷ Tanigaki, N. & Pressman, D. *Transplant. Rev.* **21**, 15-34 (1974).
- ²⁸ Barnstable, C. J. et al. *Cold Spring Harb. Symp. quant. Biol.* **41**, 443-455 (1977).
- ²⁹ Williams, A. F. *Contemp. Top. molec. Immun.* **6**, 83-116 (1977).
- ³⁰ Greenwood, F. C., Hunter, W. M. & Glover, J. S. *Biochem. J.* **89**, 114-123 (1963).
- ³¹ Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- ³² Sjöquist, J., Meloun, B. & Hjelm, H. *Eur. J. Biochem.* **29**, 572-578 (1972).
- ³³ Barclay, A. N., Letarte-Muirhead, M., Williams, A. F. & Faulkes, R. A. *Nature* **263**, 563-567 (1976).
- ³⁴ Bennett, V. & Cuatrecasas, P. *Biochim. biophys. Acta* **311**, 362-380 (1973).
- ³⁵ Springer, T. A. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2481-2485 (1976).
- ³⁶ Melcher, U. & Uhr, J. W. *Biochemistry* **16**, 145-152 (1977).
- ³⁷ Putnam, F. W., Florent, G., Paul, C., Shimoda, T. & Shimizu, A. *Science* **182**, 287-291 (1973).

letters to nature

Possible fast variability of the nucleus of Cen A at 13.5 mm

INFORMATION about short time scale events in extragalactic objects can have very important implications for the theoretical interpretation of the physical mechanisms involved. Such data are only obtained by using time-consuming observing schedules and methods, and are not

usually carried out at radio observatories. This kind of information is thus generally lacking. We report here a study of the nucleus of Cen A (NGC 5128) at a wavelength of 13.5 mm. Possible fast variability was observed.

Observations in the direction of the infrared 'hot-spot' of NGC 5128 (ref. 1) have been made at centimetre and millimetre wavelengths²⁻⁴. Kellermann⁵ has suggested an important intraday change of flux, based on few observations taken at 3.4 and 9.5 mm. Fogarty and Schuch⁶ found no variability using observations obtained monthly at

13.5 mm, in 1974. X-ray emission from the nucleus of NGC 5128 was identified by Tucker *et al.*⁵, and important variability has been claimed by other workers at X-ray wavelengths^{6,7}.

We performed 37 observations of the nucleus of NGC 5128 at $\lambda=13.5$ mm from July 1976 to March 1977, which included two periods of 15 d sequential patrol (26 July to 10 August, and 27 November to 13 December, in 1976). The observations were carried out using a 45-foot dish at Itapetinga Radio Observatory, Atibaia, Brazil. All observations were performed at the same sidereal time at the local meridian transit to avoid polarisation effects. The antenna beam at 13.5 mm is of about 4' arc, and observations were made using on-on and beamswitching techniques.

For comparison we observed Vir A in sequence, as a calibrator (for which we adopted 21.4 Jy). After 10 November 1976 we also observed the NE lobe of NGC 5128 (13 h 42 min 48 s, $-42^\circ 42' 11''$, 1950.0 coordinates). This position is situated nearly 2.5' arc NE of the nucleus such that there was only a weak beam overlap with less than about 3 dB of attenuation. This lobe was selected for comparison since close to it, in NE direction, it has been recently found some H II filamentary structures (ref. 8 and M. Dennefeld, personal communication). It corresponds to a structure identified at decimetric and metric wavelengths⁹⁻¹¹.

The measurements were normalised against Virgo A and are shown in Fig. 1. Nearly all observations were carried in the following sequence of 30 min integrations: Vir A, NE lobe, centre, Vir A. In some days the integration times were extended with 15 min extra observations. Sky attenuation was measured before and after the sequence, and the average was adopted for data correction. The error bars are $\pm 1\sigma$ referred to the worst 30-min integration measurement of the sequence. Larger error bars refer to days with bad weather, and the measurements are consequently less

reliable. In general both atmospheric and instrumental changes affected similarly all observations from a sequence.

Figure 1 suggest an 'event' (with flux reduction) in 22 November–5 December 1976, in the centre/Vir A and centre/NE lobe series of observations. This is not suggested in the NE lobe/Vir A series. Both centre/Vir A and NE lobe/Vir A data show reduced values in 11–12 November 76 and 10–11 March 77—and this is not seen in centre/NE lobe data.

It should be stressed that the observations of the centre and NE lobe were performed in nearly equal conditions, using the same corrections (elevation angle of about 70°). Vir A was observed in the opposite hemisphere at a lower elevation angle (about 45°), and the measurements suffered different corrections. An error in the estimate of the sky attenuation will not affect the relative measurement centre/NE lobe, but may affect Vir A corrected measurements, and thus centre/Vir A and NE lobe/Vir A comparisons. Thus the centre/NE lobe data are the most reliable for comparison purposes.

The coincidence in the 'event' occurrence both in the more reliable comparison centre/NE lobe and in centre/Vir A suggests that it might be of a real nature. On the other hand, due to the reasons discussed above, the reduction in centre/Vir A and NE lobe/Vir A in November 76 and March 77 could not be attributed necessarily to changes in Vir A.

The possible 'event' described here is relatively much better defined than Kellerman's² 2 d data at 3.4 mm, which have also shown a flux reduction of nearly 30%. This reduction alone bring some complications for the interpretation. But we feel the data are still few, and the effect might be only a part of a larger more complex event.

Long term data seem to confirm that there are no large time scale variations in flux from the nucleus of Cen A

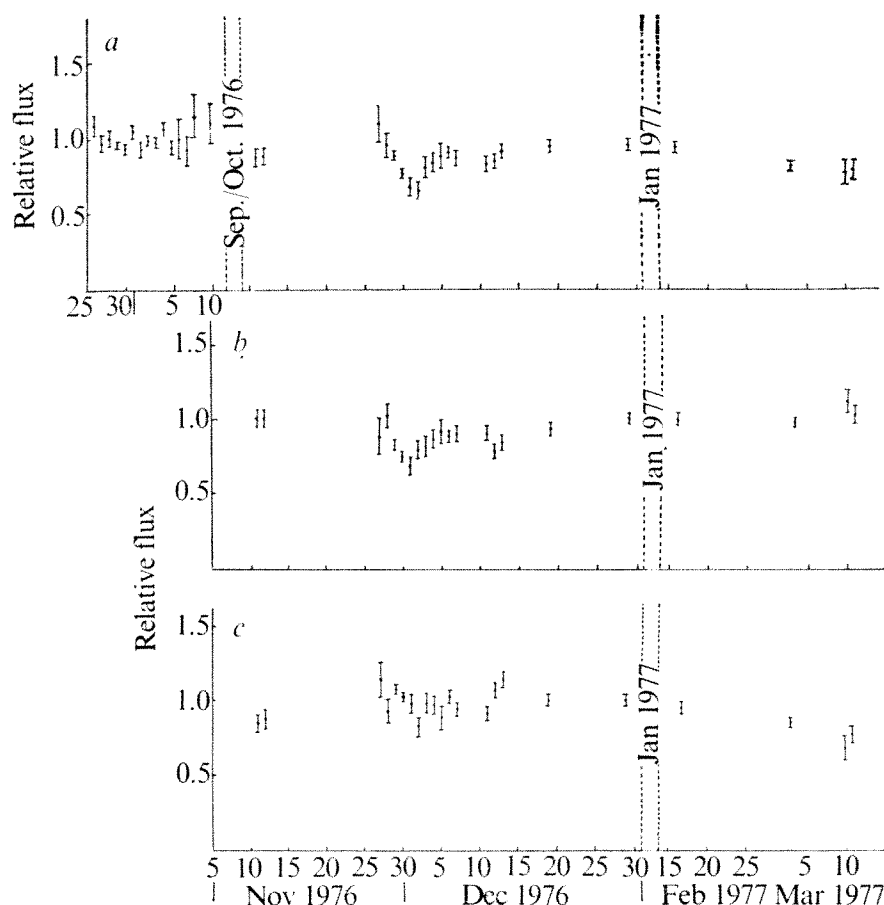


Fig. 1 Observations at 13.5 mm of the nucleus of Cen A in relation to Virgo A (a), of the nucleus of Cen A in relation to NE Lobe of Cen A (b), and of NE Lobe of Cen A in relation to Vir A (c). Ordinates are in relative units. Error bars correspond to standard errors of the mean.

(ref. 4). Short time scale events might happen within a few days, however. Complete description of the pattern will require daily observations over several months.

It should be noted that the previous map¹² of NGC5128 was derived with isotherms referred to the centre, for which variability was not taken into account. An improved version of the map at 13.5 mm is under investigation.

We thank Drs K. J. Kellermann, J. Beall and M. Dennefeld for useful discussions. This work was supported in part by Brazilian research agencies CNPq and FAPESP.

PIERRE KAUFMANN
P. MARQUES DOS SANTOS
J. C. RAFFAELLI
E. SCALISE, JR

Centro de Rádio-Astronomia e Astrofísica 'Mackenzie',
CNPq-Observatório Nacional,
São Paulo, SP, Brazil

Received 29 March; accepted 5 July 1977.

- ¹ Kunkel, W. E. & Bradt, H. V. *Astrophys. J.* **170**, L7-L10 (1971).
- ² Price, K. M. & Stull, M. A. *Nature* **245**, 83-89 (1973).
- ³ Kellermann, K. I. *Astrophys. J.* **194**, L135-L137 (1974).
- ⁴ Fogarty, W. G. & Schuch, N. J. *Nature* **254**, 124-125 (1975).
- ⁵ Tucker, W., Kellogg, E., Gursky, H., Giacconi, R. & Tananbaum, H. *Astrophys. J.* **180**, 715-724 (1973).
- ⁶ Winckler, P. F. & White, A. E. *Astrophys. J.* **199**, L139-L142 (1975).
- ⁷ Fabian, A. C., Maccagni, D., Rees, M. J. & Stoeger, W. R. *Nature* **260**, 683-685 (1976).
- ⁸ Blanco, V. M., Graham, J. A., Lasker, B. M. & Osmer, P. S. *Astrophys. J.* **198**, L63-L64 (1975).
- ⁹ Cooper, B. F. C., Price, R. M. & Cole, D. J. *Austr. J. Phys.* **18**, 589-625 (1965).
- ¹⁰ Lockhart, I. A. & Sheridan, K. V. *Proc. ASA* **1**, 344-345 (1970).
- ¹¹ Slee, O. B. & Sheridan, K. V. *Proc. ASA* **2**, 6, 1 (1975).
- ¹² Kaufmann, P., Scalise, Jr. E., Marques dos Santos, P. & Fogarty W. G. *Mon. Not. R. astr. Soc.* **169**, 15p-17p (1974).

Pi 1-2 magnetic field pulsations on dayside cleft field lines

TYPE Pi 1-2 magnetic field pulsations, $T = 1-150$ s, have a broad-band, noise-like character and a tendency to occur during active aurorae and magnetospheric substorms^{1,2}. It is well-known that type Pi 1-2 magnetic field pulsations observed in the night-morning sector during magnetospheric substorms maximise in amplitudes along the auroral oval^{1,2}. But the relation of the Pi 1-2 activity to the day sector of the auroral oval has not been clear. This relation is of particular interest because the day sector of the auroral oval seems to coincide approximately with the feet of the dayside cleft field lines; the precise relation between the dayside cleft and the dayside auroral oval, however, has not yet been established (S. I. Akasofu, personal communication). Dayside cleft field lines usually terminate between the geomagnetic latitudes of 75° and 80°. We show here that intense Pi 1-2 activity appeared at College on two occasions when the cleft came down to the College latitude, 64.7° N, at times when College was in the midday sector. Further, it is shown that an intense flux of particle precipitation into the dayside ionosphere occurred on the second occasion, 4 August 1972.

A direct comparison between Pi 1-2 activity as observed at Bar I (70.3°N, 257°E, geom.) and College (64.7°N, 257°E) on 26-27 July 1969, and observations of the dayside cleft by satellite³ is shown in Fig. 1. In the interval 2000-0100 UT, which is local midday at Bar I and College, the cleft moved towards the equator, approaching 66° at 0100 UT. Pi 1-2 activity was most intense at Bar I between 2300 and 0100 UT when Bar I was on cleft lines. Pi 1-2 was most prominent at College between 0100 and 0200 UT, but was not as intense as at Bar I (maximum amplitudes near 0.02 Hz were 16 nT at Bar I and 6 nT at College). This behaviour is consistent with a Pi 1-2 origin on cleft lines.

The cleft tends to move to smaller L values ($L = \text{McIlwain's parameter}$; the invariant latitude ($\Lambda_0 = \cos^{-1} (1/L)^{1/2}$), with increasing values of the AE index³. From this, we would expect Pi

activity to be seen at College in midday hours preferentially at times of high AE (or Kp) if the midday Pi 1-2 activity does maximise on cleft lines. To check this, we looked at College Pi data^{4,5} and found that Pi occurred in midday hours at College on about 30 d in 1966 and 1967 with Kp values ranging from 4 to 9. In contrast, Pi activity is prominent at College in night hours for Kp from 2 to 5. These characteristics seem consistent with a Pi oval that coincides approximately with the cleft lines on the day side.

On 4 August 1972, at 2240 UT, the magnetopause moved inward past Explorer 45, then at $L = 5.2$ and at 1435 MLT⁶.

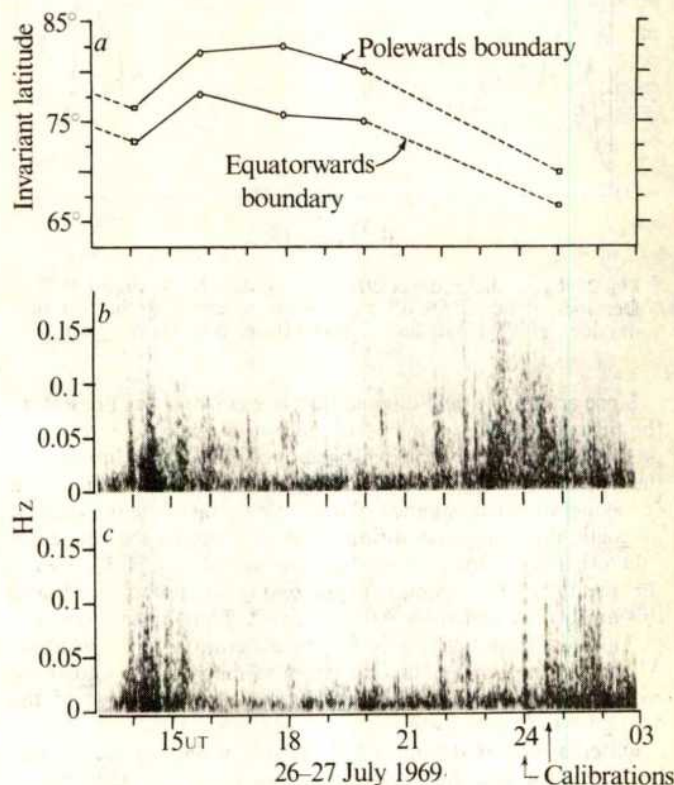


Fig. 1 Appearance of type Pi 1-2 pulsations at *b*, Bar I and *c*, College 2300-0200 UT when dayside cleft lines were in the vicinity of those sites. *a*, from Yasuhara and Akasofu ref. 3.

An induction magnetometer operating near Chatanika, $L = 5.6$, 40 km north of College, registered very strong Pi 1-2 activity at that time. The activity actually exceeded the record range of the tape recorder (30 nT at 0.02 Hz). Sonograms show that the pulsations had the broad-band form that characterises Pi 1-2 activity. The Kp index was 9₀ at the time and AE reached 2065 nT.

Since the magnetopause moved inwards past $L = 5.2$ at 2240 UT, it evidently passed $L = 5.6$ somewhat earlier, hence Chatanika was on cleft lines for the duration of the strong Pi activity observed there. Direct Chatanika radar observations showed the cleft to be located near Chatanika from 2200-2300 UT on 4 August⁷.

The Chatanika radar⁸ was in operation at the time, and the data show that prominent *E*- and *D*-regions were formed⁹ (Fig. 2). The College 30 MHz riometer went off scale in this interval, the absorption exceeding 20 dB (ref. 11). These measurements show conclusively that a field-aligned plasma stream was present on the dayside cleft field lines. Using Chatanika radar electron densities and appropriate auroral ionosphere *D*-region collision frequencies, the absorption at Chatanika was estimated to be 21.4 dB at 30 MHz, which could be produced by 70 keV electrons with a minimum precipitation rate of 3×10^6 e cm⁻³ s⁻¹ ster⁻¹ (ref. 10). Similar estimates of precipitating particle energies during this period have been given by other investigators¹².

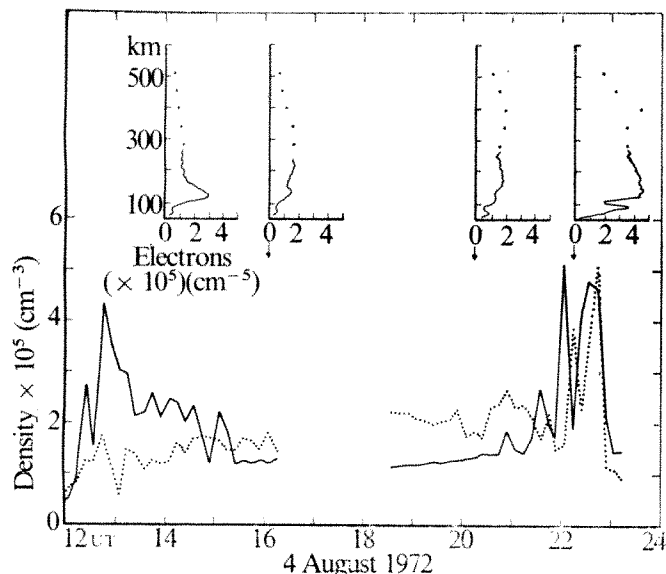


Fig. 2 Chatanika radar electron density data for 4 August 1972. Densities of up to $5 \times 10^5 \text{ cm}^{-3}$ were observed at the feet of dayside cleft field lines 2200–2300 UT (from Watkins *et al.*, ref. 7).

Since an intense field-aligned flux of electrons was present on the field lines where the Pi 1–2 pulsations were observed, it is possible to invoke the garden-hose overstability mechanism for the generation of the Pi 1–2. The garden-hose overstability is a consequence of field-aligned plasma streaming in the presence of magnetic mirroring, conditions that are met in the magnetosphere¹³. The plasma pressure becomes anisotropic with $P_{\parallel} > P_{\perp}$, the anisotropy increasing with increasing streaming speed, and this anisotropy generates Alfvén waves⁹. Thus particle energies of 1–100 keV are favourable for the generation of the unstable Alfvén waves through the garden-hose mechanism, and those energies were observed in the cleft region in the case of the 4 August 1972 activity⁶.

Observations of strong Pi 1–2 activity in the day sector may be useful for locating cleft field lines in experiments where supplementary knowledge of the cleft location is required. In recent years, the most usual ground-based technique for locating the cleft field lines in the ionosphere has been the ionosonde technique. Since magnetometer data are considerably easier to obtain than ionosonde, particularly on a continuous long-term basis, it is advisable to extend the present investigation to Pi 1–2 recording sites between 70° and 80°.

This research was supported by the NSF Atmospheric Research Section, through grant numbers ATM71-00617 and DES 74-23550.

R. R. HEACOCK
R. D. HUNSUCKER

Geophysical Institute
and Electrical Engineering Department
University of Alaska,
Fairbanks, Alaska 99701

Received 23 May; accepted 28 July 1977.

- ¹ Akasofu, S.-I. *Polar and Magnetospheric Substorms* (Reidel, Dordrecht-Holland 1968).
- ² Saito, T. *Space Sci. Rev.* **22**, 319–412 (1969).
- ³ Yasuhara, F. & Akasofu, S.-I. *J. geophys. Res.* **78**, 7286–7291 (1973).
- ⁴ Heacock, R. R. & Hessler, V. P. *High Latitude Geophysical Data, Reports UAG-C 43 to UAG-C 47* (Geophysical Institute of the University of Alaska, 1966–1967).
- ⁵ Hessler, V. P. *High Latitude Geophysical Data, Reports UAG-C 43 to UAG-C 47*, (Geophysical Institute of the University of Alaska, 1966–1967).
- ⁶ Hoffman, R. A. *et al. J. geophys. Res.* **80**, 4287–4296 (1975).
- ⁷ Wedde, T., Doupnik, J. R., Banks, P. M., Park, R. J. & Siren, J. C. *Radio Sci.* **12**, 285 (1977).
- ⁸ Leadabrand, R. L., Baron, M. J., Petricks, J. & Bates, H. F. *Radio Sci.* **7**, 747 (1972).
- ⁹ Watkins, B. J., Bates, H. F., Belon, A. E. & Hunsucker, R. D. *World Data Center-A Report UAG-28, Part II 492* (1973).
- ¹⁰ Hunsucker, R. D. *Radio Sci.* **9**, 335 (1974).
- ¹¹ Hunsucker, R. D. *World Data Center-A Report UAG-28, Part II 490* (1973).
- ¹² Doupnik, J. R., Brekke, A. & Banks, P. M. *J. geophys. Res.* **82**, 499 (1977).
- ¹³ Kan, J. R. & Heacock, R. R. *J. geophys. Res.* **81**, 2371–2376 (1976).

Linear dichroism probes to study internal electric fields

LARGE electric fields outside a highly charged macro-ion or between any ion and its counter ion atmosphere form the basis of electrolyte theory. The fields manifest themselves in a number of macroscopic properties of such systems, but few, if any, successful measurements of such fields have been possible. We report here observations which suggest that optical probes can be used as field meters in anisotropic solvents: In aqueous solutions containing flow-aligned cylindrical micelles of cetyltrimethylammonium bromide (CTAB) several anisotropic anions exhibit linear dichroism effects which can be ascribed to orientation and perturbation of these chromophores by a radially directed field of about 10^8 V m^{-1} surrounding each $(\text{CTA}^+)_n$ cylinder.

Linear dichroism ($\text{LD} = A_{\parallel} - A_{\perp}$, definition and measuring techniques in refs 1–3) and absorption (A) spectra were recorded on several substances, added in small amounts (maximum 0.1%), in aqueous CTAB (20%) solution, in a Couette device at radial optical propagation. At the concentration and temperature used, this detergent forms practically infinite cylindrical micelles, with a diameter of $\sim 50 \text{ \AA}$, which are alignable in a flow gradient^{3,4}. The observation of a non-zero LD (Table 1) is not *a priori* unequivocal evidence for an interaction between the chromophore and the fields around the macro-ion, but one has first to correct for the so-called Wiener dichroism ($= \text{WLD}$, defined in ref. 5) which is the imaginary correspondence of form-birefringence. WLD has the shape of the absorption spectrum (WLD/A being constant), but its sign depends on the dielectric constant of the medium surrounding the chromophore. In micellar CTAB, WLD is positive for chromophores close to the cationic micelles and negative for peripherally located chromophores. Thus anions have positive, and cations negative WLD⁵. The observed LD was corrected for the expected WLD (Table 1). For ions like $\text{Cr}(\text{oxalate})_3^{3-}$ or other pseudo-cubic ions⁵, WLD is sufficient to explain the observed signals. This is also true for anisotropic cations, which by avoiding the macro-ion are in regions with comparatively weak electrostatic fields. With anisotropic anions the situation is different and after eliminating WLD, a sometimes complicated pattern of bands of any sign remains (Table 1, Fig. 1).

From the treatments of Labhart⁶, Liptay⁷ and Lin⁸ on molecules in electric fields, one has for the absorption difference $A_{\chi} - A$, due to an electric dipole allowed transition $b \leftarrow a$, at the frequency ω (A_{χ} is the absorption with an angle χ between the electric field and the electric vector of the polarised light; A is the absorption in the absence of field):

$$\text{ELD}\chi(\omega) = (A_{\chi} - A)\omega = \frac{\beta E^2}{10} A(\omega) S + \frac{\omega E^2}{10\hbar} \frac{\partial}{\partial \omega} \left\{ \frac{A(\omega)}{\omega} \right\} T + \frac{\omega E^2}{10\hbar^2} \frac{\partial^2}{\partial \omega^2} \left\{ \frac{A(\omega)}{\omega} \right\} U \quad (1)$$

$$\text{where } S = (3 \cos^2 \chi - 1) [(\hat{p}_{ab} \cdot \alpha(a) \cdot \hat{p}_{ab} - \frac{1}{3} T_r(\alpha(a))) + \beta((\hat{p}_{ab} \cdot p_{aa})^2 - \frac{1}{3} |p_{aa}|^2)]$$

$$T = [(2 - \cos^2 \chi) T_r(\Delta \alpha(b, a)) + (3 \cos^2 \chi - 1) \hat{p}_{ab} \cdot \Delta \alpha(b, a) \cdot \hat{p}_{ab} + 2\beta[(2 - \cos^2 \chi) p_{aa} \cdot \Delta p(b, a) + (3 \cos^2 \chi - 1) (\hat{p}_{ab} \cdot p_{aa}) \hat{p}_{ab} \cdot \Delta p(b, a)]]$$

$$U = [(2 - \cos^2 \chi) |\Delta p(b, a)|^2 + (3 \cos^2 \chi - 1) |\hat{p}_{ab} \cdot \Delta p(b, a)|^2]$$

where $\beta = 1/kT$, \hat{p}_{ab} is a unit vector along the transition moment p_{ab} ; $\alpha(a)$ and $p_{aa} = \sum e r_i$ are the polarisability tensor and the permanent dipole moment, respectively, of the ground state. $\Delta \alpha(b, a)$ and $\Delta p(b, a)$ are the difference in polarisability and the difference in dipole moment, respectively, between the ground and the excited states. If dipole orientation effects⁹ predominate over

Table 1 Observed linear dichroism (LD) of various chromophoric ions in the aligned micellar CTAB(20%)/H₂O system

Chromophoric probe ion	Symmetry	Wavelength of extrema (nm) LD	<i>A</i>	LD <i>A</i>	WLD <i>A</i>	ELD* <i>A</i>	θ
<i>p</i> -Aminobenzenesulphonate ⁻	C _{2v}	297 251.5	295† 251.5	-0.042 +0.034	+0.02 +0.02	-0.06 +	$\pi/288$ 088
Methyl orange (4'-dimethylaminoazobenzene-4-sulphonate) ⁻	C _{2v}	418	425	-0.029	+0.02	-0.05	088
Methyl red (4-dimethylaminobenzene-azobenzene-2'-carboxylate) ⁻	C _{2v}	515 410	512 410†	-0.012 +0.015	+0.020 +0.020	-0.03 +	08 08
Benzoate ⁻	C _{2v}	276.5 268.5 262.0 255 <245	276.8 269.8 263.8 256	+0.046‡ +0.040‡ +0.037‡ +	+0.02 +0.02 +0.02 +0.02	+0.03 +0.02 +0.02 -0.09	$\pi/28$ $\pi/28$ $\pi/28$ 08 08
Co(III) (ethylenediaminetetraacetate) ⁻	C ₂	553 380	538 380	+0.012 -0.014	+0.02 +0.02	0 -0.03	08 0**
<i>trans</i> [Co(ethylenediamine) ₂ Cl ₂] ⁺	D _{2h}	610 460 <325	610 460 325	-0.014 -0.01 -0.014	-0.017 -0.017 -0.017	0 0 0	†† †† ††
Cr(oxalate) ₃ ³⁻	D ₃	570 415 270	570 417 270	+0.023 +0.029 +0.032	+0.020 +0.020 +0.027	0 0 0	†† †† ††
Co(ethylenediamine) ₂ CO ₃ ⁺	C ₂	520 358	525 360	-0.010 -0.010	-0.017 -0.017	0 0	† **

Concentrations of added impurities were varied according to $A = 0.1 - 1.5$ (at $d = 0.1$ cm) without any noticeable variation in the LD/ A spectrum.

*Recorded LD adjusted for WLD estimated according to ref. 5.

†Shoulder.

‡Value referring to resolved component.

§Band polarisation confirmed in LD study in stretched polymer sheet.

¶d-d transition ${}^1T_{1g} \leftarrow {}^1A_{1g}$ in O_h^{12} .

**d-d transition ${}^1T_{2g} \leftarrow {}^1A_{1g}$ in O_h ; isotropic but may in C_{2v} get a certain allowance along the C₂-axis, that is, parallel to \hat{p} .

††Unpolarised isotropic transition.

‡‡No dipole moment.

§§Assignment according to ref. 11.

effects due to electronic perturbations (that is, T , U and $\alpha(a)$ can be neglected) we have

$$\frac{A_{\parallel} - A_{\perp}}{A} = \frac{A_{\parallel=0} - A_{\parallel=\pi/2}}{A} = \frac{1}{10} \left(\frac{|p_{aa}| |E|}{kT} \right)^2 (3 \cos^2 \theta - 1) \quad (2)$$

where θ is the angle between p_{ab} and p_{aa} . After scaling by $-1/2$ equation (2) applies to the geometry of the CTAB system (\parallel = the direction of the micelle cylinders). In Table 1, θ values are given when \hat{p}_{ab} is available from spectroscopic assignments and p_{aa} from geometrical considerations. For an ion the dipole moment is referred to the centre of mass as origin. A generally poor agreement indicates that the assumptions behind equation (2) are not justified here. But, we get an accurate order of magnitude of the field (or at least the minimum field) since the field appears as a squared term. Thus $(A_{\parallel} - A_{\perp})/A = +0.03 \pm 0.01$ is consistent with $pE/kT = 0.39 \pm 0.06$ for a polar molecule with $\theta = 0$. If the permanent dipole moment is 5 D, which is reasonable for *p*-aminobenzenesulphonate at a neutral pH, we get $E = 2.5 \times 10^8 \text{ V m}^{-1}$ ($\pm 20\%$).

A more satisfactory treatment should rest on a semi-empirical evaluation of S , T and U by electric dichroism spectroscopy. In Fig. 2 we show the $A_{\parallel=\pi/2} - A$ spectrum of methyl red in the presence of an applied external electric field. As predicted by equation (1) the signal was found to be proportional to E^2 . The contribution from the T , 'polarisability', term is predominant and a curve with the shape of $\partial A/\partial \omega$ is obtained. Returning to the spectrum of methyl red at the surface of (CTA⁺)_n micelles (Fig. 1), we may now rationalise it, with the aid of the 'artificial' spectrum, in terms of an electrostatic field, radially directed from the (CTA⁺)_n, and with a strength of $1.45 \times 10^8 \text{ V m}^{-1}$. A higher value results if the incomplete alignment of the micelles is taken into account.

The present findings also agree with the field expected from electrostatic theory. We assume that the observed phenomena take place for ions at a distance r from the charged surface of the macroion (r being less than the thickness of the ionic atmosphere). From

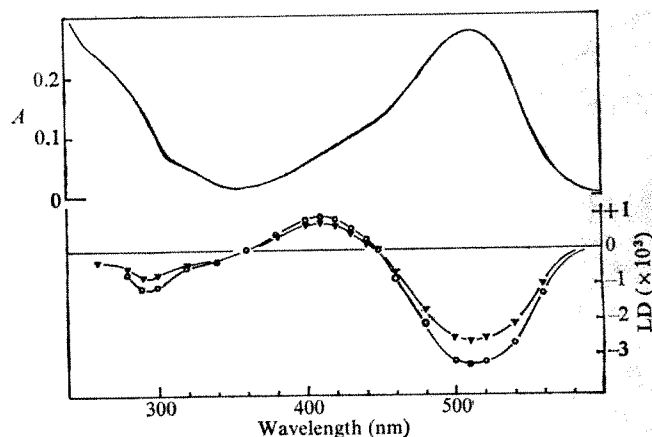
the difference in potential between bound and free counterions one may approximate the field as¹⁰

$$E = \frac{l \ln(R/r) \gamma e}{r d \epsilon} \quad (3)$$

where γ is the apparent degree of dissociation of counterions, R is the apparent radius of the macromolecule and r the radius of its free volume, d the average distance between neighbouring ionised groups and ϵ the dielectric constant of the solvent (water). In the present system we take $\gamma \leq 0.5$, $R/r = 0.2$ and $d = 5 \times 10^{-10} \text{ m}$, so in the region 5–10 Å from the cylinder we have fields ranging between 8×10^8 and $4 \times 10^8 \text{ V m}^{-1}$.

Platt¹³ suggested the term electrochromism to designate all changes in optical absorption or emission of molecules in condensed phases due to external electric fields, thus including energy

Fig. 1 Linear dichroism $LD = A_{\parallel} - A_{\perp}$ and absorption (A) of methyl red in 20% CTAB, 80% H₂O at a Couette flow of $G = 2,600 \text{ s}^{-1}$ (▽) and $5,000 \text{ s}^{-1}$ (○). Optical path-length: 0.10 cm.



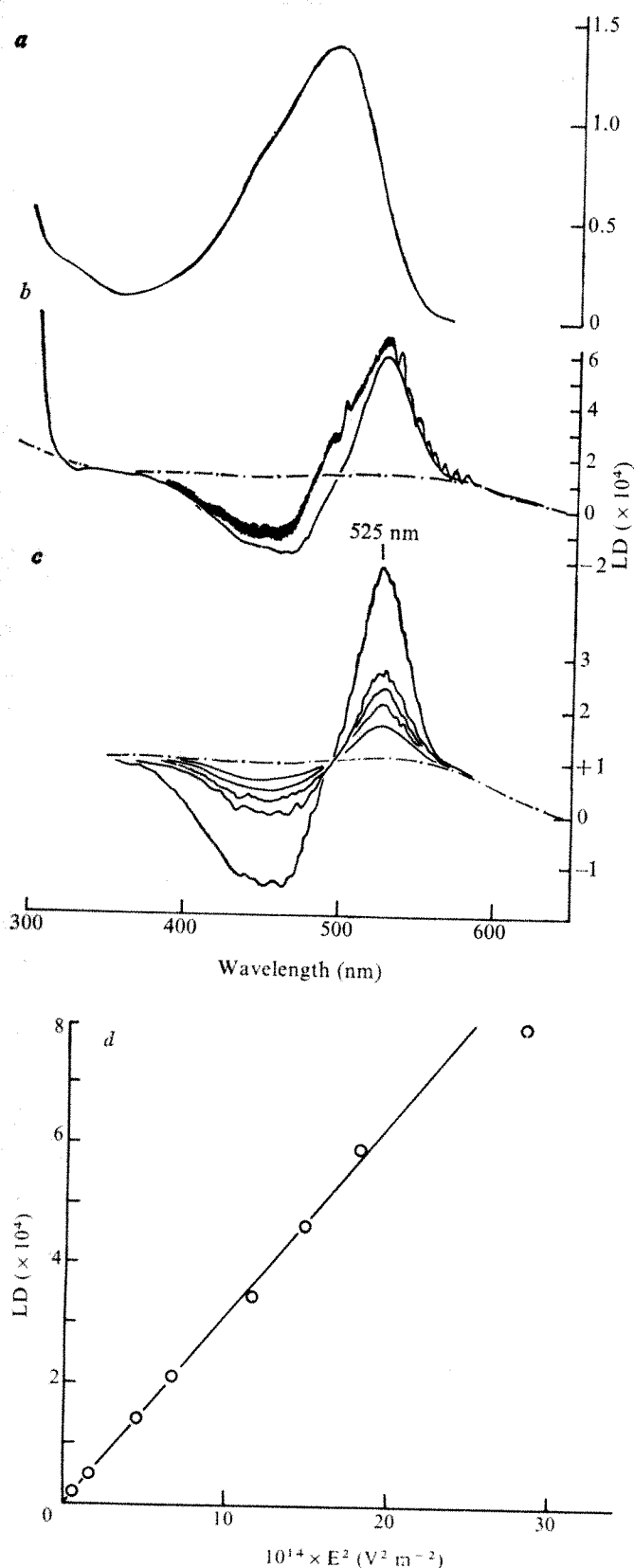


Fig. 2 *a*, Ψ Absorption and, *b*–*c*, electric linear dichroism (ELD = $A_{\lambda} - \pi/2 - A$) of methyl red in polystyrene sheet (thickness 0.0065 cm) obtained as the absorption difference with and without a longitudinal electric field (a field sinusoidally oscillating at 470 Hz between 0 and a given amplitude value, limited by flash-over around 4,000 V, was used together with lock-in electronics). *c*, Curves recorded immediately after applying the field (no orientation) at 0 (—), 1.6, 2.0, 2.3, 2.5 and 3.8×10^7 V m $^{-1}$ (certain orientation) at two spectral band widths: 2 nm (noisy curve) and 8 nm. Fringes due to interference in film, visible in the former spectrum. *d*, Steady state ELD signal at 525 nm, from the sample in *ac* plotted versus E^2 .

shifts as well as anisotropic phenomena (Kerr effect, electric dichroism). Much interest has recently centred around the internal fields in biomembranes¹⁴, solid polymers¹⁵ and so on, and the use of dye¹⁶ probes to study isotropic manifestations, such as shifts and intensity changes, of the solvent field has also been discussed. Here we report the possibility of discriminating the field effects by using anisotropic 'solvatochromism'.

This work was supported by the Swedish Natural Research Council (Project K-3216).

BENGT NORDÉN

*Inorganic Chemistry 1,
University of Lund,
Chemical Center,
P.O.B. 740,
S-220 07 Lund 7, Sweden*

Received 4 April; accepted 25 July 1977.

- ¹ Davidsson, Å. & Nördén, B. *Chem. Scr.* **9**, 49–53 (1976).
- ² Nördén, B. & Tjerneld, F. *Biophys. Chem.* **4**, 191–198 (1976).
- ³ Johansson, L. B.-Å., Lindblom, G. & Nördén, B. *Chem. phys. Lett.* **39**, 128–133 (1976).
- ⁴ Scheraga, H. A. & Backus, J. K. *J. Am. chem. Soc.* **73**, 5108–5112 (1951).
- ⁵ Nördén, B., Davidsson, Å. & Johansson, L. B.-Å. *Nature* **261**, 400–402 (1976).
- ⁶ Labhart, L. *Helv. Chim. Acta* **44**, 447–467 (1961).
- ⁷ Liptay, W. Z. *Naturforsch.* **20**, 272–289 (1965).
- ⁸ Lin, S. H. *J. chem. Phys.* **62**, 4500–4524 (1975).
- ⁹ Fredericq, E. & Houssier, C. *Electric Dichroism and Electric Birefringence* (Clarendon, New York, 1973).
- ¹⁰ Oosawa, F. *Polyelectrolytes* (Marcel Dekker, New York, 1971).
- ¹¹ Jaffé, H. H. & Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy* (Wiley, New York, 1962).
- ¹² Douglas, B. E., Haines, R. A. & Brushmiller, J. G. *Inorg. Chem.* **3**, 1194–1198 (1963).
- ¹³ Platt, J. R. *J. chem. Phys.* **34**, 862–863 (1961).
- ¹⁴ Reich, R., Scheerer, R. & Sewe, K.-U. *Ber. Bunsenges. physik. Chem.* **80**, 245–246 (1976).
- ¹⁵ Hong, S. D. & Stein, R. S. *J. polym. Sci.* **13**, 1447–1459 (1975).
- ¹⁶ Kuhn, H. & Schweig, A. *Chem. phys. Lett.* **1**, 255–258 (1967).

Quasi-Fermi level measurement in an illuminated GaP photoelectrolysis cell

No experimental procedure is known for measuring directly the position of the minority carrier quasi-Fermi level, ϕ , at the surface of a semiconductor electrode¹. ϕ is an important parameter in studies of photoelectrolysis (p.e.) (ref. 2), of surface states, of catalysis, of the anomalous intensity dependence of the photovoltage of Schottky barriers³, and of the efficiency and stability against corrosion of low-cost photoelectrochemical solar cells. Hence a means for determining ϕ should be of wide interest. I describe here a technique which results from an effort to measure changes in the potential versus standard calomel electrode (SCE), V_{Au} , of a thin porous Type B gold film⁴ evaporated on the surface of n-GaP in a $\langle Au/GaP/K_2SO_4/Pt \rangle$ p.e. cell when the cell is illuminated with photons having energy $h\nu \geq E_G$, the 2.23 eV energy gap of GaP. Changes in V_{Au} of $\sim +1.0$ V have been observed. It is shown below that $V_{Au} \sim \phi$, the minority hole quasi-Fermi level in the illuminated n-GaP anode. This series of measurements provides other valuable information. The valence band (VB) of the Au/GaP anode is shown to > 0.21 V below, or anodic to, the OH^-/H_2O redox potential, so that oxidation of OH^- is possible. But, p.e. with liberation of H_2 and O_2 is shown to be highly improbable. The results provide evidence that the Type B gold film does not protect the GaP surface against electrochemical corrosion. The variation with time of V_{Au} provides a sensitive indicator of the existence and rate of GaP corrosion. The technique for determining ϕ should be applicable to semiconductors, both n- and p-type, other than GaP.

The single crystal n-GaP (Metals Research) anode was prepared with a sintered Au + 12%Ge alloy ohmic contact on the rear surface and the front surface was etched in aqua regia. A ring of electrically insulating Sealit (Fisher Scientific) 1 mm wide was painted on the front surface at its perimeter, leaving a clear area in the centre. A 16 nm thick Au film was rapidly evaporated (3 s) to cover both the Sealit ring and the clear area. Electrical

contact to the Au film was by means of a Au-foil ring placed between the gold-covered Sealit and the polyethylene cell wall, which had a 0.6-cm diameter hole through which illumination reached the 0.12-cm² exposed Au surface.

A schematic view of the cell and electrical connections is given in Fig. 1. Illumination intensity L approximates 2 am1 suns ≈ 150 mW cm⁻². Potentials were measured between the Au ring and SCE (V_{Au}), between the GaP ohmic contact and SCE (V_{GaP}), and between the GaP ohmic contact and the Pt cathode (V_{cell}). V_{Au} , V_{GaP} and V_{cell} were first noted for the open-circuited (o.c.) cell, dark and then illuminated. Measurements of these potentials were then repeated for the short-circuited (s.c.) cell, along with observations of current I and time T . No external potential was applied at any time to the cell. N₂ was bubbled through the electrolyte during the measurements.

For the o.c. dark cell, $V_{Au} \sim V_{GaP} \sim +0.09$ V (SCE); for the o.c. illuminated cell $V_{Au} = -0.03$ V, $V_{GaP} = -1.0$ V (initially). We interpret this illuminated result to mean that the initial energy difference between the electron Fermi level, $E_a^i = V_{GaP}$, and $\phi_p = V_{Au}$, equals 0.97 V. The result also shows that illumination has caused a -0.13 V cathodic shift in the Fermi level of the Au film. The photovoltage generated through separation of electron-hole pairs by the electric field of the surface Schottky barrier causes V_{GaP} to shift to -1.0 V versus SCE.

The s.c. results, more characteristic of normal cell operation, are given in Fig. 2. The outstanding feature of the data is the immediate rise of V_{Au} from $+0.08$ V to $+0.92$ V (SCE) when the Au/GaP anode is illuminated. The OH⁻/H₂O redox potential is at $+0.71$ V (SCE) for the pH 4.6 electrolyte so that oxidation of OH⁻ ions by the photoholes is initially possible. There is a sustained rise of I during the first few minutes of illumination together with the associated parallel shift in the cathodic direction of V_{Au} and V_{GaP} . Current I continues to rise even when V_{Au} becomes cathodic to the OH⁻/H₂O potential, indicating that the major part of I is due to oxidation of some species other than OH⁻. There is an initial difference of 0.99 V for $V_{Au} - V_{GaP}$, equal to the initial o.c. potential difference, and the reduction of this difference with time as V_{Au} falls off more rapidly with time than does V_{GaP} .

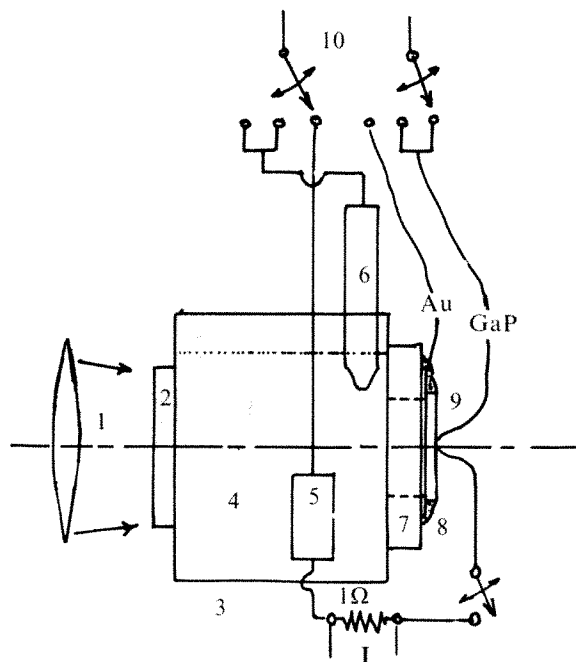


Fig. 1 Schematic view of cell for measuring V_{Au} . 1, Light from 150-W quartz-I₂ lamp; 2, quartz cell window; 3, polyethylene cell, 2.5 cm wide; 4, 0.1 M K₂SO₄ at pH 4.6; 5, large Pt black cathode; 6, Luggins capillary and SCE; 7, polyethylene mount for anode; 8, Sealit cement; 9, Au/n-GaP anode; 10, DMM having $R_{in} > 10^{10} \Omega$.

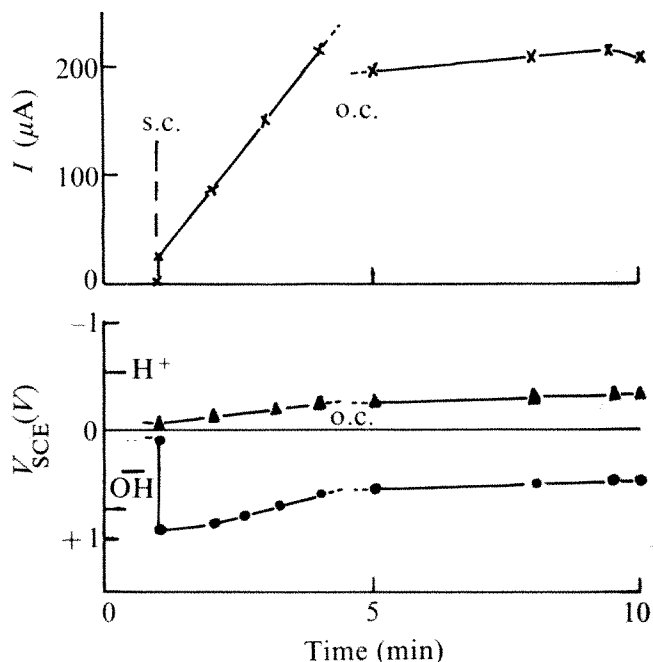


Fig. 2 Time variation of gold-film potential (●), bulk n-GaP potential (▲) and cell current (×) during the first 10 min of s.c.-illuminated operation. Note the gold-film potential is anodic to the OH⁻/H₂O redox potential for the first 2 min of operation so that oxidation of OH⁻ by the photoholes is possible.

Agitation of the electrolyte at $T = 14$ min seems to have ruptured the Au film; V_{Au} immediately fell to $+0.08$ V and the current to 37 μ A. Visual inspection of the Au surface from outside the cell showed several dark patches on the Au/GaP surface. Inspection later of the cleaned GaP surface revealed deep corrosion craters directly below where the dark patches had been noted earlier.

Tests of other gold-covered anodes revealed a pattern of behaviour similar to the above, with the exception that the gold surface films seemed to be intact.

Before the results are discussed, the concept of the quasi-Fermi level will be introduced and defined, followed by a brief examination of key features of the Type B film hypothesis⁴.

Because of the low rate of charge transfer between CB and VB of a semiconductor, non-equilibrium concentrations of electrons, $n_0 + \Delta n$, in the CB, and of minority carrier holes, $p_0 + \Delta p$, in the VB, are generated by illumination absorbed in the surface space charge region. Each distribution has in general a different mean free energy and a corresponding quasi-Fermi level. Unless L is very large, $\Delta n < n_0 = 2 \times 10^{17}$ cm⁻³, so that the electron mean free energy and Fermi level remain virtually unchanged by the illumination. But $\Delta p \gg p_0$, the minority hole concentration in the n-GaP, so that Φ_p can be changed drastically by the illumination. Thus

$$\Phi_p \equiv A + kT/e \{ \ln [(p_0 + \Delta p)/n_1] \} \quad (1)$$

$$\Delta p \sim L \tau_p \quad (2)$$

where n_1 is carrier concentration and A the electron Fermi level in an intrinsic GaP semiconductor. τ_p is the hole lifetime near the n-GaP surface.

The model⁴ for the Type B film assumes the existence of a 2 nm thick semi-insulating oxide film between the Au film and the GaP substrate. Photoholes from the n-GaP can tunnel through the oxide film into the Au film. Positive charge and potential build up in the Au film until the rate at which holes

enter the film equals the rate at which holes oxidise redox species, such as OH^- , in the electrolyte. To maintain the net flow of holes and the current in the s.c. cell, Φ_p will be more anodic than V_{Au} , and V_{Au} more anodic than the redox potential of the oxidised species. If the oxide is thin and easily tunnelled through, indicated by an abrupt rise of V_{Au} when L is turned on, one expects $V_{\text{Au}} \sim \Phi_p$. Also in this case the maximum value of $V_{\text{Au}} - V_{\text{GaP}}$ for the o.c. and s.c. cells should be almost equal, in agreement with the results above.

For the s.c. results it is possible to demonstrate a set of relationships between the cathodic shifts of V_{Au} and V_{GaP} and the sustained rise of I during the first few minutes of cell operation. (1) The cathodic shift of V_{GaP} is equal to the shift of the Pt cathode potential as increasing amounts of H are reduced and absorbed on the Pt surface by the photoelectron current originating in the CB of the n-GaP. (2) For constant L , equation (1) indicates that $V_{\text{Au}} - V_{\text{GaP}}$ should remain constant with time. This is not observed, however, instead V_{Au} shifts more rapidly in the cathodic direction than does V_{GaP} . (3) We attribute this observed difference in rate to electrochemical corrosion of the GaP surface beneath the Au film, according to the following sequence: GaP corrosion \rightarrow increased surface recombination rate for electrons and holes \rightarrow reduction in $\tau_p \rightarrow$ reduction in $\Delta p \rightarrow$ reduction in $\Phi_p \rightarrow V_{\text{Au}} - V_{\text{GaP}}$ decreases with increasing time. (4) The sustained rise in I during the first 3 min may be due to a corrosion-generated local increase of H^+ under the Au film⁵ which in turn increases the corrosion rate and I . The intensity L establishes an upper limit for I so that I peaks and then begins to fall off as corrosion reduces τ_p and Δp .

If the above analysis is valid, then the observation of a reduction in $V_{\text{Au}} - V_{\text{GaP}}$ with time can be used as a tool for determining the existence and rate of electrochemical corrosion under the protective Au film. It could thus serve to evaluate photoelectrochemical solar cells and their rate of corrosion in various electrolytes.

Data of Fig. 2 also provide information about the flat-band potential, ϵ_{fb} , of the Au/GaP anode and the possibility of p.e. The VB edge of the Au/GaP anode is more than 0.21 eV below the $\text{OH}^-/\text{H}_2\text{O}$ redox potential since Φ_p must be above the VB edge; Δp in equation (1) cannot exceed the density of hole states in the VB, ϵ_{fb} is approximately 2.23 eV above the VB edge.

During the first few minutes of s.c. operation, when V_{Au} is anodic to the $\text{OH}^-/\text{H}_2\text{O}$ potential, oxidation of OH^- by holes in the Au film is possible. In the absence of an external potential p.e. is not possible since $V_{\text{Au}} - V_{\text{GaP}} \approx \Phi_p - E_i^n < 1.23$ V, the p.e. threshold, at all times. Equation (1) shows that a 10^4 -fold increase in L and Δp would be required to make $V_{\text{Au}} - V_{\text{GaP}} \approx 1.23$ V. A Pd film evaporated on to the surface of the Au film⁴ will not alter this conclusion (in agreement with some preliminary results).

Finally, we may conclude that in almost neutral aqueous electrolytes a n-GaP surface cannot be protected from electrochemical corrosion through use of a porous gold film evaporated on to its surface.

WILLIAM E. PINSON*

Infrared Photo Limited,
41 Palsen Avenue, Ottawa,
Ontario, Canada K2G 2W3

Received 20 June; accepted 12 July 1977.

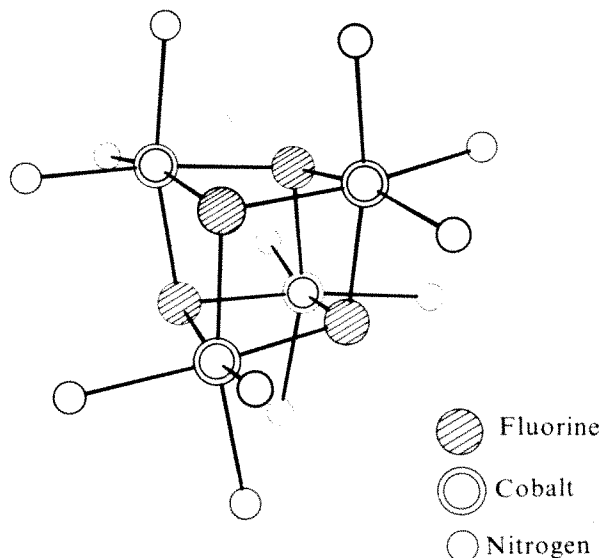
* Visiting professor, Physics Department, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5.

The first cubane-type fluoro-bridged cluster

CUBANE-TYPE clusters containing the unit M_4A_4 have been the subject of many investigations. Several of these systems have been characterised by X-ray structure determination. Such clusters are important from a bioinorganic point of view, for example, Fe_4S_4 clusters in iron-sulphur proteins¹. From a catalytic point of view, clustered compounds are becoming increasingly important as models for heterogeneous catalysts, and even as catalysts by themselves². Monoatomic ligands (for example, $\text{A} = \text{S}, \text{Cl}, \text{P}, \text{Br}, \text{Se}$) have been reported as bridging ligands in cubane-type clusters, but larger groups are known in which C, N, and O act in a similar way³. No cubane-type clusters have been previously reported with the highly electronegative F^- ion as a bridging ligand. We describe here the synthesis and structure of the first fluoro-bridged cubane-type cluster, having the formula $[\text{M}_4\text{F}_4(\text{L})_{12}] (\text{BF}_4)_4$, in which $\text{M} = \text{Mn}, \text{Cd}, \text{Co}$ and $\text{L} = N\text{-ethylimidazole}$ or $N\text{-propylimidazole}$. On reaction of $\text{M}(\text{H}_2\text{O})_6 (\text{BF}_4)_2$ in ethanol-triethyl orthoformate with an ethanolic solution of the ligand, finely-divided white powders (Mn, Cd) or purple crystals (Co) separate, analysing as $\text{M}(\text{ligand})_4\text{BF}_4$. In these circumstances the F^- ions are spontaneously generated by a slow decomposition of the BF_4^- ion. Such a decomposition of tetrafluoroborates, yielding fluoro complexes, has been reported in a few other cases, yielding polymeric fluoro-bridged systems⁴, dimers $(\text{M}_2\text{F}_2)^5$ and also monomers MF_2 (ligand)₄ (ref. 6).

X-ray powder diagrams showed all compounds to be mutually isomorphous. Infrared spectra indicate the presence of uncoordinated BF_4^- ions. Ligand-field spectra of the Co(II) compounds indicate a six-coordinate octahedrally-based geometry for Co(II) , whereas magnetic measurements at low temperature (2–80 K) show the presence of a weak anti-ferromagnetic exchange ($-J \sim 1 \text{ cm}^{-1}$) between the Co(II) ions. The strong far-infrared absorptions around 300 cm^{-1} suggest the presence of bridging M-F-M units. From these physical measurements a cubane-type structure was proposed. To verify this

Fig. 1 Cubane-type cluster of $\text{Co}_4\text{F}_4 (N\text{-ethyl imidazole})_{12}^{4+}$ with the first coordination sphere around cobalt. Mean distances and angles are: $\text{Co-F} = 2.14(2) \text{ \AA}$; $\text{Co-N} = 2.19(3)$; $\text{Co-Co} = 3.29(2)$; $\text{F-F} = 2.71(1)$; $\angle \text{F-Co-F} = 78.5(5)^\circ$; $\angle \text{Co-F-Co} = 100.5(5)^\circ$.



¹ Gerischer, H. *Proc. Int. Conf. Photochem. Conversion Storage sol. Energy Univ.* Western Ontario, London, Ontario, Canada (August, 1976).

² Manassen, J., Cahen, D., Hodes, G. & Sofer, A. *Nature* **263**, 97–100 (1976).

³ Gerischer, H. *J. electroanal. Chem.* **58**, 263–274 (1975).

⁴ Nakato, Y., Abe, K. & Tsubomura, H. *Ber. Bunsenges. physik. Chem.* **80**, 1002–1007 (1976).

⁵ Gerstner, M. E., Harris, L. A. & Wilson, R. H. *Abst. 442 RNP, Philadelphia Meeting Electrochem. Soc.* (May, 1977).

proposal a crystal-structure determination was undertaken.

An octahedrally shaped crystal of $\text{Co}(\text{N-ethylimidazole})_2\text{BF}_4$ having the approximate edges of 0.2 mm was selected. The crystal belongs to the cubic space group Pa3 with $a = 25.797(1)$ Å. The experimental density of about 1.4 g cm^{-3} indicates that 32 CoL_2BF_4 units are present in the unit cell. One unit occupies an eightfold position (the threefold axis); the other one is in a general 24-fold position. The cluster, consisting of four CoL_2BF_4 units, has trigonal symmetry. At the present stage of refinement $R_F = 0.13$. The cluster is drawn in Fig. 1. The trigonal clusters can be considered as being built up by two interpenetrating tetrahedrons. The F_4 tetrahedron is symmetric: all F-F distances are equal within experimental error. The Co_4 tetrahedron has a larger edge and is slightly elongated in the trigonal direction: $\text{Co-Co} = 3.28\text{--}3.31$ Å. This relatively large Co-Co distance suggests at best a weak Co-Co bonding. The Co-F and Co-N distances have normal values compared to other octahedrally coordinated Co(II) compounds. The BF_4^- ions are located outside the cluster; one BF_4^- is on the trigonal axis, whereas the other anion is in a general position. Due to disorder the orientation of the BF_4^- anions cannot be determined accurately.

The Co-F-Co angles of about 101° are important in understanding the low-temperature magnetic behaviour of this compound. According to De Jongh and Block⁹ bond angles larger than 90° should result in antiferromagnetic behaviour, in agreement with the experiments.

J. C. JANSEN
H. VAN KONINGSVELD
J. REEDIJK

Delft University of Technology,
Department of Chemistry,
Delft-2208,
The Netherlands

Received 4 July, accepted 8 August 1977.

¹ Holm, R. H. *Endeavour* 34, 38-45 (1975).

² Demitras, G. C. & Muetterties, E. L. *J. Am. chem. Soc.* 99, 2796-7 (1977).

³ Bertrand, J. A. & Eller, P. G. *Progr. inorg. Chem.* 21, 29-53 (1976).

⁴ Guichelaar, M. A., van Hest, J. A. M. & Reedijk, J. *Inorg. nucl. Chem. Lett.* 10, 999-1004 (1974).

⁵ Jansen, J. C. & van Koningsveld, H. *Cryst. Struct. Commun.* 5, 441-445 (1976).

⁶ Smit, S. & Groeneveld, W. L. *Inorg. nucl. Chem. Lett.* 11, 277-80 (1975).

⁷ Babel, D. Z. *Anorg. Allg. Chem.* 369, 117-30 (1969).

⁸ Clarke, P. J. & Milledge, M. J. *Acta Crystallogr. B* 31, 1543-53 (1975).

⁹ de Jongh, L. J. & Block, R. *Physica* 79B, 568-93 (1975).

Hydrothermal manganese in the Galapagos Rift

HYDROTHERMAL emanations originating at mid-ocean ridges have been thought¹⁻⁵ to provide a substantial source of manganese to the ocean but the evidence supporting this hypothesis has been indirect. Anomalous manganese concentrations have been measured in naturally occurring systems where seawater is in direct contact with lava flows⁶⁻⁸. Laboratory studies have shown that seawater tends to leach manganese from basalts at elevated temperatures and pressures⁹⁻¹¹. Anomalous high manganese accumulation rates have also been determined for sediments adjacent to active ridge systems, most notably the East Pacific Rise¹²⁻¹⁴. No measurements of manganese concentrations in seawater near mid-ocean ridges, or in hydrothermal fluids emanating from these ridges, have yet been made, however. We report here the results of the first such direct measurements, which show that manganese is being injected into the deep sea by hydrothermal circulation of seawater

through newly-formed oceanic crust.

We analysed water samples collected in the Galapagos Rift during two cruises of the RV Melville (Pleiades expedition, Legs 1 and 2). These samples were collected from hydrocasts and with a remote-controlled sampler attached to the Deep Tow vehicle of the Scripps Marine Physical Laboratory¹⁵. Unfiltered aliquots of these samples were acidified to pH 2 with redistilled 6M HCl. Manganese was determined by addition of radioactive ⁵⁴Mn,

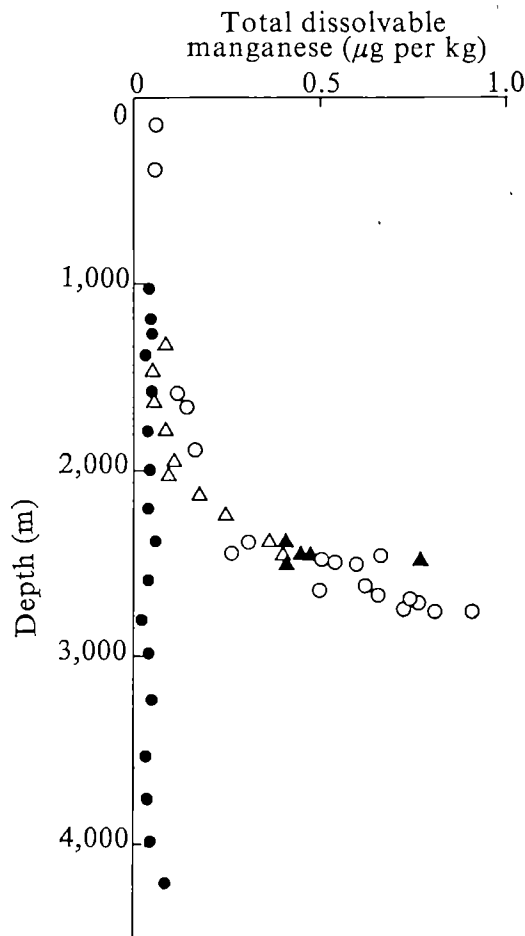


Fig. 1 The concentrations of TDM plotted against depth for samples collected from hydrocasts at two Pacific locations. ●, Samples collected during the Geosecs program at station 343 ($16^\circ 31.6' \text{N}$, $123^\circ 1.4' \text{W}$). △ And ○, samples from eight Pleiades Expedition stations taken at the Galapagos Rift survey site ($0^\circ 35.5'$ to $0^\circ 48.0' \text{N}$, $86^\circ 4.7'$ to $86^\circ 10.2' \text{W}$; see map ref. 15) △, Pleiades Leg 1; ○, Pleiades Leg 2. ▲, Deep Tow samples, although results for samples 7-0 and 8-3 are not plotted. The size of the symbols approximates the analytical uncertainty of the measurements.

adjustment of the pH to 8, extraction of manganese into 8-hydroxyquinoline in chloroform, and back extraction into 3M HNO_3 (ref. 1). Manganese concentration in the 3M HNO_3 solution was measured using an atomic absorption spectrophotometer with a graphite furnace and the yield was determined by counting the 835 keV ⁵⁴Mn γ ray. From these data and the volumes of the initial sample and concentrate, the manganese concentration of the initial sample was calculated. This value, which is believed to include all dissolved and particulate manganese except that fraction in the lattice of silicate minerals, is called 'total dissolvable manganese' (TDM)¹.

Figure 1 shows how TDM concentrations vary with depth at two separate locations. The shaded circles represent samples collected during the Pacific Geosecs program at station 343 ($16^\circ 31.6' \text{N}$, $123^\circ 1.4' \text{W}$). Of the locations at which we have made these measurements, station 343 is the closest geographi-

cally to the Galapagos Rift. Below 1,000 m, the TDM profile at this station is typical, with concentrations ranging from 0.017 to 0.087 μg per kg. Also shown in Fig. 1 are the TDM concentrations found for the samples collected from hydrocasts over the Galapagos Rift. They include samples taken at one station during Leg 1 and seven stations during Leg 2. These samples show TDM concentrations ranging from 0.046 to 0.90 μg per kg, with a maximum at the depth of the ridge crest in the study area. We believe that these anomalously high concentrations are the result of the injection of hydrothermal plumes into this area. Furthermore, the fact that the TDM concentration increases rapidly with increasing depth implies scavenging of hydrothermal manganese from the water column.

In addition to the high manganese concentrations found in the hydrographic profiles, we have made direct measurements of manganese in hydrothermal plume samples collected in the Galapagos Rift by the Deep Tow sampling system. The hydrothermal origin of these plume samples has been unequivocally demonstrated by measurements of excess ^3He and ^{222}Rn (ref. 16) as well as by the hydrographic evidence¹⁸. The TDM results for the Deep Tow samples are listed in Table 1, together with the ^{222}Rn results¹⁶ for the same samples. Sample 8-3, which had a ^{222}Rn anomaly of 80 d.p.m. per 100 kg, had a small positive $\delta^3\text{He}$ anomaly, and sample 7-0, which had a ^{222}Rn anomaly of 191 d.p.m. per 100 kg, had a large $\delta^3\text{He}$ anomaly. These data show that there is a one-to-one correspondence between the presence of very high TDM concentrations, the hydrographic identification of plumes, and the presence of excess quantities of helium and radon. The TDM concentration in plume sample 7-0 is 50 times the concentration in the surrounding bottom water. TDM is thus an exceptionally sensitive tracer of hydrothermal fluids: dilutions of the order of one part in 10^6 parts bottom water should be measurable.

Table 1 Total dissolvable manganese measurements on samples collected with the Deep Tow vehicle on the Galapagos Rift (0°48'N, 85°55'W), Pleiades Expedition Leg I

Tow no.-bottle no.	TDM (μg per kg)	Excess ^{222}Rn ¹⁶ (d.p.m. per 100 kg)
Samples with potential temperature spikes		
7-0	24	191
8-3	2.1	80
Near-bottom samples with no temperature spikes		
7-1	0.41	39
7-2	0.41	49
8-1	0.77	—
8-5	0.48	—
8-7	0.48	—

Sample 7-0 was collected from a hydrothermal plume with a potential temperature anomaly of $+0.20^\circ\text{C}$ relative to the surrounding bottom water¹⁶. Assuming that hydrothermal water in contact with basalt has a temperature of 100°C (ref. 17), and that a negligible fraction of the TDM in sample 7-0 was scavenged before sampling, we calculate that the manganese concentration in the undiluted hydrothermal solution is 12 mg per kg. This value agrees well with predictions based on measurements of dissolved manganese in seawater basalt systems⁸⁻¹¹.

Our measurements provide the first direct evidence that manganese is injected into the deep water column as a result of leaching from newly-formed crust by hydrothermally circulating seawater. Although further measurements are required to establish the areal and temporal variability of this process, our data suggest that hydrothermal sources represent an important part of the manganese cycle in seawater.

We thank R. Collier, J. M. Edmond, J. Corliss, F. N. Spiess, P. F. Lonsdale, H. Craig, J. Dymond, and the participants of Pleiades Expedition Legs 1 and 2 of the RV *Nelville* who made the collection of these data possible. We also thank A. E. Bain-

bridge and the Geosecs Operations Group for collecting the Geosecs samples. This work was supported by the USNSF.

G. KLINKHAMMER

Graduate School of Oceanography,
University of Rhode Island

M. BENDER

Graduate School of Oceanography,
University of Rhode Island,
Kingston, Rhode Island 02881

R. F. WEISS

Scripps Institution of Oceanography,
University of California, San Diego,
La Jolla, California 92093

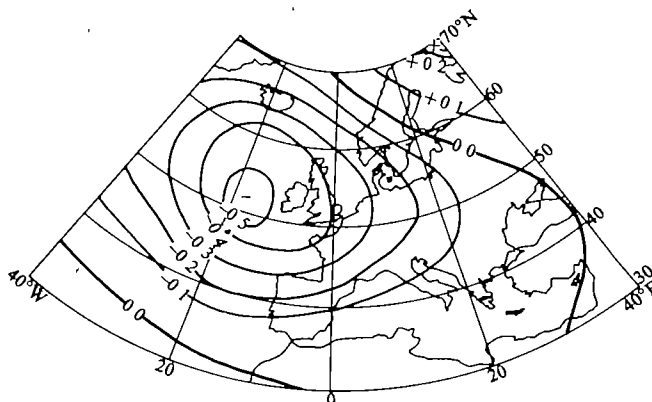
Received 9 May; accepted 19 July 1977.

- 1 Bender, M., Klinkhammer, G. & Spencer, D. *Deep-Sea Res.* (in the press).
- 2 Seyfried, W. & Bischoff, J. *J. Earth planet Sci. Lett.* 34, 71-77 (1977).
- 3 Elderfield, H. *Mar. Chem.* 4, 103-132 (1976).
- 4 Lyle, M. *Geology* 4, 733-736 (1976).
- 5 Corliss, J. B. *J. geophys. Res.* 76, 8128-8138 (1971).
- 6 Elderfield, H. *Mar. Geol.* 13, M1-M6 (1972).
- 7 Ferguson, J. & Lambert, I. B. *Econ. Geol.* 67, 25-37 (1972).
- 8 Claffison, J. *Nature* 255, 138-141 (1975).
- 9 Bischoff, J. L. & Dickson, F. W. *Earth planet. Sci. Lett.* 25, 385-397 (1975).
- 10 Mottl, M. J., Corr, R. F. & Holland, H. D. *Geol. Soc. Am. Abs. Program.* 6, 879 (1974).
- 11 Hajash, A. *Contr. Miner. Petrol.* 53, 205-226 (1975).
- 12 Bostrom, K. & Peterson, M. N. A. *Econ. Geol.* 61, 1258-1265 (1966).
- 13 Bender, M. *et al. Earth planet Sci. Lett.* 12, 425-433 (1971).
- 14 Dymond, J. & Veeh, H. H. *Earth planet Sci. Lett.* 28, 13-22 (1975).
- 15 Weiss, R. F., Lonsdale, P. F., Lupton, J. E., Bainbridge, A. E. & Craig, H. *Nature* 267, 600-603 (1977).
- 16 Lupton, J. E., Weiss, R. F. & Craig, H. *Nature* 267, 603-604 (1977).
- 17 Ross, D. A. *Science* 175, 1455-1457 (1972).

Solar influence on North Atlantic mean sea level pressure

A PRINCIPAL component analysis (PCA) of mean sea level (MSL) pressure data for the North Atlantic-European sector of the Northern Hemisphere has revealed evidence of a solar-terrestrial relationship. The principal components represent independent atmospheric circulation anomaly patterns and the coefficients give the variation in time of the contribution of each anomaly pattern^{1,2}. The data set described here comprises 101 winter MSL pressure anomalies (relative to the 1874 to 1974 winter average) on a 25-point, 20° longitude by 10° latitude grid covering the area 40°W - 40°E , 30°N - 70°N . Winters (December-February) are dated by the year of the January. The suitability of this grid for calibrating proxy data for the European sector is being investigated as a preliminary stage to the derivation of Holocene climatic information³. The data were obtained from the UK Meteorological Office,

Fig. 1 Second principal component of winter MSL pressure anomalies (mbar). Based on data for the period 1874-1974.



and are likely to contain inhomogeneities due to different analysis techniques⁴ and so on. Some errors have become apparent in the course of the analysis and in general are confined to negligible low variance principal components. Missing data were replaced by the 101-yr mean for the grid point. Similar principal components were obtained by repeating the analysis for 30-yr sub-intervals. A slight latitudinal shift in the patterns occurred and there was some change in the relative importance of the principal components. Detailed results of the PCA are to be published elsewhere.

The second principal component (PC2) accounts for 20% of the variance of the original data set. The pattern is dominated, when its coefficient is positive, by a large negative anomaly area to the south of Iceland and west of the UK (Fig. 1). Positive anomalies occur over north-east Scandinavia. A positive contribution of PC2, therefore, represents intensified cyclonic development centred around 50°N 20°W, corresponding to a southward displacement

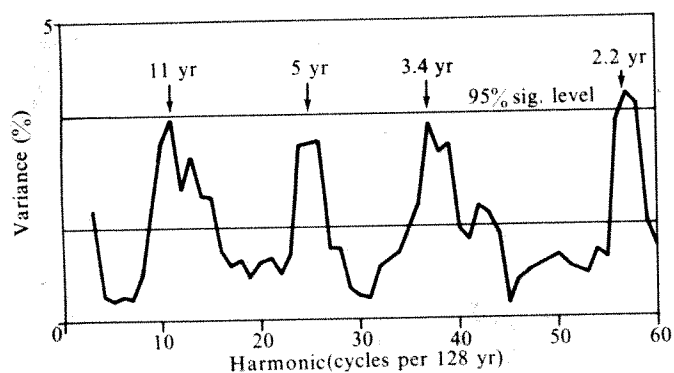


Fig. 2 Variance spectrum of the coefficients of PC2.

of the Iceland Low and enhanced southerly airflow over the North Sea. The pattern is inverted if its coefficient is negative.

The variance spectrum of the time series of the coefficients of the second principal component is shown in Fig. 2. Spectral peaks occur centred at 11, 5.0, 3.4 and 2.2 yr. The 11 and 2.2-yr peaks touch and surpass respectively the 95% significance level for an *a priori* choice of period, assuming a white noise null continuum³. The 2.2-yr peak may be associated with the quasi-biennial oscillation, but it is close to the Nyquist frequency. The other three peaks I tentatively assign to the 11-yr sunspot cycle and its harmonics; they are jointly significant at the 95% level⁵. A conservative estimate of the area enclosed by these three peaks is that 25% of the time variance of PC2 is associated with these cycles. This represents 5% of the total variance in the original data set.

Further evidence that the 11-yr cycle in the coefficients of PC2 is associated with solar variation is found by comparing the spatial pattern of PC2 with the pattern of 24-h 500-mbar height change following samples of individual solar flares⁶. Schuurmans' map (Fig. 3) shows an area of negative 500-mbar height anomalies (which can be interpreted as an anomalous upper air trough) over the mid North Atlantic and positive anomalies (an anomalous upper air ridge) over western Europe, with enhanced upper air southerlies over the eastern North Atlantic. The area of negative MSL pressure anomalies in PC2 is situated beneath the region of upper air divergence just ahead of the anomalous upper air trough. Such a region is favourable for cyclonic development and intensification⁷. This close correspondence between PC2 and the upper air circulation anomaly patterns associated with

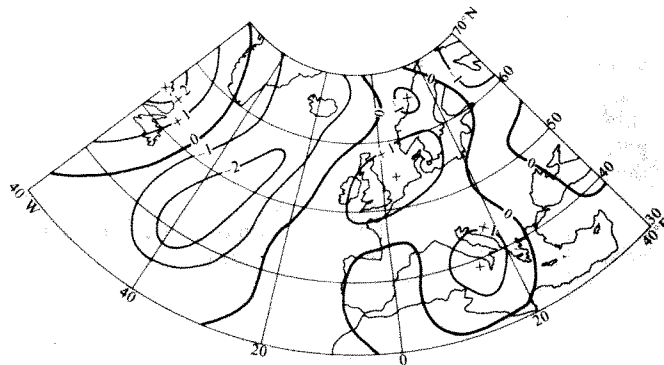
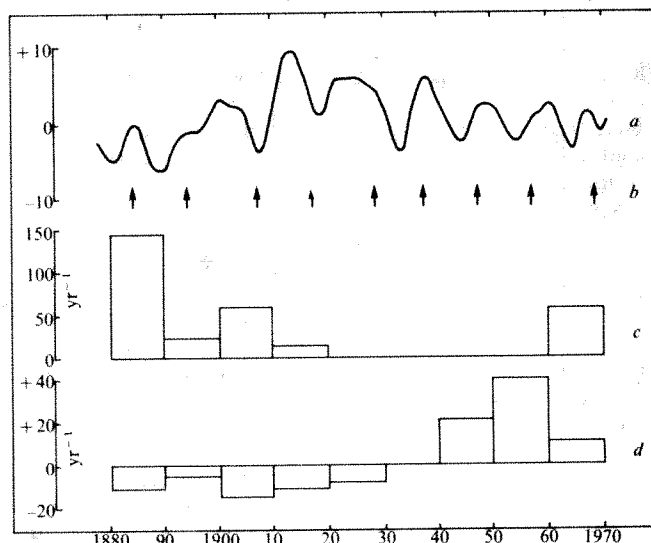


Fig. 3 500 mbar height change (gpdm) in the 24 h following a solar flare (mean of 81 events). (After Schuurmans⁶.)

high solar activity lends added support to the reality of the solar-terrestrial relationship. As solar flare activity is enhanced at sunspot maximum, the longer period solar effect on the atmospheric circulation seen in PC2 arises through the modulation throughout the 11-yr sunspot cycle of the short-term solar flare effects on the circulation. The physical mechanism by which low energy solar particles emitted in flare events can affect the atmospheric circulation has yet to be identified. The pattern of PC2 is very similar to the MSL pressure difference between sunspot maximum and minimum presented by Parker⁸, based on January data since 1750. An 11-yr cycle is also present in the S index, a measure of southerliness over the UK⁹. This variation in southerliness over the eastern Atlantic is a common feature of all the MSL pressure patterns associated with solar variation discussed here.

The coefficients of PC2, passed through a low pass binomial filter designed to reduce the amplitude of wavelengths less than 10 yr by 50% or more⁵, are shown in Fig. 4. The dates of sunspot maxima are arrowed¹⁰. An in-phase relationship is present during the 1880s and throughout the period 1920–60. It breaks down, however, between 1890 and 1920 and possibly during the 1960s. The relationship between North Atlantic sea temperature and sunspot num-

Fig. 4 *a*, Coefficients of PC2, smoothed with a 9 weight low pass binomial filter⁵. *b*, Dates of sunspot maxima¹⁰. *c*, Volcanic dust veil index, 10-yr averages¹⁴ updated to 1969 (H. H. Lamb, personal communication). *d*, Relative sunspot numbers, 10-yr average anomalies relative to 1949–71 mean¹⁰.



bers, discussed by Muir¹¹ and Colebrook^{12,13} is also weakest about the turn of the century. This apparent variability in the strength of the North Atlantic solar-terrestrial relationship requires further investigation before its predictive potential can be realised. There are three possible explanations for the long-term variability. First, sunspot numbers may not be a good indicator of the particular component of the solar output affecting the atmospheric circulation. Second, the volcanic dust loading of the stratosphere was minimal during the period 1920-1960¹⁴ (Fig. 4c) and this may have resulted in a stronger solar control on the atmospheric circulation during that period. At other times, the volcanic control may mask the solar effects. Third, the amplitude of the sunspot cycle was far greater after 1920 than before (Fig. 4d).

The principal component analysis of the MSL pressure data is to be extended to other sectors of the Northern Hemisphere Proxy data for areas where the solar-terrestrial relationship is strongest are to be used to lengthen, the record of this 11-yr climatic variation and to identify the cause of the longer-term variation in its strength.

P.M.K. is supported by a NERC Research Fellowship.

P. M. KELLY

Climatic Research Unit,
School of Environmental Sciences,
University of East Anglia,
Norwich, UK

Received 30 May; accepted 25 July 1977

- ¹ Kutzbach, J. E. *J. appl. Met.* 6, 791-802 (1967).
- ² Craddock, J. & Flood, C. *Q. J. R. met. Soc.* 95, 576-593 (1969).
- ³ Kelly, P. M. *Palaeogeog. Palaeoclim. Palaeoecol.* (in the press).
- ⁴ Williams, J. & van Loon, H. *Mon. Weath. Rev.* 104, 1354-1361 (1976).
- ⁵ W.M.O. T.N. No. 79 *Climatic Change* (W.M.O., Geneva, 1966).
- ⁶ Schuurmans, C. J. E. *Meded. Verh. K. ned. met. Inst.* 92 (1969).
- ⁷ Sutcliffe, R. C. & Forsdyke, A. G. *Q. J. R. met. Soc.* 76, 189-217 (1950).
- ⁸ Parker, B. N. *Met. Mag.* 105, 33-44 (1976).
- ⁹ Kelly, P. M. *Phys. Bull.* 27, 305-307 (1976).
- ¹⁰ Lamb, H. H. *Climate: Present, Past and Future* 1 (Methuen, London, 1972).
- ¹¹ Muir, M. S. *Nature* 266, 475-476 (1977).
- ¹² Colebrook, J. M. *Nature* 263, 576-577 (1976).
- ¹³ Colebrook, J. M. *Nature* 266, 476 (1977).
- ¹⁴ Lamb, H. H. *Phil. Trans. R. Soc.* 226, 425-533 (1970).

Copper exclusion as a mechanism of heavy metal tolerance in a green alga

ORGANISMS isolated from environments polluted by heavy metals are often tolerant of those metals¹⁻⁵. Tolerance is usually accompanied by metal uptake equal to or greater than that of non-tolerant organisms^{2,6-10}; the accumulated metals seem to be chemically detoxified and/or physically sequestered to render them inactive^{2,6,7,11-13}. Because heavy metal tolerant algae¹⁰, plants², yeast⁷ and invertebrates^{1,10} have been found not to restrict metal uptake, metal exclusion has been considered a rare mechanism of tolerance⁹. I report here, however, that a copper-tolerant strain of the common unicellular green alga *Chlorella vulgaris* excludes copper.

Two strains of *Chlorella vulgaris* Beijerinck 1890 var. *vulgaris* Fott et Nováková were isolated from the River Hayle, which drains disused copper mines in Cornwall, England. The non-tolerant strain came from a site upstream of mining influence where copper was not detectable in the water ($<0.002 \text{ mg l}^{-1}$). The tolerant strain came from a polluted site with a total copper concentration in the water of $0.12 \pm 0.03 \text{ mg l}^{-1}$ (mean \pm s.e.m. of four samples taken during 1975-76.) To eliminate any non-genetic variation between the isolates, clones of each strain were subcultured approximately 30 times on Bold's basal medium agar¹⁴.

The effect of copper on the growth rates of the tolerant and non-tolerant strains is shown in Fig. 1. Although copper

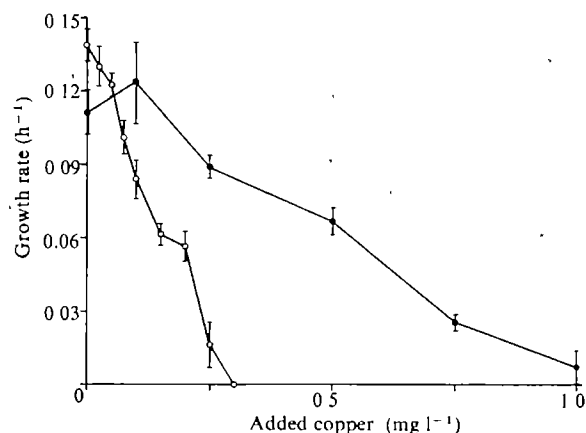


Fig. 1 The effect of copper added to the medium on the growth rate of tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris*. Cultures were grown axenically in defined medium with constant light (8,000 lx supplied by 10 40-W warm white fluorescent tubes) and temperature ($24 \pm 1^\circ \text{C}$). The medium contained: 0.20 mM NaNO_3 ; 0.04 mM MgSO_4 ; 0.07 mM CaCl_2 ; 0.25 mM NaCl ; 0.006 mM K_2HPO_4 ; 0.024 mM KH_2PO_4 ; 5.0 mM PIPES (piperazine-*N,N'*-bis [2-ethane sulfonic acid]) at pH 6.2; plus 0.01 ml l^{-1} of each of the standard Bold's basal medium micronutrient solutions¹⁴ but without EDTA. The pH was 6.2 at the beginning and end of growth. Cultures were of 50-ml volumes in 250-ml conical flasks and were aerated by shaking at 60 oscillations min^{-1} on a reciprocal shaker. Copper was added as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Growth was followed as turbidity using an Evans Electroselenium nephelometer with a green filter (OGR1). Growth rates were computed from a least squares fit to the linear phase of the growth curves and are expressed as the reciprocal of the doubling times in h. Each point is the mean of three cultures, bars represent the standard errors of the mean.

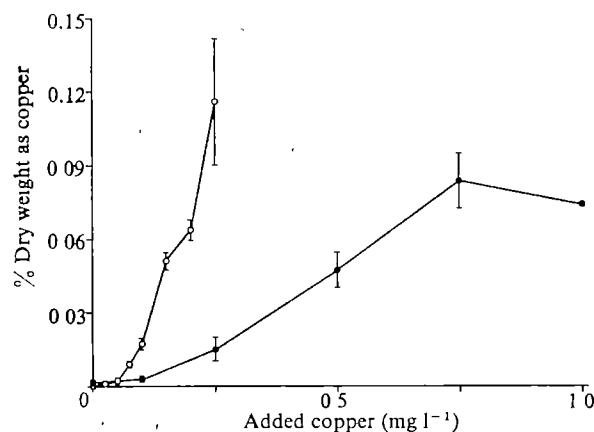


Fig. 2 The amount of copper, as percentage dry weight, accumulated by the tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris* against the amount of copper added to the medium. Stationary phase cells were centrifuged at 500g for 30 min, washed three times with 50-ml volumes of distilled water, and filtered on to washed and pre-weighed Gelman membrane filters (0.45 μm pore diameter). After drying at 70°C for 24 h, filters were weighed to determine dry weight yield, boiled in 10 ml Aristar HNO_3 to near dryness, taken to 5 ml with distilled water, and analysed for copper with an Evans Electroselenium 240 atomic absorption spectrophotometer fitted with a Shandon Southern Instruments A3470 flameless attachment (carbon rod). Results are expressed as percentage dry weight as copper, computed as the amount of copper accumulated by the algae divided by the total dry weight yield of each culture, $\times 100$. At 0 mg l^{-1} copper, the total yields of the two strains were equivalent. With increasing copper, the yields were reduced; for both strains, the minimum yields, at the highest copper concentrations, were 60% of those of the 0 mg l^{-1} copper controls. Each point is the mean of three cultures, except those points at copper concentrations at which not all cultures grew, namely, the non-tolerant strain at 0.25 mg l^{-1} copper (mean of two cultures) and the tolerant strain at 1 mg l^{-1} copper (one culture). Bars represent standard errors of the mean.

reduced the growth rates of both strains, the tolerant strain was much less sensitive. A copper concentration of as little as 0.05 mg l^{-1} decreased the growth rate of the non-tolerant strain, and 0.30 mg l^{-1} was completely inhibitory. The tolerant strain, however, was not affected by 0.10 mg l^{-1} , and one of three cultures at 1.00 mg l^{-1} survived and grew.

Other reported effects of heavy metals on unicellular algae—increased lag period^{15–17}, increased cell size^{15,16,18} and decreased yield of cell number^{4,18} and dry weight¹⁹—were observed with both strains. The appearance of giant cells, up to 20 times the normal volume, reflects an uncoupling of growth from cell division; as pointed out previously^{15,18,20}, this phenomenon indicates that heavy metals may specifically inhibit division in algae. Also, early in the growth phase, dividing cells failed to separate, resulting in multicellular aggregates, which persisted until late log phase. Thus it seems that heavy metals also affect cell separation.

Figure 2 shows that the non-tolerant strain accumulated 5–10 times more metal at comparable external copper concentrations than did the tolerant strain. The growth rate of each culture as a function of the amount of copper accumulated by the algae is shown in Fig. 3. The lack of difference between the two strains implies that they respond identically to the same amount of cellular copper. Other growth parameters, for example, lag period and yield, are

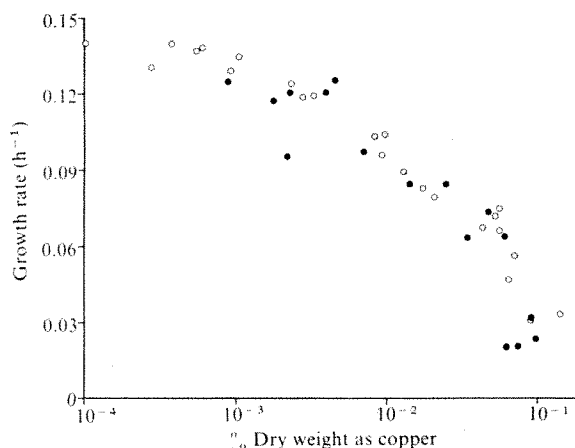


Fig. 3 The relationship between the growth rate of each culture and the amount of copper, as percentage dry weight, accumulated by the tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris* grown at various external copper concentrations. Growth rate is the reciprocal of the doubling time in h.

also identical in the two strains at equivalent cellular copper concentrations. Thus copper exclusion is likely to be the only mechanism of tolerance in this alga.

There have been a few reports of metal tolerant organisms that exclude metals. Micro-organisms commonly accelerate the vaporisation of mercury^{21,22}, although Davies¹⁸ reported that the mercury tolerance of the marine alga *Dunaliella tertiolecta* was primarily due to internal detoxification. Bryan and Hummerstone⁸ found a zinc-tolerant marine polychaete, *Nereis diversicolor*, to be impermeable to zinc, cadmium and manganese; copper tolerance in this animal may also result in part from decreased permeability²³. Other zinc-tolerant organisms, including the freshwater bryophyte *Scapania undulata*²⁴, and several species of freshwater filamentous algae (my unpublished results), also seem to exclude zinc.

Because algae form the base of many aquatic food chains, a mechanism of tolerance involving the exclusion of metal may be important to the ecology of polluted environments. A metal that is not accumulated by primary producers is less likely to be concentrated in higher trophic levels.

I was supported in part by a studentship from the Huntingdon Research Centre, Huntingdon, Cambridge.

PATRICIA L. FOSTER

Department of Applied Biology,
University of Cambridge,
Pembroke Street, Cambridge, UK

Received 30 June; accepted 4 August 1977.

- Bradshaw, A. D. *Nature* **169**, 1098 (1952).
- Antonovics, J., Bradshaw, A. D. & Turner, R. G. *Adv. ecol. Res.* **7**, 1–85 (1971).
- Russel, G. & Morris, O. P. *Nature* **228**, 288–289 (1970).
- Stokes, P. M., Hutchinson, T. C. & Krauter, K. *Can. J. Bot.* **51**, 2155–2168 (1973).
- Harding, J. P. C. & Whitton, B. A. *Br. phycol. J.* **11**, 417–426 (1976).
- Turner, R. G. in *Ecological Aspects of Mineral Nutrition of Plants* (ed. Rorison, I. H.) *Br. ecol. Soc. Symp.* **9**, 399–410 (Blackwell, Oxford, 1969).
- Ashida, J. A. *Rev. Phytopath.* **3**, 153–174 (1965).
- Bryan, G. W. & Hummerstone, L. G. *J. mar. biol. Ass. U.K.* **51**, 845–863 (1971); **53**, 839–857, 859–872 (1973).
- Hutchinson, T. C. & Stokes, P. M. in *Water Quality Parameters, ASTM STP* **573**, 320–343 (Am. Soc. for Testing and Materials, 1975).
- Brown, B. E. *Water Res.* **10**, 555–559 (1976).
- Turner, R. G. & Marshall, C. *New Phytol.* **70**, 539–545 (1971).
- Silverberg, B. A. *Phycologia* **14**, 265–274 (1977).
- Brown, B. E. *Freshwat. Biol.* **7**, 235–244 (1977).
- Nichols, H. W. & Bold, H. C. *J. Phycol.* **1**, 34–38 (1965).
- Steemann Nielsen, E. & Kamp-Nielsen, L. *Physiol. Pl.* **23**, 828–840 (1970).
- Nuzzi, R. *Nature* **237**, 38–40 (1972).
- Bartlett, L., Rabe, F. W. & Funk, W. H. *Water Res.* **8**, 179–185 (1974).
- Davies, A. G. *J. mar. biol. Ass. U.K.* **56**, 39–57 (1976).
- Greene, J. C., Miller, W. E., Shiroyama, T., Soltero, R. A. & Putnam, K. *Proc. Symp. on Terrestrial and Aquatic Ecological Studies of the Northwest, Eastern Washington State College, Cheney, Washington, March 26–27*, 327–336 (1976).
- Kanazawa, T. & Kanazawa, K. *Pl. cell. Physiol. Tokyo* **10**, 495–502 (1969).
- Tomomura, K., Maeda, K., Futai, F., Nakagami, T. & Yamada, M. *Nature* **217**, 644 (1968).
- Ben-Bassat, D., Shelef, G., Gruner, N. & Shuval, H. I. *Nature* **240**, 43–44 (1972).
- Bryan, G. W. in *Effects of Pollutants on Aquatic Organisms* (ed. Lockwood, A. P. M.) *Soc. exp. Biol., Sem. Ser.* **2**, 7–34 (1976).
- McLean, R. O. & Jones, A. *Freshwat. Biol.* **5**, 431–444 (1975).

Genetic variation in wild populations of rain-forest trees

In the tropical rain forest, many tree species grow close together, and they are apparently equivalent in their edaphic requirements and spatially interchangeable. Most species occur at low density although they may be geographically widespread. No other plant community contains as many species of a single life form as do certain mixed dipterocarp forests of the aseasonal wet lowlands of Malesia^{1–3}. Such a population structure and level of diversity makes it hard to see how speciation has occurred. Spatial isolation of individuals and the abundant, normally hermaphrodite flowers simultaneously available for fertilisation in the canopy of the dipterocarp emergents suggest the possibility of self-fertilisation, but many of the understorey species are dioecious. As part of a comparative study of the reproductive biology of rain forest tree species, intra-specific genetic variation of isozymes has been demonstrated for the first time in an emergent and an understorey species of rain forest trees. Polymorphism is common in both species and there is evidence for short-range spatial heterogeneity in gene frequency and also in leaf morphology.

The species studied were *Shorea leprosula* Miq. an emergent species, and *Xerospermum intermedium* Radlk. an understorey species. *S. leprosula* is a dipterocarp which occurs widely in Thailand, Sumatra and Malaysia. *X. intermedium* is a member of the Sapindaceae which includes *Nephelium lappaceum*, the rambutan. Except in Malaysia and Borneo, where it is common on well-watered slopes, its distribution and abundance throughout South-East Asia is not yet known. Samples were collected from three sites, mostly from the Pasoh Forest Reserve which is in the southern part of Peninsular Malaysia.

Electrophoretic analysis of aqueous extracts of leaves was performed in starch or acrylamide slab gels and, after separation, gels were treated to identify the presence of esterases, leucine amino-peptidase, glutamic-oxalacetic transaminase, acid and alkaline phosphatase and also an

unidentified enzyme which proved useful in *S. leprosula*. Initial difficulties in resolving enzymes, especially in *X. intermedium*, were overcome by incorporating polyvinyl pyrrolidone, diethyldithiocarbamate and mercaptoethanol in the extraction buffer at pH 8.6; technical details will be reported elsewhere. Control extracts were incorporated in all runs and the R_F values were adjusted to the value in the reference sample to allow comparisons between gels. In most systems there was evidence of age-related changes in gene activity and so comparisons are confined within single age groups, for example, seedlings or mature trees, and refer to the presence or absence of a band at defined R_F positions.

In addition to the electrophoretic evidence the study has included for *X. intermedium* an analysis of variation in leaf morphology between trees in relation to their distance apart. Evidence of a somewhat different kind has been provided by comparisons of electrophoretic patterns in ten species of *Shorea* with morphological differences between them.

In *S. leprosula* in which 80–250 individuals were sampled per enzyme system, there is striking evidence of genetic polymorphism at alternative R_F positions. Table 1 shows that more than half the total number of positions, pooled over all systems, show variation while the frequency of the rarer state is 0.10 or above. This indicates high levels of polymorphism and hence of heterozygosity. We cannot yet determine the number of loci involved. Tests of independence of presence or absence of bands for all possible pairs generally indicate independence but not always so, probably due to determination of more than one band by a single locus. Column B of Table 1 allows for such correlations and the actual number of loci probably corresponds more closely to the bands referred to in column B than to those in A. Thus *S. leprosula* resembles many other outbreeding species in being highly heterozygous.

Table 1 Combined record of variation in *Shorea leprosula* shown by electrophoretic systems

	A Total no.	B Adjusted no.
R_F positions	40	36
% with variable band presence	55	50

In *X. intermedium* fewer systems were studied and the number of bands per system was less than in *S. leprosula*. In samples from 56 trees, six putatively distinct loci were identified and two of these, both determining esterases, were polymorphic. Comparisons from wild individuals as well as test crosses suggested the occurrence of a pair of alleles at each locus, one active and the other null, with a frequency of 0.97 and 0.49 for the active alleles in the 'fast' and 'slow' positions, respectively. Each locus conformed to the Hardy-Weinberg distribution and each apparently segregated independently of the other. Thus the genetic situation in this species seems to be essentially the same as in *S. leprosula*.

Spatial heterogeneity was evident in *S. leprosula*. Samples derived from groups of trees separated by approximately 700 m are likely to differ significantly at one or more R_F positions. There is also evidence that the degree of difference is correlated with distance apart and this may be related to limited dispersal of pollen and fruit. For *X. intermedium* there are similar indications; heterozygotes often occur between or close to alternative heterozygotes. In this species, variation in leaf morphology has been studied in samples of generally ten leaflets from 68 trees growing in an area 600 m². Analysis of variance within

and between trees for leaf length, breadth, vein number, ratio of vein number to length and ratio of leaf length to breadth revealed highly significant heterogeneity between trees. The distance between any pair of trees can be computed from the coordinates of a rectangular grid applied to a map of the trees' location. The regressions of differences for all possible pairs for the leaf characters on distance apart proved highly significant ($P < 0.001$). How far genetic variation contributes to the variation in the leaf characters is not known but this evidence is not inconsistent with that from the electrophoretic study.

The electrophoretic patterns of 10 species of *Shorea* were compared for esterases, glutamic-oxalacetic transaminase and alkaline phosphates. An index of similarity (I) refers to the ratio of bands in common to total bands identified⁴. Combining the systems the total band number ranges from

Table 2 Values of I based on gel-band patterns in comparisons of species of the genus *Shorea* with *S. leprosula*

Species	Total no. of bands	I
<i>S. curtisii</i>	32	0.500
<i>S. parvifolia</i>	33	0.424
<i>S. acuminata</i>	29	0.413
<i>S. dasyphylla</i>	29	0.275
<i>S. macroptera</i>	31	0.258
<i>S. lepidota</i>	27	0.222
<i>S. macrantha</i>	29	0.137
<i>S. hemsleyana</i>	29	0.103
<i>S. ovalis</i>	35	0.000

27 to 35. Table 2 shows the paired comparisons between *S. leprosula* and nine other species. There is good agreement between I value and taxonomic affinity². Thus *S. leprosula*, *S. parvifolia* Dyer and *S. curtisii* Dyer ex King are taxonomically close and agree in having high I values. Indeed for *S. leprosula* and *S. curtisii* we have the highest I value of 0.5 and for these two species hybrids have been identified in the field. Comparison with *S. ovalis* (Korth.)Bl. provides a zero value consistent with its taxonomic position in a separate section of the genus. It is likely that such electrophoretic evidence will make a valuable contribution to the taxonomy of dipterocarps and probably other groups where specific distinctions are often hard to define.

In *S. ovalis*, a tetraploid, 40 seedlings were collected from the base of four possible parent trees and examined by electrophoresis; altogether 36 R_F positions were available for comparison and of these only two, both esterases, were variable. The low level of variation in this species may be related to self-fertilisation (H. T. Chan, work in preparation).

We infer from the evidence reported here that both the emergent species *S. leprosula* and the understorey species *X. intermedium* have an outbreeding system, combined possibly with short-range heterogeneity in gene frequency which may be related to predominantly short-range pollen and fruit dispersal. The latter conditions contribute to physical isolation between populations and hence may promote speciation. Our findings are being compared with evidence relating to cytology, fertility, pollination mechanisms and breeding structure to set the evolutionary problems of rain forest trees in better perspective.

We thank the Leverhulme Trust Fund, the Royal Society, the Natural Environment Research Council and the Carnegie Trust for support.

YIK-YUEN GAN*

FORBES W. ROBERTSON

Department of Genetics,

PETER S. ASHTON

Department of Botany,
University of Aberdeen

ENGKIK SOEPADMO
DAVID W. LEE

Department of Botany,
University of Malaya, Kuala Lumpur,
Malaysia

Received 22 May; accepted 27 July 1977.

Present address: Department of Biology, University of Agriculture Malaysia
Selangor, West Malaysia.

¹ Ashton, P. S. *Oxford Forest Mem.* 25, (1964).

² Whitmore, T. C. *Tropical Rain Forests of the Far East* (Oxford University Press, Oxford 1975).

³ Symington, C. F. *Malay. Forest Rec.* 16, 2 (1943).

⁴ Sarich, V. M. *Nature* 265, 24 (1977).

Freezing avoidance by deep supercooling in hydrated lettuce seeds

Dry seeds of several species survive exposure to extremely low temperatures, including immersion in liquid air for up to 60 d (refs 1–3), but most young seedlings are very susceptible. Fully hardened twigs of several northern tree species tolerate freezing in liquid nitrogen (-196°C) (ref. 5), and deep supercooling seems to be an important mechanism for the avoidance of freezing in both xylem ray parenchyma and flower buds of many other hardy but less cold resistant, deciduous, woody species^{6,7}. Resistance to freezing stress in seeds has been suggested to be related to water content^{1,4,8}, but little attention has been paid to either the relationship between stage of germination and degree of freezing resistance or the nature of the resistance. We report here that hydrated lettuce seeds (achenes) can avoid injury by deep supercooling and that the level of resistance can be determined precisely by differential thermal analysis (DTA). Furthermore, we show that the

deep supercooling is dependent on the intact structure of the endosperm.

Experiments were conducted with seed of Grand Rapids lettuce. Moisture content was controlled by leaving seeds to imbibe water for specific intervals; by leaving them to imbibe in different concentrations of polyethylene glycol (molecular weight 6,000, PEG 6000) for specific intervals, and by storing them at controlled relative humidities in closed vials on top of various concentrations of H_2SO_4 . Moist seeds were dried rapidly between filter papers and samples were prepared for freezing and for determination of water content. Seeds that had imbibed in polyethylene glycol were washed three times with distilled water before preparation. Dry weight was determined after drying at 105°C for 24 h. Moisture content was calculated as percentage water of fresh weight. Supercooling nucleation points were determined by DTA (ref. 7) with an automatically controlled, liquid- N_2 -cooled Cryoson freezing system, with a constant freezing rate of 20°C per h. Exotherms were recorded on potentiometric recorders, at 0.5 mV, connected to the samples with 0.5 mm copper-constant thermocouples. Between 1 and 10 seeds were used with each recording channel. Samples (10 to about 100 seeds) for survival tests were removed from the freezer at predetermined temperatures and kept at 2°C for 2–4 h before ability to germinate was tested in light at 24°C . Seeds were counted after 3 d and the percentage of germinated and growing seeds was used as a criterion for viability.

Lettuce seeds with 5–13% water content were not injured by freezing to -196°C , but with 13–16% moisture, the threshold of freezing tolerance declined to -40°C (Fig. 1a). No exotherms were detected by DTA in either case but two types of exotherms were found in intact seeds

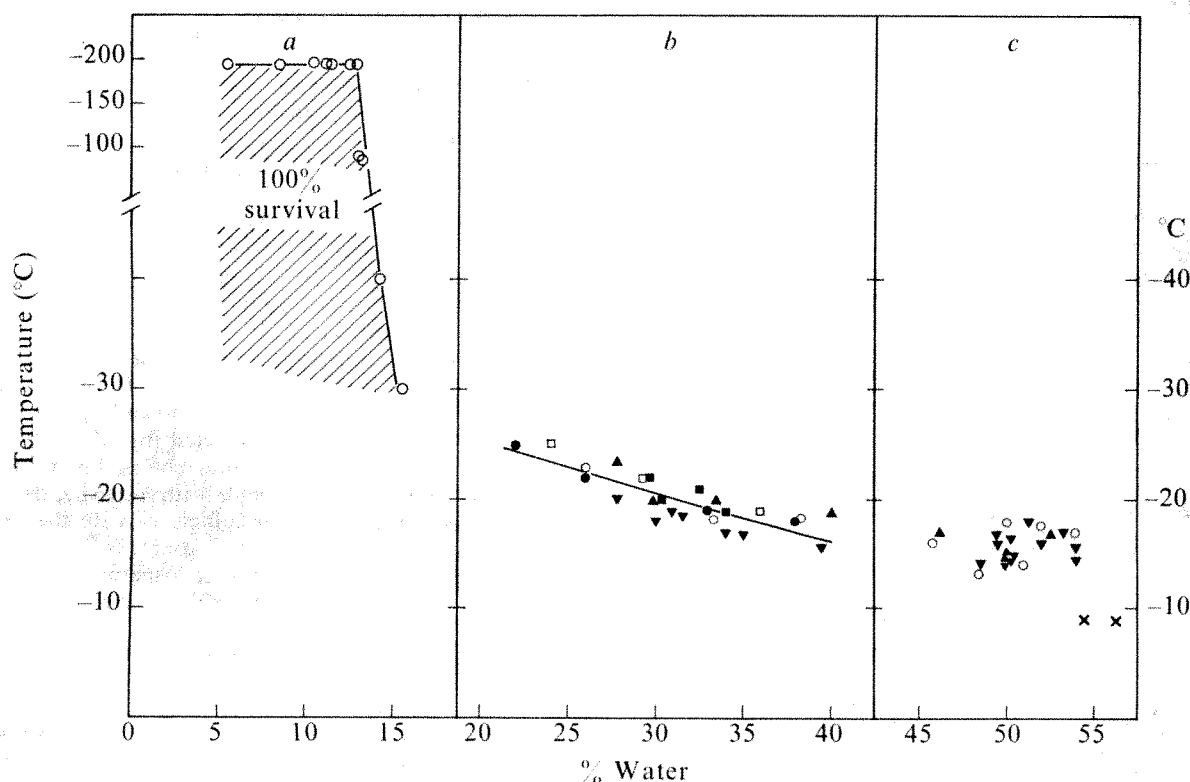


Fig. 1 Relationship between seed water content and hardness in Grand Rapids lettuce seeds. *a*, Limited for 100% survival of seeds stored at 18°C or at 4°C in controlled humidities for 10–14 d. Hardiness at -196°C was tested by keeping the seeds in liquid nitrogen for 30 min. *b*, The mean temperature of the secondary exotherms in seeds imbibed at 30°C in various concentrations of PEG 6000 (0.06–0.12 molal). Open symbols: 5 min red (R) light, $4.2 \mu\text{W cm}^{-2}$ at 660 nm, given after 2 h imbibition in darkness; closed symbols: darkness. \square , \blacksquare , 12 h imbibition; \circ , \bullet , 24 h; ∇ , 72 h; \blacktriangle , 96 h. Regression equation: $y = -0.43x + 33.9$, $r = -0.84$, $P < 0.001$. *c*, Mean temperature of secondary exotherms in seeds imbibed at 30°C in water for 4–72 h. \circ , R; \blacktriangle , R plus 5 min far-red (FR), $24 \mu\text{W cm}^{-2}$ at 730 nm; \blacktriangle , darkness; \times , germinated seeds.

containing more than 20–25% water (Fig. 2). The first appeared as a single peak when the temperature of the sample was $-10 \pm 2^\circ\text{C}$ regardless of the number of seeds in the sample, indicating that the nucleation point for this peak is remarkably uniform. This exotherm is not related to the injury of intact seed and we suggest that it represents freezing of extracellular or bulk water in the outer layers, similar to extracellular freezing of other plant tissue⁷. Each seed frozen showed one deep supercooling exotherm. These secondary exotherms represent the killing point of individual seeds. In seeds imbibed in PEG 6000 the mean exotherm temperature was linearly correlated ($r = -0.84$, $P < 0.001$) with the moisture content within the limits of 20–40% (Fig. 1*b*). A plateau was reached at 40–50% moisture where the mean exotherm temperature of intact seeds remained at $-16 \pm 2^\circ\text{C}$ ($r = 0.004$), irrespective of imbibition time (4–72 h). There was no effect of red (5 min, $4.2 \mu\text{W}$

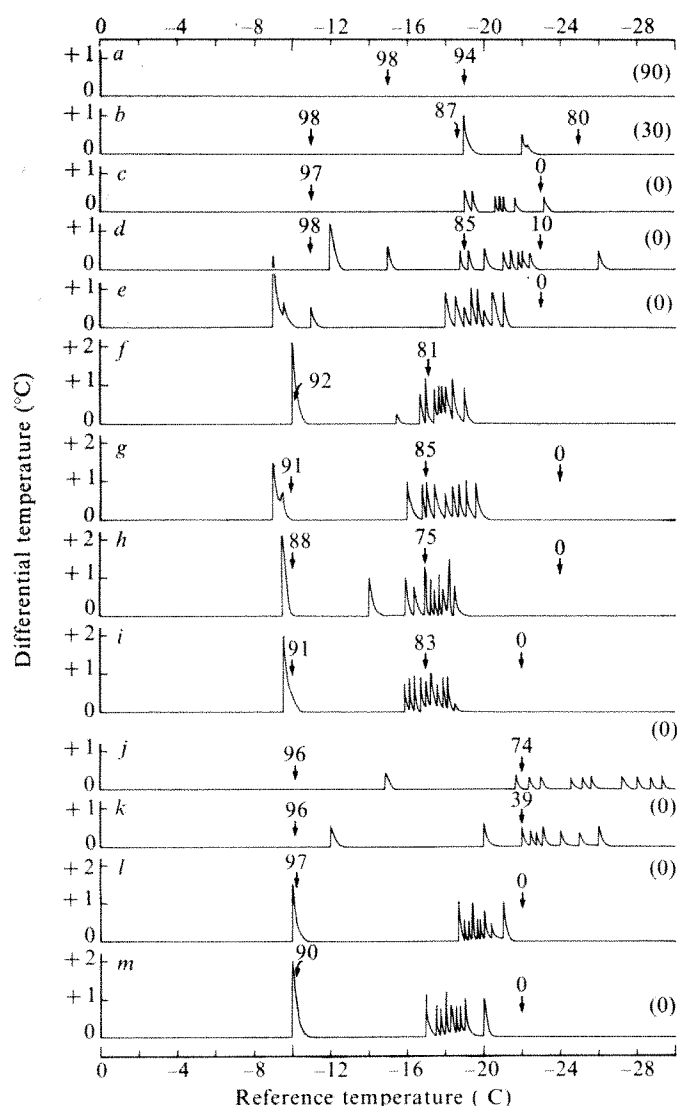


Fig. 2 Differential thermal analysis of lettuce seeds with moisture contents at various levels. Ten seeds in each sample. Figures and arrows show the percentage of survival (germination) in samples removed at temperatures indicated, those in parentheses show survival of samples frozen down to -35°C . Freezing rate 20°C per h in all cases. To obtain the various moisture levels seeds were imbibed at 30°C in darkness either in water for various times (*a–i*) or in various molal concentrations of PEG 6000 (*j–m*) for 24 h. *a*, 0 or 5 min imbibition, 5.7–16% water content; *b*, 10 min, 20%; *c*, 20 min, 26%; *d*, 30 min, 32%; *e*, 60 min, 41%; *f*, 4 h, 49.7%; *g*, 8 h, 49.5%; *h*, 12 h, 48.8%; *i*, 24 h, 51.2%; *j*, 0.12 molal, 21.9% water; *k*, 0.10 molal, 26.0%; *l*, 0.08 molal, 33.3%; *m*, 0.06 molal, 38.2%.

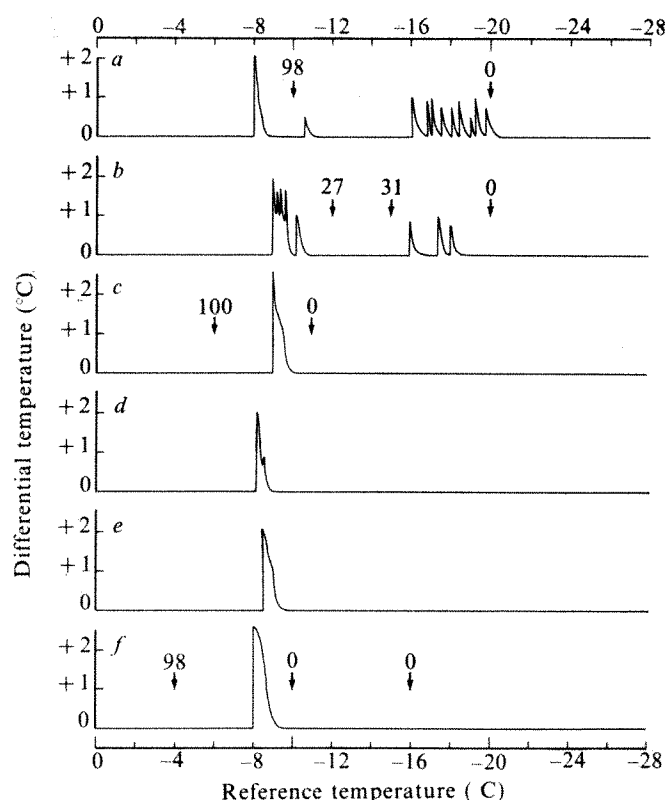


Fig. 3 Differential thermal analysis of lettuce seeds. *a*, Intact seeds; *b*, seed coats removed; *c*, excised embryos; *d*, endosperms; *e*, intact seeds stuck four times with a needle; *f*, germinated seeds. Figures and arrows are as in Fig. 2. Seeds coats and endosperm were removed with a scalpel under a stereoscope in light after an imbibition at 30°C for 16 h. Seeds stuck with a needle were imbibed in water for 4 h at 30°C . There were ten seeds or embryos in each sample.

$\text{cm}^{-2} \text{nm}^{-1}$ at 660 nm) or far red (5 min $24 \mu\text{W cm}^{-2} \text{nm}^{-1}$ at 730 nm) irradiation on the position of the secondary exotherms up to 8 h after exposure of seeds.

The freezing pattern changed considerably, however, after disruption of the endosperm–seed coat envelope. As soon as the radicle emerged the secondary exotherms disappeared and the first exotherm represented the killing point of the seed (Fig. 3). Similarly, embryos excised from the seeds after imbibition for 2–12 h produced only one exotherm and did not survive below this nucleation temperature (Fig. 3). The occurrence of secondary exotherms depends on the intact structure of the endosperm. If fruit coats were excised carefully without injury to the endosperm envelope, embryos did supercool as detected by the presence of secondary peaks at the nucleation points, but if the endosperm was damaged, for example with a needle, deep supercooling ceased and death was coincident with the first peak (Fig. 3).

Therefore the integrity of the endosperm envelope seems to facilitate deep supercooling which concurrently imparts freezing avoidance to -12° to -18°C in intact, imbibed lettuce seeds. Deep supercooling of lettuce seeds is not, however, an exclusive property of live seeds: it also occurred with intact, imbibed seeds which had been killed by low or high temperatures. Work with apple stems has shown that intact dead twigs yield exotherms if rehydrated to 20% water but not if ground to a fine powder⁸.

The mechanism by which the intact endosperm imparts freezing avoidance to the embryo is not clear. Perhaps disruption of the endosperm facilitates intimate contact of relatively pure water in the coats with the water in the embryo, thus providing ice crystal formation in all parts of the seed at the first exotherm. Germinated

seeds contain slightly more water than imbibed non-germinated seeds, and perhaps endosperm injury increases embryo water content. But even embryos excised from seeds imbibed in 0.10 molal PEG 6000 and kept in 0.12 molal PEG 6000 during freezing did not yield secondary exotherms.

The mechanism of freezing resistance in lettuce seeds seems to resemble those found in other tissues and species at various stages of cold hardiness. We suggest that lettuce seed offers a useful system for the study of mechanisms of freezing tolerance and avoidance.

OLAVI JUNTILA
CECIL STUSHNOFF*

Institute of Biology and Geology,
University of Tromsø,
9001 Tromsø, Norway

Received 13 June; accepted 21 July 1977.

*Permanent address: Department of Horticultural Science and Landscape Architecture, University of Minnesota, St Paul, Minnesota 55108.

- 1 Kieselbach, T. A. & Ratcliff, J. A. *Nebr. Agric. Exp. St Bull.* 163, 1-16 (1918).
- 2 Lipman, C. B. & Lewis, G. N. *Plant Physiol.* 9, 392-394 (1934).
- 3 Lipman, C. B. & Lewis, G. N. *Plant Physiol.* 11, 201-205 (1936).
- 4 Levitt, J. *Responses of Plants to Environmental Stresses* (Academic, New York, 1972).
- 5 Sakai, A. *Nature* 185, 392-394 (1960).
- 6 George, M. F. & Burke, M. J. *Curr. Adv. Plant Sci.* 8, 349-360 (1976).
- 7 Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J. W. & Li, P. H. *A. Rev. Plant Physiol.* 27, 507-528 (1976).
- 8 Lockett, M. C. & Luyet, B. J. *Biodynamica* 7, 67-76 (1951).
- 9 Quamme, H., Weiser, C. J. & Stushnoff, C. *Plant Physiol.* 51, 273-277 (1973).

Guard cell malic acid metabolism during stomatal movements

THE mechanism by which stomata open and close has long been a puzzle, but only now are we beginning to make major advances in our understanding of their metabolism. It has been concluded that guard cells can fix CO_2 and incorporate it into starch¹⁻³. It has been shown that the bulk of, if not all, the CO_2 fixed by guard cells is converted to oxaloacetic acid (OAA) by phosphoenolpyruvate carboxylase and the OAA, in turn, is reduced to malic acid^{3,4}. Some amination of OAA producing aspartate has also been detected⁴. Although there is indirect evidence suggesting that aspartate and/or malate can provide carbon for starch synthesis in the guard cells⁴ direct evidence for this is lacking. We have investigated some characteristics of guard cell CO_2 fixation which directly results in organic acid formation and we also have direct evidence that malic acid is a precursor of starch formation.

Microautoradiography showed that guard cells in epidermal strips of *Commelina communis* L. were able to fix CO_2 in the light or the dark irrespective of whether stomata were open or closed. Although some localisation of ^{14}C could be detected in the region of the epidermal cells the label was concentrated within the stomatal complexes. These observations were confirmed by CO_2 fixation kinetics experiments. Figure 1 shows similar rates of ^{14}C uptake by epidermal tissue of *C. communis* when floated on a $\text{NaH}^{14}\text{CO}_3$ solution in the light or the dark. Slightly greater uptake rates of ^{14}C were obtained in the light when epidermal tissue of *C. diffusa* was cut into small pieces and immersed in $\text{NaH}^{14}\text{CO}_3$ solution⁵.

Microautoradiography also showed that guard cells became more heavily labelled if the epidermis remained attached to the mesophyll. Whether this was due to some channelling of ^{14}C -labelled products from the mesophyll to the guard cells or whether the mesophyll in some way enhances guard cell CO_2 fixation is not known.

When L-(U- ^{14}C)malic acid was fed to epidermal strips of *C. communis* while stomata closed from an aperture of $8\ \mu\text{m}$ in response to darkness, or while they opened from fully closed in response to CO_2 -free air and light, guard cell

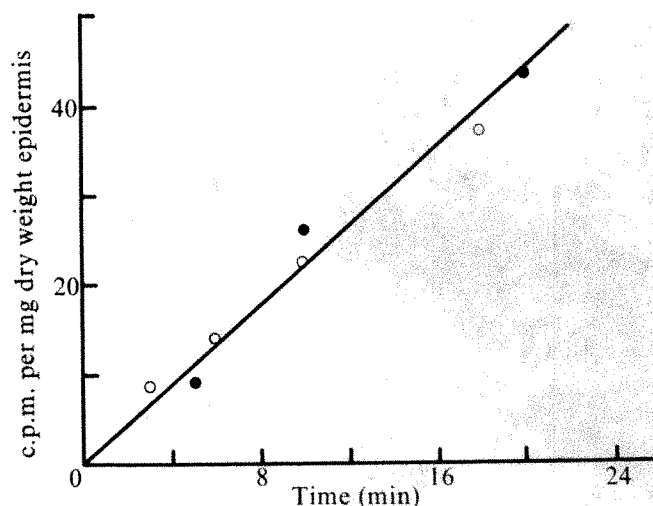


Fig. 1 CO_2 fixation kinetics of abaxial epidermal strips of *C. communis* in light (○) and darkness (●). Epidermal strips were floated, cuticle uppermost, on 3 ml 'suspension medium' containing 0.33 M sorbitol, 0.05 M tricine buffer (pH 8.0), 0.002 M sodium nitrate, 0.002 M EDTA, and 0.005 M potassium hydrogen phosphate. At zero time 0.2 ml of 0.05 M $\text{NaH}^{14}\text{CO}_3$ was injected into the suspension medium and the solution quickly swirled to mix, giving a CO_2 concentration of 4.5×10^{-5} M. At the indicated times tissue was immersed in 2.0 ml 20% trichloroacetic acid to stop the reaction and drive off excess $^{14}\text{CO}_2$. Radioactivity in the tissue was then determined by liquid scintillation counting.

starch became labelled in both cases (Fig. 2). Incorporation of label into starch, however, was greatest when the stomata were closing. Microautoradiographs also verified that label from malic acid was incorporated into starch, mainly located in areas corresponding to stomatal positions in the epidermis (blackened areas in Fig. 3). Thus, incorporation of ^{14}C from labelled malic acid into starch is not restricted to closing movements in epidermal strips. But, good correlations for guard cell malic acid accumulation⁶

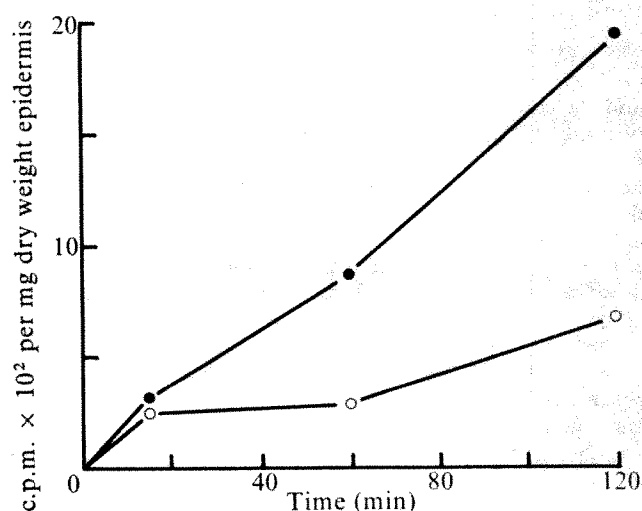


Fig. 2 Incorporation of ^{14}C from L-(U- ^{14}C)malic acid into starch during 2-h exposure. The abaxial epidermis of *C. communis* was floated, cuticle uppermost, on 20 ml of 10 mM sodium citrate buffer containing 10 mM sodium chloride and 0.086 mg (25 μCi) U- ^{14}C -malic acid, pH 5.5. In one treatment the epidermal strips possessed closed stomata which were stimulated to open to $7\ \mu\text{m}$ after 2 h incubation in light and CO_2 -free air (○) and in the other, stomata were initially open to about $8\ \mu\text{m}$ and were made to close to about $4\ \mu\text{m}$ after 2 h incubation in darkness and normal air (●). At the times indicated, samples of tissue had their ethanol and water soluble products extracted leaving the insoluble starch and the radioactivity in the starch was determined by liquid scintillation counting.

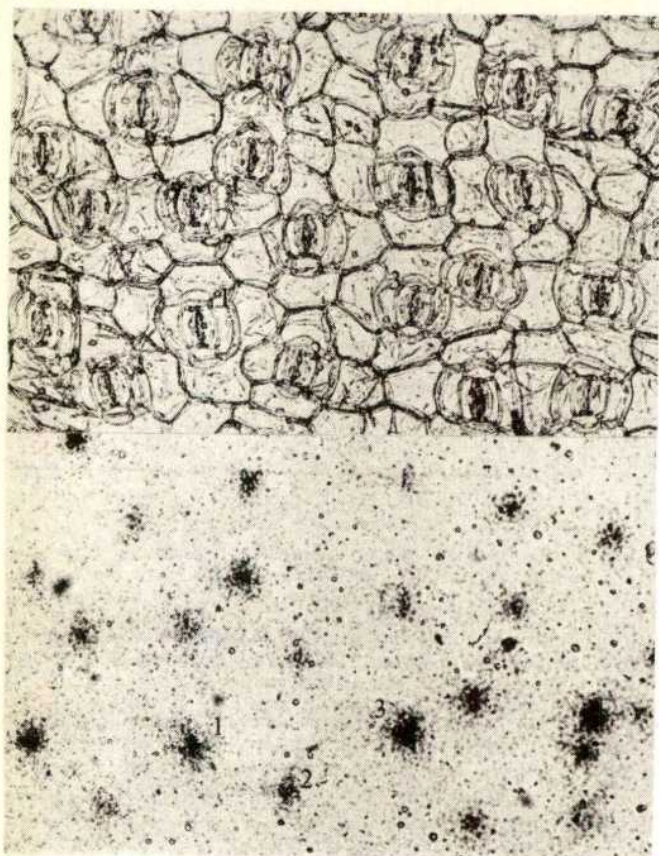


Fig. 3 Microautoradiograph of an epidermal strip of *C. communis* exposed to darkness and normal air for 2 h to close the stomata and after treatment as described in the legend to Fig. 2. Blackened areas located predominantly over the stomatal complexes indicate ¹⁴C label in starch. (Stomata 1, 2 and 3 correspond to the numbered, blackened areas.) The dried epidermis was placed on a glass slide and a piece of Ilford Pan F film firmly taped on top with the emulsion in contact with the tissue. On top of the film was placed another slide and the whole was clamped together with screw-clips. After exposure for 29 d the piece of film was detached from the tape and tissue and developed in Kodak DX-80 developer.

and starch disappearance⁶ have been observed with stomatal opening. We have also found high levels of starch in guard cells of *C. communis* when stomata were closed; this level decreased as stomata opened although starch could still be detected at wide stomatal apertures. This suggests that there is a dynamic flux of carbon between malic acid and starch with the direction of starch production outpacing its breakdown when stomata are closing. Results of an investigation of pathways for malic acid-starch interconversions will be published elsewhere.

In conclusion we have established that malic acid is a precursor of starch synthesis in guard cells of *C. communis*. But, the mechanism of the control of guard cell CO₂ fixation and hence malic acid synthesis, and the control of the production of starch from malic acid in relation to stomatal movements, remain obscure.

J.C.R. thanks the SRC for a research studentship.

C. M. WILLMER
J. C. RUTTER

University of Stirling,
Department of Biology,
Stirling, Scotland, UK

Received 17 January; accepted 28 June 1977.

⁴ Willmer, C. M. & Ditttrich, P. *Planta* 117, 123–132 (1974).

⁵ Allaway, W. G. *Planta* 110, 63–70 (1973).

⁶ Meidner, H. & Mansfield, T. A. *Physiology of Stomata* 141–145 (McGraw-Hill, New York, 1968).

Orientation columns in macaque monkey visual cortex demonstrated by the 2-deoxyglucose autoradiographic technique

In the past fifteen years physiological studies of the primary visual cortex in higher mammals have provided evidence for two independent systems of functional subdivisions, ocular dominance columns and orientation columns¹. These two systems are closely related to two important functions of visual cortex: combining at a single-cell level the information that originates in the two eyes, and rearranging the spatial information from the lateral geniculate body so that cells after the initial stage of visual processing come to respond to specifically oriented lines in the visual field.

The ocular dominance columns have been demonstrated anatomically by three different staining techniques^{2–4}. We describe here the use of a new method⁵ which makes possible the anatomical demonstration of the orientation columns.

The original evidence that cells are aggregated according to their response characteristics came from microelectrode recordings¹. In a penetration perpendicular to the cortical surface all cells are dominated by the same eye and all give optimal responses to the same stimulus orientation, whereas in an oblique or tangential penetration there is an alternation of the dominant eye, from left to right and back, and, at the same time, a series of regular changes in optimal stimulus orientation in steps of 10° or less, clockwise or counterclockwise. Reversals in direction of rotation occur sporadically, on the average every few mm, and the orderly sequences of small orientation shifts are occasionally broken by abrupt large shifts of up to 90°. A full cycle of either type, one eye and then the other or a rotation through 180°, generally requires a horizontal movement along the cortex of 1 mm or less². The constancy of eye dominance and of optimal stimulus orientation during perpendicular penetrations indicates that the two sets of subdivisions are arranged perpendicular to the surface and the layers. Because of their cross-sectional shape in brain sections perpendicular to the surface, the subdivisions have been called 'columns', and a complete set of columns (left plus right eyes, or a full 180° rotation) is called a hypercolumn.

Inspection of cortical sections stained by conventional methods gives no hint of these vertical subdivisions. The only obvious segregation of cells is the horizontal system of layers, and this segregation has certain physiological correlates. Layer IVc, at about mid-cortical thickness, is the site of termination of the geniculate afferents and contains cells that differ in their physiological properties from cells in the other layers in two respects: like the geniculate afferents, they have no orientation preference; and they are almost all strictly monocular. In contrast, cells in the layers above and below IVc almost all show clear orientation specificity and about half are binocular, though any given cell is likely to respond best to one or the other eye.

In the last few years considerable progress has been made in working out the geometry of the columnar subdivisions. Three independent anatomical methods have made it possible to see the ocular dominance columns in layer IVc^{3–6} where they form a set of parallel bands which are for the most part straight, but in places form loops and whorls and occasionally show bifurcations and blind endings. The columns must therefore have the form of parallel sheets rather than being pillar-like. For the orientation columns,

¹ Shaw, M. & MacLachlan, G. A. *Can. J. Bot.* 32, 784–794 (1954).

² Nishida, K. *Physiologia Pl.* 16, 281–298 (1963).

³ Willmer, C. M., Pallas, J. E., Jr & Black, C. C., Jr. *Pl. Physiol.* 52, 448–452 (1973).

microelectrode recordings, especially multiple parallel penetrations, likewise suggest an arrangement in parallel sheets, and the frequent reversals in direction of rotation would be compatible with a swirling pattern.

Until very recently no method has been available for demonstrating the orientation columns anatomically. Several years ago Sokoloff and his group⁷ developed a procedure for labelling active brain tissue. The method depends on the fact that brain cells, which use mainly glucose as a source of energy, take up 2-deoxyglucose as though it were glucose and metabolise it as far as 2-deoxyglucose-6-phosphate, but no further. This compound cannot easily pass out of the cell, and tends to accumulate. Physiologically active regions of brain may then be identified by the use of radioactively labelled deoxyglucose and autoradiography. This method was used by Sokoloff's group in monkeys in which one eye was stimulated during a 45-min period immediately following intravenous injection of deoxyglucose⁸. (The other eye was occluded in some monkeys and had been previously removed in others.) The result, a striking demonstration of the ocular dominance columns in the striate cortex, differed from the results obtained by the other anatomical techniques in demonstrating the columns through the full cortical thickness, rather than showing just the parts in layer IVc.

We have used the deoxyglucose method to reveal the orientation columns. Our procedure is based on that of Sokoloff and his group, to whom we are indebted for first-hand instruction in the method. We injected a lightly anaesthetised juvenile macaque monkey with ^{14}C -2-deoxyglucose, $150 \mu\text{Ci kg}^{-1}$ in $100 \mu\text{Ci ml}^{-1}$ saline, and then stimulated for 45 min by moving back and forth in front of the animal a black screen on which were pasted a set of irregularly spaced vertical white stripes. Stripe widths were $0.5\text{--}1^\circ$, and movement was $2\text{--}4^\circ \text{ s}^{-1}$. Both eyes were held open, protected by contact lenses, refracted at the screen distance (1 m) and aligned with a variable prism over one eye so that the foveas were superimposed. To be sure of the alignment and as a check on the state of the animal we also recorded from single binocular cells in striate cortex and superimposed the receptive fields.

At the end of the stimulus period the animal was given an additional dose of anaesthesia followed by a lethal dose of intravenous KCl. It was then decapitated, and the skull was cleaned of skin, gradually immersed over a 4-min period in liquid Freon-22 at -125°C , and stored at -80°C . Small blocks of brain were later sectioned at $20 \mu\text{m}$ in a cryostat at -26°C , and the sections were picked up on a cover slip and immediately dried at 98°C . These sections were then pressed against X-ray film for 2–3 weeks and the film was finally developed. Some of the sections used for autoradiography were also later stained for Nissl substance.

An autoradiograph made from a section perpendicular to the striate cortex is shown in Fig. 1. Vertical regions of dense label can be seen extending through the full thickness of cortex. About midway through the thickness is a dense uniformly labelled horizontal band. By comparing the autoradiographs with neighbouring sections stained for Nissl substance it can be shown that this band corresponds to layer IVc. This agrees with the observation from microelectrode recordings, that cells in layer IVc show no orientation specificity. No such uniform labelling was seen in IVc in the results of Sokoloff's group⁸ or in our experiments, when ocular dominance columns were labelled by this method.

The vertical labelled regions in Fig. 1 are on the average about 0.6 mm apart and occupy a considerable fraction of the repeat distance, as expected from the fact that each cell responds not just to a single orientation but to a range of orientations to either side of the optimum, with some cells highly selective and others less so. For example, in a sharply tuned cell the orientation that evokes a half-

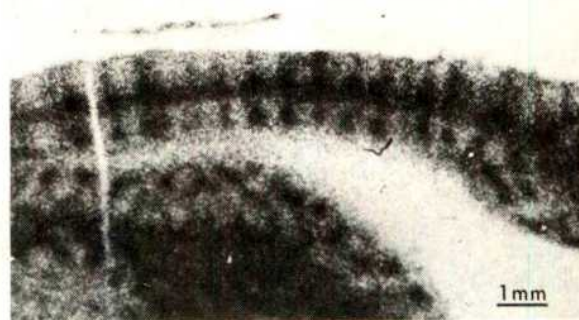


Fig. 1 Autoradiograph of a ^{14}C -2-deoxyglucose section through monkey striate cortex, perpendicular to the surface. The stimulus consisted of moving vertical, irregularly spaced white stripes presented to the entire visual field of both eyes for 45 min. Labelled regions are vertical in cross section, about 0.6 mm apart, and extend through the full cortical thickness. Layer IVc, situated at about mid-depth and identified from neighbouring Nissl-stained sections, is uniformly labelled, as expected from the absence of orientation specific cells in that layer.

maximal response may be $10\text{--}15^\circ$ from the optimal. The widths of the labelled regions must depend on many factors including the sharpness of tuning curves (response plotted against stimulus orientation), and the relationship between cell activity and deoxyglucose uptake, and between uptake and the grain density of the autoradiographs.

A tangential section through the dorsolateral surface of the occipital lobe is shown in Fig. 2. The white matter is grazed in two places which appear as pale ovals. These are surrounded by layers VI and V, cut almost tangentially, and here the labelled regions can be seen face-on, forming a complex pattern of rings, loops, and branching stripes. Their separation is strikingly constant, averaging roughly $570 \mu\text{m}$. Only in a few places is there a suggestion of parallel stripes. Surrounding this area is layer IVc, which is again uniformly labelled, and just outside IVc is IVb, where the aggregations of label are particularly dense. Layers II and III are more lightly labelled but also show distinct aggregations. (The dense bands at the extreme left, perpendicular to the surface, are microtome knife artefacts.)

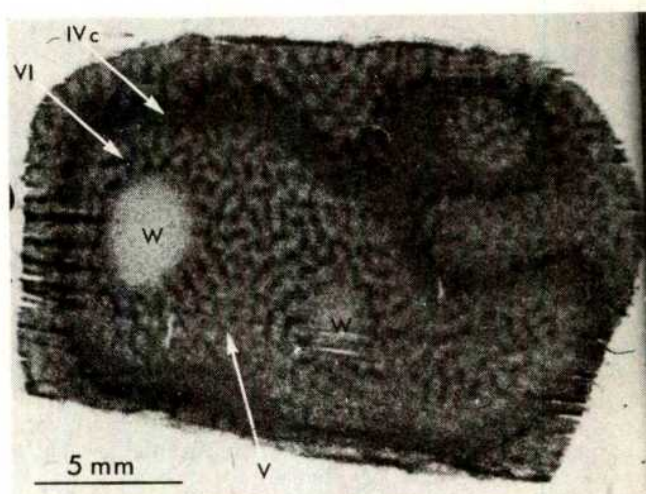


Fig. 2 Autoradiograph of a tangential section through the dorsolateral surface of the occipital lobe (striate cortex) of the same monkey. Dark areas are radioactively labelled. The section grazes white matter (W) in two places, which are seen as pale ovals. Surrounding these are densely labelled sets of orientation columns in layer VI. Outside this is layer V, lightly labelled, and then IV c, which is uniformly labelled with no hint of columnar subdivisions. The upper layers again show densities related to columns. (Dense bands perpendicular to the surface at the extreme left are artefacts produced by the microtome knife.)

More superficial sections from this block, tangent to the upper layers (II–III) rather than to white matter, show an almost identical pattern, as expected from the fact that the labelled regions are perpendicular to the surface.

So far we have examined too few brains to be sure that the pattern of the orientation columns is always as complex in form as that shown here. Ocular dominance columns were examined in the same region as that shown in Fig. 2, by transneuronal autoradiography following an injection of ^3H -proline into one eye two weeks before the deoxyglucose experiment. These showed a more regular pattern of stripes, with a spacing only slightly coarser than that of the orientation hypercolumns ($770\text{ }\mu\text{m}$ compared with $570\text{ }\mu\text{m}$). There was no obvious tendency for the two sets of columns to be related in any simple way: they were not consistently orthogonal, and were certainly not parallel. This result will be published separately⁹.

The anatomical demonstration of the orientation columns provides still another verification of the columnar organisation of the striate cortex. It is reassuring to find such agreement between morphology and physiology, and unusual to find the physiology actually leading the way to a structural description.

DAVID H. HUBEL
TORSTEN N. WIESEL
MICHAEL P. STRYKER

Department of Neurobiology,
Harvard Medical School,
Boston, Massachusetts 02115

Received June 23; accepted August 8 1977.

- ¹ Hubel, D. H. & Wiesel, T. N. *J. Physiol. Lond.* **160**, 106–154 (1962); **165**, 559–568 (1963); *ibid.* **195**, 215–243 (1968); *J. comp. Neurol.* **158**, 267–294 (1974).
- ² Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **158**, 295–306 (1974).
- ³ Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **146**, 421–450 (1972).
- ⁴ Wiesel, T. N., Hubel, D. H. & Lam, D. M. K. *Brain Res.* **79**, 273–279 (1974).
- ⁵ Hubel, D. H., Wiesel, T. N. & LeVay, S. *Phil. Trans. R. Soc. B* **278**, 377–410 (1977).
- ⁶ LeVay, S., Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **159**, 559–576 (1975).
- ⁷ Sokoloff, L. in *Brain Work. The Coupling of Function, Metabolism and Blood Flow in the Brain* (eds Ingvar, D. H. & Lassen, N. A.) 385–388 (Academic, New York, 1975).
- ⁸ Kennedy, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4230–4234 (1976).
- ⁹ Hubel, D. H., Wiesel, T. N. & Stryker, M. P. *J. comp. Neurol.* (in the press).

Instability of the eye in the dark and proprioception

THE extraocular muscles of most mammals contain stretch receptors which are not necessarily in the form of muscle spindles. The cat, for example, has no muscle spindles, but the oculomotor muscles contain structures, with spiral endings, that are in parallel with the muscle fibres and respond to stretch¹. Most proprioceptive fibres run extraorbitally in the ophthalmic branch of the Vth nerve^{2,3}. The cell bodies of these fibres seem to be located in the mesencephalic root of the Vth nerve⁴. There is electrophysiological evidence of proprioceptive projections to the cerebellum^{5,6}, superior colliculus^{7,8} and visual cortex⁹. The function of ocular proprioception is uncertain, although there is evidence that in man it may be an indicator of eye position in the dark¹⁰. In the cat it seems to have a role in maintaining the binocularity of single neurones in the visual cortex^{11,12}. Because most proprioceptive fibres run into the ophthalmic branch of the Vth nerve, we have investigated eye movements in the alert animal before and after section of this branch. We report here that, in the absence of visual and vestibular stimuli, the eye ipsilateral to the section becomes unstable and performs slow pendular oscillations. Asymmetries are observed in the horizontal vestibular nystagmus recorded in total darkness from the two eyes after unilateral section.

Cats chosen for their docility were anaesthetised with pentobarbital. A plastic cylinder was fixed to the skull and Ag–AgCl electrodes were implanted for recording horizontal movements of both eyes. The cat was trained to stay

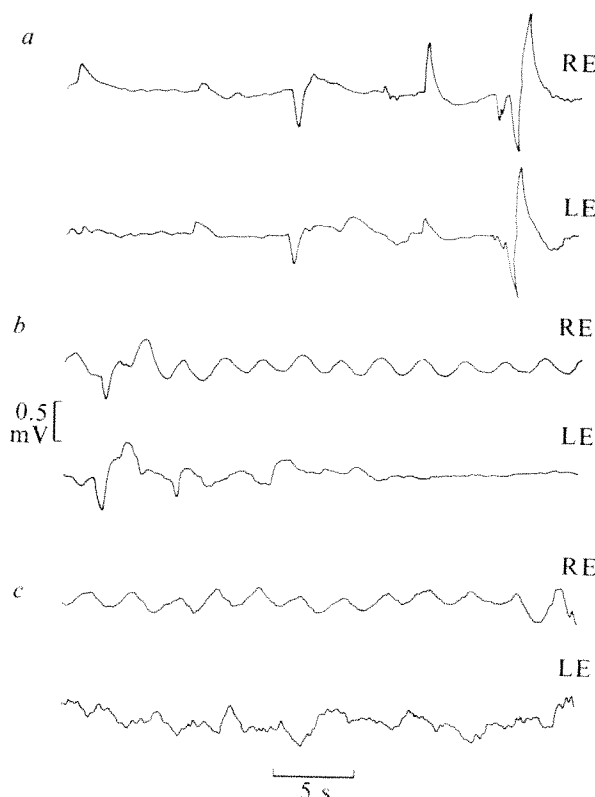


Fig. 1 *a*, Electro-oculographic a.c. recordings of horizontal movements of the right eye (RE) and left eye (LE) of a normal cat in the dark. *b*, The same, recorded 1 d after section of the ophthalmic branch of the right Vth nerve and *c*, 1 week after section of the ophthalmic branch of the left Vth nerve. Upward trace deflections indicate rotation of the eyes to the right, downward deflections, rotations to the left. The vertical bar corresponds to 5° horizontal eye rotation. Band pass: 0.1–20 Hz.

in a comfortable box with its head fixed by the plastic cylinder to a holder. The holder could either prevent any head motion or allow head rotations about a vertical axis. The cats were trained, over several days, to perform horizontal eye movements, tracking a visual target with the head either fixed or free to rotate horizontally. Eye movements were calibrated in each session by setting two pointers 30 cm from the eyes and displacing them 15° on either side of the cat's medial plane. The cat's attention was then called to either pointer while an experimenter checked the correctness of fixation, and the corresponding position of the recording trace was marked.

The cat box and the head holder were fixed to a table that could be oscillated sinusoidally about a vertical axis at various amplitudes and velocities.

When sufficient eye movements were recorded, the animal was again anaesthetised and the ophthalmic branch of the right Vth nerve was cut at the exit from the orbit or at the entrance into the semilunar ganglion. A small aperture was made into the parieto-temporal bone and the brain was lifted gently to reveal the three branches of the Vth nerve. Care was taken not to touch the oculomotor nerves. The operation was repeated on the left side after 2 or 3 weeks. After the experiments the animal was killed and the completeness of the section was checked.

We present here only results concerning eye movements in the dark, either spontaneous or elicited by head or body oscillations. The records of horizontal eye movements of normal cats that are awake in darkness contain periods with frequent spontaneous saccades, and periods with very few saccades, but with slow drifts mainly conjugate in the two eyes.

After section of the ophthalmic branch of the Vth nerve

no gross changes in the patterns of visually triggered eye movements were observed.

Seven out of nine cats displayed pendular movements of the eye ipsilateral to the section of the ophthalmic branch of the Vth nerve in the absence of visual stimuli (Fig. 1). The upper traces were recorded before the operation, the middle traces after section of the ophthalmic branch of the right Vth nerve and the bottom traces after further section of the ophthalmic branch of the left Vth nerve. The oscillations may have different amplitudes and frequencies in different cats, as comparison of Figs 1 and 2 (from two different cats) suggests. The frequency of the oscillations, however, was stable for each cat. The highest and lowest frequencies observed were 0.6 and 0.08 oscillations per s, the latter having a larger amplitude. There was no correlation between the occurrence of the oscillations and electroencephalogram activity, but the oscillations were more frequent when the cat was not performing frequent voluntary saccades. The two cats in which regular pendular movements were not observed were particularly restless even when deprived of visual and acoustic stimuli, and the rate of saccades was especially high in their eye movement recordings in the dark.

There were eye oscillations in most cats for at least 1 or 2 weeks after the operation. In three cats eye oscillations were observed a month after the second operation.

The patterns of eye movements induced by sinusoidal oscillations of the head or of the entire body became asymmetric in the two eyes after unilateral section of the ophthalmic branch of the Vth nerve. Alterations were induced in the eye ipsilateral to the section and consisted mainly of a decreased amplitude of the fast components of the vestibular nystagmus recorded in the dark. The patterns of vestibular nystagmus after unilateral section were equal in the two eyes when records were made in the light and were similar to those recorded before section. Details of these results will be reported elsewhere.

As controls, two cats underwent a complete unilateral operation except for the section of the nerves. Neither pendular motions in the dark nor asymmetries in the vestibular nystagmus of the two eyes were observed in these cats.

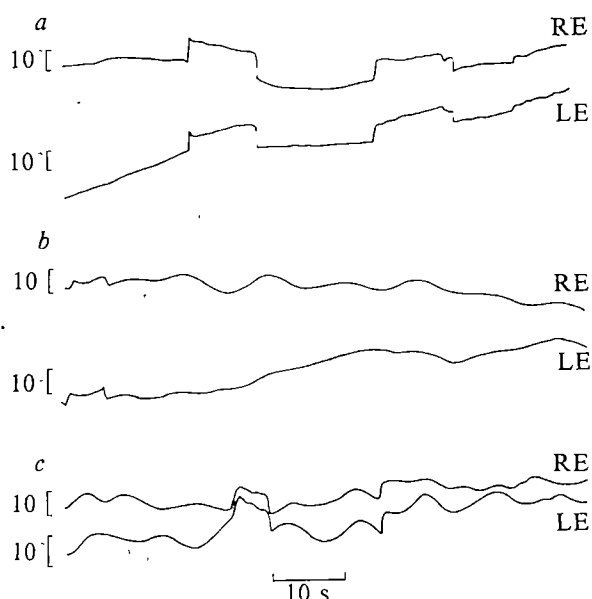


Fig. 2 *a*, Electro-oculographic d.c. recordings of horizontal movements of the right eye (RE) and left eye (LE) of a normal cat in the dark. *b*, The same, recorded 2 d after section of the ophthalmic branch of the right Vth nerve and *c*, 4 d after section of the ophthalmic branch of the left Vth nerve. Upward trace deflections indicate rotations of the eyes to the right.

The most obvious interpretation of the eye oscillations seen after section of the ophthalmic branch of the Vth nerve is that they result from the opening of the loop of the servo-system device responsible for the maintenance of eye position. Position errors can be signalled by two mechanisms: vision, indicating retinal error, and proprioception indicating relative stretch of the oculomotor muscles. It is not surprising that the interruption of the second source of information brings about oscillations of the eye in the dark.

A. FIORENTINI

L. MAFFEI

Laboratorio di Neurofisiologia del CNR,

Via S. Zeno, 51,

56100—Pisa, Italy

Received 13 June, accepted 2 August 1977.

- 1 Bach-y-Rita, P. & Ito, F. *J. Physiol. Lond.* 186, 663 (1966).
- 2 Batini, C. & Buisseret, P. *Arch. Ital. Biol.* 112, 18 (1974).
- 3 Batini, C., Buisseret, P. & Buisseret-Dalmas, C. *Brain Res.* 85, 74 (1975).
- 4 Buisseret, P., Guérin, J. P., Horscholle-Bossavit, G. & Tye-Dumont, S. *J. Physiol., Paris* 65, 369 A (1972).
- 5 Fuchs, A. & Kornhuber, H. H. *J. Physiol. Lond.* 200, 713 (1969).
- 6 Baker, R., Precht, W. & Lindäs, R. *Brain Res.* 38, 440 (1972).
- 7 Abraham, V. C. & Rose, P. K. *J. Neurophysiol.* 38, 10 (1975).
- 8 Rose, P. K. & Abraham, V. C. *Brain Res.* 97, 95 (1975).
- 9 Buisseret, P. & Maffei, L. *Expl. Brain Res.* 28, 421 (1977).
- 10 Skavenski, A. A. *Vision Res.* 12, 221 (1972).
- 11 Maffei, L. & Bisti, S. *Science* 191, 579 (1976).
- 12 Maffei, L. & Fiorentini, A. *Brain Res.* 105, 73 (1976).

Leukocytes are consistently associated with degenerating embryos in IUD-bearing rhesus monkeys

IN spite of intensive research during the past 15 yr the precise mechanism whereby an intrauterine device (IUD) exerts its contraceptive action is unclear. There is wide agreement that the effect is intra- rather than extrauterine, and is probably elicited after arrival of the conceptus in the uterine cavity and before its attachment to the endometrium¹. The most plausible suggestion seems to be that the device acts as a foreign body and provides a sterile inflammatory reaction in the endometrium which in some unknown way renders the uterine cavity hostile to preimplantation embryos^{1,2}. The presence of large numbers of inflammatory cells, consisting mainly of neutrophils and, to a lesser extent, macrophages, is a constant feature of the endometrial reaction in IUD-bearing women and other mammals. In some species there is a high positive correlation, in the presence of an IUD, between the concentration of leukocytes in the uterine fluid and the suppression of implantation (for example, in the rabbit³). So far, however, there has been no direct proof of a consistent association between leukocytes and unimplanted embryos in IUD-fitted primates. This paper provides evidence for such an association in the rhesus monkey.

Our study stems from the successful development of a method for the recovery of uterine embryos from normal monkeys⁴. It consists of flushing fluid through a large-bore, fenestrated needle inserted into the uterine lumen above the cervix and aspirating it through a second, fundal needle into a receiving syringe; it has a yield rate of about 50% of preimplantation embryos in animals examined between days 15 and 20 of the menstrual cycle.

Normal females without IUDs were first subjected to the flushing procedure for the recovery of uterine embryos and then fitted with a shortened tailless Margulies coil inserted surgically through the fundus. After two successive menstrual periods the animals were mated and flushed once, as before, between days 15 and 20 of the cycle. The contents of the flush was deposited immediately into a siliconised watch glass and searched under a stereomicroscope. Embryos were transferred to cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer, photographed and processed for

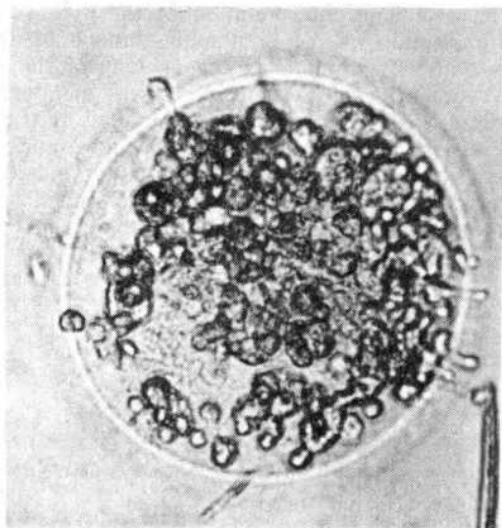


Fig. 1 Blastocyst from IUD-monkey (no. 476), showing leukocytes on the surface and apparently penetrating the zona pellucida ($\times 256$).

electron microscopical examination³. The remainder of the flush was centrifuged and the cellular composition of the pellet was determined by means of a haemocytometer and cytocentrifuge. During initial recovery attempts in IUD-animals, the flush was found to contain far more cells and debris than observed in non-IUD flushes (Table 1) and tended to clot, making the search for embryos difficult; heparin added to the receiving syringe reduced this tendency.

Sixteen females, from all of which embryos had been recovered in the non-IUD condition, have been flushed after being fitted with a device yielding six embryos in a total of 46 attempts (Table 1). Five of these embryos, the sixth being lost in processing, have been prepared and representative sections were examined by light microscopy and with an electron microscope. All five were considered to be abnormal or degenerating and each showed polymorphonuclear leukocytes both outside and within the embryonic tissue (Figs 1–3). In two of them macrophages were also present. In another, judged to be an azonal morula, all blastomeres were fragmenting, with leukocytes infiltrated between them. Two azonal embryos were blastocysts with

leukocytes present in the blastocoel cavities (Fig. 2). Another blastocyst possessed the zona pellucida and, again, leukocytes were found within the blastocoel and interspersed among cells of the inner cell mass. In two cases leukocytes were seen apparently passing through the zona pellucida (Fig. 3). The trophoblast layer showed irregular cells with disorganised cell junctions and an array of cytoplasmic membranous material not observed in normal monkey blastocysts³. The fifth embryo comprised only five or six shrunken cells within an intact zona. Electron microscopy revealed several polymorphs and macrophages in direct contact with embryonic cells. The polymorphs appeared to be typical, mature neutrophils and the macrophages had extensive plasma membrane invaginations. Occasional eosinophils were also present.

By contrast, of a total of three unfertilised ova and 30 embryos so far recovered from non-IUD monkeys, none had any polymorphs or other types of leukocyte attached to the surface, and only eight embryos were considered to be degenerating.

Cytological analysis of all IUD-flushes (irrespective of embryo recovery) showed that the number of cells varied from 4.37×10^5 to 2.39×10^6 , most of them being neutrophils (Table 1). Flushes from non-IUD fitted uteri gave

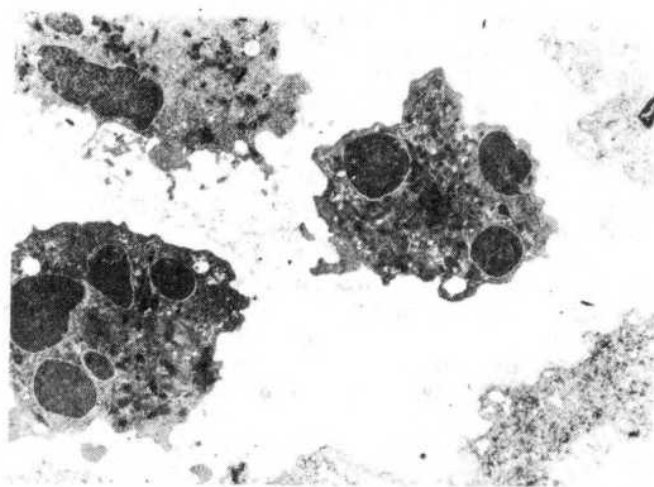


Fig. 2 Electron micrograph of a portion of an azonal blastocyst from an IUD-monkey (no. 616). Neutrophils are present in the blastocoel. T, Trophoblast layer ($\times 4,750$).

Table 1 Details of embryos and cells in uterine flushes from IUD- and non IUD-bearing rhesus monkeys

No. of female	Day of recovery*	Embryo stage	Total cell counts and differential distributions (%)						
			Totals	Neutrophils	Eosinophils	Monocytes	Lymphocytes	Macrophages	Endometrial
IUD flushes with embryo recovery (46 attempts in 16 females)									
476	16	5-6 cell, with zona	—	—	—	—	—	—	—
616	16	Blastocyst, azonal	5.08×10^5	65.7	6.7	8.4	0	7.2	12.0
617	19	Blastocyst, azonal	1.02×10^6	51.2	4.7	20.8	0.4	12.9	10.0
618	19	Morula, azonal	7.58×10^5	58.1	5.9	8.8	0.2	11.6	15.4
476	20	Blastocyst, with zona	9.39×10^5	33.2	11.2	10.7	1.9	27.6	15.4
613	15 (?)	Morula, azonal†	5.89×10^5	42.7	8.0	8.8	0	31.4	9.1
IUD flushes without embryo recovery (means \pm s.e.; $n = 17$)									
—			$1.18 \pm 0.13 \times 10^6$	48.6 ± 3.6	5.0 ± 0.8	9.6 ± 1.1	1.4 ± 0.5	20.3 ± 2.5	15.3 ± 3.0
Non-IUD flushes without embryo recovery (means \pm s.e.; $n = 7$)									
—			$2.32 \pm 0.62 \times 10^5$	11.3 ± 4.6	2.2 ± 1.4	6.0 ± 2.5	5.4 ± 2.0	0.8 ± 0.5	74.3 ± 9.1

*Day 1 counted as the first day of menstrual bleeding.

†Embryo lost in processing—no definitive identification made.

values between 1.44×10^4 and 3.92×10^5 (mean: $2.32 \pm 0.62 \times 10^5$), most being epithelial cells. By contrast, Joshi *et al.*⁹ reported no significant increase in polymorphs in the uterine flushings of IUD-fitted baboons. Their method of collecting and evaluating the flushes differed, however, substantially from our own. In particular, their use of centrifuge speeds of 2,500g is likely to cause leukocyte destruction, which can be avoided with speeds of less than 750g as employed by ourselves.

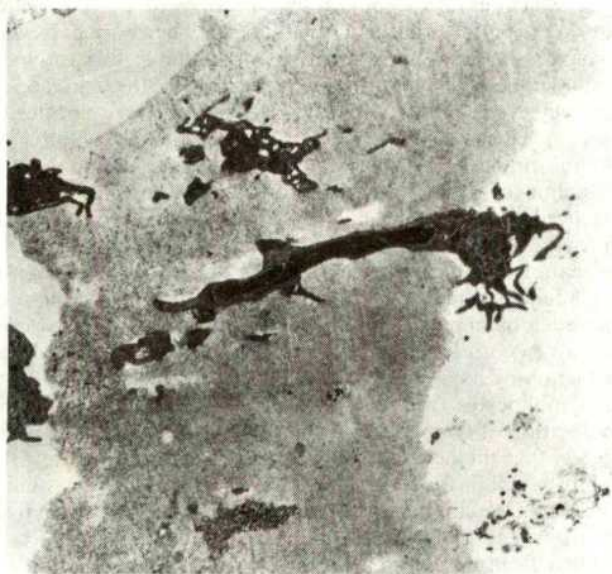


Fig. 3 Electron micrograph of the zona pellucida (Z) of an embryo from an IUD-monkey (no. 476). A leukocyte is seen passing through the zona into the blastocoel (B) ($\times 5,550$).

Our findings show that, in IUD-bearing rhesus monkeys, embryos can enter the uterine cavity at about the expected time after conception. They are, however, invariably abnormal or degenerating as well as invested with luminal leukocytes induced by the device. Also, compared with the yield in normal females (about 50%), far fewer embryos can be recovered at corresponding cycle days from IUD-fitted animals (about 13%).

It is feasible that the greatly increased cell content of the flush in IUD-monkeys may have made the detection of a recovered embryo impossible or that the embryo, when flushed out, was already too degenerated to be recognisable. It is also possible that in spite of normal fertilisation and early cleavage, embryos of IUD-fitted monkeys remain 'blocked' at or near the tubouterine junction and are therefore not recoverable by uterine flushing. The conclusion, implicit in our findings, that in monkeys with IUDs the blastocyst stage may be reached is indirectly supported by the finding of increased serum levels of human chorionic gonadotrophin in a proportion of IUD-bearing women examined during the second part of the menstrual cycle^{7,8}. Unfortunately no comparable data are available for rhesus monkeys.

Whether the embryos observed in our study were entirely normal before their investment and penetration by leukocytes is not known. It is possible that the highly cellular milieu of the uterine environment, perhaps derived from dead or dying leukocytes, is embryotoxic and that the main function of recently infiltrating live leukocytes is in the final destruction and removal of dying or damaged embryos. Nonetheless, the demonstrated association between degenerating uterine embryos and their investment with leukocytes in IUD-fitted monkeys is sufficiently constant and striking to suggest that it is more than coincidental.

The financial support of the Ford Foundation (grant no. 630-0576B) is gratefully acknowledged.

P. R. HURST
KATHRYN JEFFERIES
P. ECKSTEIN
KARIN DAWSON
A. G. WHEELER

Department of Anatomy,
Medical School,
University of Birmingham,
Birmingham, UK

Received 13 June; accepted 9 August 1977.

- Duncan, G. W. & Wheeler, R. G. *Biol. Reprod.* **12**, 143-175 (1975).
- Parr, E. L. & Shirley, R. L. *Fert. Steril.* **27**, 1067-1077 (1976).
- El-Sahwi, S. & Moyer, D. L. *Fert. Steril.* **22**, 398-408 (1971).
- Hurst, P. R., Jefferies, K., Eckstein, P. & Wheeler, A. G. *Biol. Reprod.* **15**, 429-434 (1976).
- Hurst, P. R., Jefferies, K., Eckstein, P. & Wheeler, A. G. *J. Anat.* (in the press).
- Joshi, S. G., Kraemer, D. C. & Chenault, C. B. *Contraception* **2**, 339-351 (1970).
- Landesman, R., Coutinho, E. M. & Saxena, B. B. *Fert. Steril.* **27**, 1062-1066 (1976).
- Beling, C. G., Cederqvist, L. L. & Fuchs, F. *Am. J. Obstet. Gynec.* **125**, 855-858 (1976).

Active thymus derived suppressor lymphocytes in human cord blood

A FOETUS carries antigens that are foreign to its mother, but the mother does not routinely reject it as an allograft. The fate of the foetus may thus depend on preventing or blocking the ability of the immune system of the mother to respond to alien foetal antigens. Clearly, this is a complex process which may involve several mechanisms¹⁻⁶. One mechanism implicates the active blocking of the immunological capacity of the mother to respond to her foetus^{5,6}. We reported previously that T lymphocytes taken from cord blood of human newborns inhibits their own mothers' lymphocytes from entering mitosis^{6,7}. We have studied the subpopulations of T lymphocytes in human cord blood and ascertained their ability to inhibit both the division of lymphocytes and the production of immunoglobulin by lymphocytes obtained from the mother. We report here that active T suppressor cells occur in cord blood, that these T suppressor cells bear IgG receptors; and that the T cells bearing IgG receptors inhibit both mitosis of and immunoglobulin production by lymphocytes from the mother.

Blood (10-30 ml) was collected in heparin from the umbilical cords of healthy, singleton human males immediately after delivery but before expulsion of the placenta. Venous blood (25 ml) was collected in heparin from their mothers' arm within 1 h after delivery and from non-pregnant females and males (ages 21-43 yr, all healthy). These blood samples were the source of lymphocytes prepared by using Ficoll-Hypaque gradients. Division of the lymphocytes into T-cell enriched and B-cell enriched fractions was as described before⁷. The T cell population was over 97% pure, and the B cell population over 94% pure as judged by uptake of neutral red dye and expression of surface immunoglobulins⁷. T lymphocytes were separated into subpopulations bearing Fc IgG receptors (T_G) or IgM Fc bearing receptor cells (T_M) using the method of Moretta *et al.*^{8,9}. The purified T_G and the T_M subpopulations each contained less than 1% contaminating cells.

To study the effects of lymphocytes from each newborn on the growth of lymphocytes from his mother, equal numbers of cells from each mother and her baby ($1-2 \times 10^6$ from each) were combined and suspended in RPMI 1640 medium containing 20% foetal calf serum, antibiotics and glutamine (RPMI growth media) with phytohaemagglutinin P (PHA-P) (50 μ g per ml of medium). This concentration of PHA-P had been found to be optimal for stimulating lymphocytes from both the mother and baby¹⁰. When kept separate, both mothers' and newborns' cells divided equally well with an average viability of 60%-80% as determined by Trypan blue dye exclusion. After incubation for 3 d the cultures were treated with colchicine and the chromosomes prepared and stained as reported before^{7,11}. Cells from a male baby (positive for

Y chromosome) were always mixed with his mother's cells (lacking a Y chromosome)

Table 1 shows that when unseparated lymphocytes or enriched subpopulation of T_G cells from a newborn baby were cultured with equal numbers of lymphocytes from his mother, the vast majority of cells undergoing mitosis were of baby origin. In all six pairs studied in which the newborn's T_G cells were mixed with his mother's lymphocytes, a mean \pm s.e.m. of $90.7 \pm 1.4\%$, whereas 9% or less were from the mother ($P < 0.01$). In contrast, when T_G depleted fractions of cells were taken from a newborn and mixed with his mother's lymphocytes, only about half of the cells undergoing mitosis were of baby origin (Table 1). Thus in all six pairs involving T_G depleted cell populations, a mean of $49.3 \pm 2.3\%$ of the total lymphocytes in mitosis were from newborns as determined by the presence of the Y chromosome (unfractionated T lymphocytes from cord blood gave a value of $90.7 \pm 1.4\%$, whereas 9% or less were from the mother ($P < 0.01$). In contrast, when T_G depleted fractions of cells were taken from a newborn and mixed with his mother's lymphocytes, only about half of the cells undergoing mitosis were of baby origin (Table 1). Thus in all six pairs involving T_G depleted cell populations, a mean of $49.3 \pm 2.3\%$ of the total lymphocytes in mitosis were from newborns as determined by the presence of the Y chromosome. When the T_M subpopulation of a newborn's cord blood lymphocytes were mixed with lymphocytes from his mother, roughly half of the cells undergoing mitosis were

Table 1 T_G subpopulation of T lymphocytes from human newborns suppresses mitosis of lymphocytes from their mothers

Case	Newborn lymphocyte subpopulation mixed with total maternal lymphocytes	Metaphase cells with Y chromosome (%)
P.A.	Total	94
P.A.	T_G	91
P.A.	T_G depleted	57
P.A.	T_M	59
C.L.	Total	88
C.L.	T_G	84
C.L.	T_G depleted	55
C.L.	T_M	52
L.O.	Total	89
L.O.	T_G	100
L.O.	T_G depleted	46
L.O.	T_M	49
R.O.	Total	89
R.O.	T_G	90
R.O.	T_G depleted	49
R.O.	T_M	53
W.H.	Total	96
W.H.	T_G	89
W.H.	T_G depleted	47
Mc.	Total	88
Mc.	T_G	95
Mc.	T_G depleted	42
	Expected result from 1:1 mixture	50

Lymphocytes from newborns and their mothers were obtained after Ficoll-Hypaque gradient centrifugation. The T cells were separated from these lymphocytes by E rosetting, and then distinct subpopulations of T_G or T_M cells were isolated on the basis of binding and rosetting with ox red blood cells (RBC) bearing the Fc portion of human IgG or IgM, respectively. Briefly, purified T cells were mixed with ox erythrocytes (ORBC) coated with rabbit IgG antibodies, pelleted and incubated on ice for a minimum of 10 min. Rosetting cells (T_G) were separated from non-rosetting cells (T_G depleted fraction) and purified by pelleting through two density gradients. The collected cell suspension contained less than 1% non-rosetting cells. RBC were removed by treatment with ammonium chloride buffer and the residual lymphocytes were suspended in 199 medium supplemented with 20% foetal calf serum, antibiotics and glutamine (199 growth medium), and pelleted. T_G enriched and T_G depleted cells were then resuspended in 199 growth medium and incubated overnight. T_G depleted cells were mixed with ORBC coated with rabbit IgM antibodies, pelleted and placed on ice for 1 h. The resulting rosettes purified on a Ficoll-Hypaque gradient were dissociated in a vortex mixer and purified human IgM at a concentration of $125 \mu\text{g ml}^{-1}$ added to block free IgM receptors. T_M cells were then separated from ORBC by density gradient centrifugation. Equal numbers of cells ($1-2 \times 10^6$) from male newborns and their mothers were mixed and cultured with PHA-P, $50 \mu\text{g ml}^{-1}$. After incubation for 3 d the cells were treated with colchicine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). Usually 50-100 cells in metaphase were examined from each culture for the presence or absence of the Y chromosome. In the culture conditions used, lymphocytes from mothers survived as well as lymphocytes from newborns and mean mitotic indexes of both pairs of cells were equivalent. Mixing adult (instead of newborn) male lymphocytes with lymphocytes from mothers (or other adult females) resulted in % of metaphase cells having a Y chromosome \pm s.e.m. of $52 \pm 8\%$.

of baby origin ($53.2 \pm 2\%$). Clearly, the subpopulation of T cells that suppressed mitosis of mothers' lymphocytes is that bearing Fc receptors for IgG. Further, these studies showed that lymphocytes collected from an infant's cord blood are primed to inhibit division of the mother's lymphocytes and do not need to be activated.

We then studied the effects of subpopulations of T lymphocytes obtained from each newborn on the ability of lymphocytes from his mother to make immunoglobulin. Experiments with polyclonal B cell differentiation induced by PWM had indicated both the need of T cell help and the optimal synthesis of immunoglobulin, which usually occurred by the seventh day^{9,12}. After culturing an enriched T cell population from a newborn baby with B lymphocytes from his mother, we found that significantly less immunoglobulin was synthesised than when the enriched T lymphocytes came from the mother. Hence, in all three pairs tested (Table 2), when T cells from the newborn were mixed with B cells from the mother, a 46% or greater reduction in immunoglobulin production (average $55.3 \pm 5.8\%$) occurred. These results could be influenced, in part, by the inability of 'immature' newborn T cells to provide sufficient help for adult B cells¹³. This is not the likely explanation for our results, however, as T cells from newborns or from adults provide equivalent amounts of help for synthesis of immunoglobulin by B cells of the newborn (Table 2). That T cells from a newborn apparently could not inhibit immunoglobulin production by its own B cells is consistent with our observation that lymphocytes from a newborn baby cannot abrogate the proliferation of lymphocytes from other newborns⁷. This result suggests that the newborn's lymphocytes, themselves, resist the inhibitory action for which they are responsible. Other studies showed that the suppression of immunoglobulin production by B cells from the mother resided in the T_G subpopulation of cells obtained from cord blood (Table 3). Hence, when equivalent numbers of a newborn's T_G lymphocytes were mixed with B cells from his own mother or from an alien mother, immunoglobulin synthesis was inhibited 63% or 65%, respectively.

Table 2 T lymphocytes from human newborns suppress immunoglobulin production by lymphocytes from their mothers

Case	Source of lymphocytes	IgG made (ng per 1×10^6 B cells)	% Reduction
	B cells	T cells	
Y.O.	Mother	Mother	116
Y.O.	Mother	Newborn	66
Y.O.	Newborn	Newborn	62
Y.O.	Newborn	Mother	69
L.O.	Mother	Mother	498
L.O.	Mother	Newborn	232
L.O.	Newborn	Newborn	58
L.O.	Newborn	Mother	47
T.R.	Mother	Mother	82
T.R.	Mother	Newborn	28
T.R.	Newborn	Newborn	ND
T.R.	Newborn	Mother	ND

Cells from newborns and their mothers were placed on Ficoll-Hypaque gradients and separated into B cell and T cell populations^{7,9}. Equal numbers of B cells (1×10^6) and T cells were incubated in culture with PWM ($50 \mu\text{g ml}^{-1}$, the concentration optimal for stimulating B and T cells⁷) in 5 ml of growth medium. After 7 d the culture supernatant fluids, free of cells, were analysed for IgG concentration by measuring the ability of various dilutions of supernatant fluid to block IgG-anti IgG binding. The reagents, their purity and the methodology for doing this assay were as before^{12,16}. The lower limit of immunoglobulin detection was 4 ng of IgG per ml. ND, none detected.

These results clearly show that T lymphocytes bearing Fc receptors for IgG (T_G) taken from the cord blood of human male newborns effectively suppress the division of their mothers' lymphocytes and lower the ability of B cells from the mother to make IgG *in vitro*. In contrast, the T-cell enriched lymphocyte population taken from human cord blood, then either depleted of T_G cells or bearing only IgM receptors (T_M) cannot suppress mitoses of maternal lymphocytes. These data are consistent with and extend the results of Moretta *et al.*, who showed that T_G lymphocytes obtained from adults dose-dependently suppressed

Table 3 T_G subpopulation of T lymphocytes from the human newborn suppresses production of immunoglobulin by lymphocytes of its mother

Source of lymphocytes		T cell population	IgG made (ng per 1×10^6 B cells)	% Reduction
B cells	T cells			
Mother: BA	Mother: BA	Total	303	
Mother: BA	Newborn: BA	T _G	114	63
Mother: BA	Newborn: WE	T _G	108	65

Adult human peripheral blood or cord blood placed on Ficoll-Hypaque gradients was separated into B and T lymphocyte fractions. T cells from newborns were divided into distinct populations of T_G cells on the basis of rosetting with ox RBC. 1×10^6 B cells from a mother were mixed with either 1×10^6 total T cells from the same mother or 1×10^6 T_G cells from her baby or an alien baby and cultured with PWM in 5 ml of RPMI growth medium. After 7 d the cultured supernatant fluids, free of cells, were analysed for IgG concentration (see Table 2 legend).

the differentiation and proliferation of B cells after stimulation with PWM in the presence of helper T_H cells⁹. No significant suppressor activity by peripheral blood lymphocytes collected from adults occurred unless T_G cells were first activated by interaction with immune complexes. In contrast, we found that T suppressor cells (T_G) in human cord blood are already active and able to suppress mitosis of maternal lymphocytes and production of immunoglobulin by maternal lymphocytes induced to differentiate with PWM. This indicates that lymphocytes from the baby are probably primed *in vivo* for their suppressor effect. Recently Maissin and his associates¹⁴ detected circulating antigen-antibody complexes in the sera of most pregnant women, suggesting a possible mechanism whereby T_G cells of a newborn may be activated. Further interaction of maternal and neonatal cells may generate T_G cells. Also suggestive of the biological importance of T_G cells in maternal-foetal interrelationships is the finding that three to fourfold more T_G cells are found in cord blood than in adults' blood (> 30% in the former compared with < 10% in the latter¹³).

How does this suppression work? Olding, Murgita and Wigzell using double culture chamber systems have shown that cord blood lymphocytes from a newborn effectively suppress the proliferation of lymphocytes from their mothers or other non-pregnant adults across a cell-semipermeable membrane, suggesting that inhibition of maternal lymphocyte proliferation occurs via a soluble suppressor factor(s)¹⁵. They also found that suppressor activity associated with newborn lymphocytes was completely abrogated by irradiation with 6,000 rad indicating the requirement for cell division in the *in vitro* release of suppressor factor. These findings are similar to those of Moretta *et al.*, who showed that treatment of activated T_G adult lymphocytes with 3,000 rad inhibited their ability to suppress polyclonal B cell differentiation and immunoglobulin production induced by PWM⁹.

Many factors in maternal-foetal interrelationships probably participate in preventing the automatic rejection of the foetus by its mother. Our studies suggest a mechanism by which foetal tissues can survive in a hostile immunological environment. We show that thymus derived suppressor lymphocytes, which are identified on the basis of bearing an Fc receptor for IgG, occur in infants' cord blood and perform an active process in inhibiting potentially harmful maternal lymphocytes from dividing. Further, the newborn's T suppressor cells are already primed in cord blood and after coming in contact with mothers' cells would render such cells unlikely to respond to the foetal foreign antigens. When this protective mechanism first develops is not clear, but necessarily it must arise after the foetal lymphoid system develops. The presence of foetal T_G cells late in pregnancy and their ability to release soluble suppressor factor(s) raise questions as to the level and fate of these cells during spontaneous abortions as well as the possible future use of suppressor factors as a therapeutic adjunct.

This research was supported by USPHS grants AI 07007, NS 12428, USNSF grant I-364, and grants CA 16673 and 13148 from the NCI.

MICHAEL B. A. OLDSTONE
ANTOINETTE TISHON

Department of Immunopathology,
Scripps Clinic and Research Foundation,
La Jolla, California 92037

LORENZO MORETTA*

Cellular Immunobiology Unit,
University of Alabama in Birmingham,
Birmingham, Alabama

Received 20 May; accepted 18 July 1977.

*Present address: Instituto di Microbiologia, Università de Genova, 16132 Genova, Italy

- Faulk, W. P. & Temple, A. *Nature* **262**, 799-802 (1976).
- Goodfellow, P. N., Barnstable, C. J., Bodmer, W. F., Snary, D. & Crumpton, M. J. *Transplantation* (in the press).
- Jenkinson, E. J. & Billington, W. D. *Transplantation* **18**, 286-289 (1974).
- Currie, G. A., van Doorninck, W. & Bagshawe, K. D. *Nature* **219**, 191-192 (1968).
- Hellström, K., Hellström, I. & Braun, J. *Nature* **224**, 914-915 (1969).
- Olding, L. B. & Oldstone, M. B. A. *Nature* **249**, 161-162 (1974).
- Olding, L. B. & Oldstone, M. B. A. *J. Immun.* **116**, 682-686 (1976).
- Ferrarini, M., Moretta, L., Abrile, R. & Durante, M. L. *Eur. J. Immun.* **5**, 70-72 (1975).
- Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M. & Cooper, M. D. *J. exp. Med.* (in the press).
- Olding, L. B., Benirschke, K. & Oldstone, M. B. A. *Chin. Immun. Immunopath.* **3**, 79-89 (1974).
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. & Hungerford, D. A. *Exp. Cell Res.* **20**, 613-618 (1960).
- Waldmann, T. A. *et al. Lancet* **ii**, 609-613 (1974).
- Hayward, A. R. & Lawton, A. R. *J. Immun.* (in the press).
- Masson, P. L., Delire, M. & Cambiaso, C. L. *Nature* **266**, 542-543 (1977).
- Olding, L. B., Murgita, R. A. & Wigzell, H. *J. Immun.* (in the press).
- Lerner, R. A., McConahey, P. J., Jansen, I. & Dixon, F. J. *J. exp. Med.* **135**, 136-149 (1972).

Evidence for a common evolutionary origin of gastrin and cholecystokinin

THE gastrointestinal hormones gastrin and cholecystokinin (CCK) are structurally and functionally related in that they have identical COOH-terminal pentapeptide sequences, which represent the biologically active portions of the molecules. The rest of each molecule only modifies its activity quantitatively and determines relative potencies for various targets. This has suggested that the two hormones have evolved from a common ancestral molecule^{1,2}. To test this hypothesis we have investigated the occurrence of gastrin and CCK in different vertebrate species by immunocytochemistry and radioimmunochemistry. We now present evidence that in amphibians and teleosts gastrin and CCK are no longer recognisable as separate hormones.

To avoid problems with immunocytochemical cross-reactivity, antisera specific for the NH₂-terminal and middle sequence of gastrin were prepared. In submammalian species such antisera failed to demonstrate endocrine cells, although they were potent in demonstrating mammalian gastrin-producing cells. In contrast, antisera against the common COOH-terminal sequence of the two hormones reacted well with gastrointestinal endocrine cells of mammals, birds, reptiles, amphibians and bony fishes. This suggests that the biologically active COOH-terminal sequence is the only region of the gastrin molecule that has been preserved during evolution. Hence, submammalian gastrin cells can be demonstrated only with antisera that also react with the COOH-terminus of CCK. Therefore a specific CCK antiserum (no. 4698) that reacts with sequence 25-30 of porcine CCK-33, but fails to bind any gastrin (sulphated as well as non-sulphated) was used. Thus cells reactive with the COOH-terminus-specific antiserum but not with the CCK antiserum are called gastrin cells, whereas cells reactive with both antisera can be expected to contain a region similar to sequence 25-33 of CCK-33. In this way



Fig. 1 Frog antral mucosa. Staining with CCK antiserum no. 4698 (PAP technique). Numerous immunoreactive epithelial cells are visible ($\times 280$). Specimens from gastrointestinal mucosa of mammals (man, pig and dog), birds (chicken and duck), reptiles (*Testudo graeca*), amphibians (*Rana esculenta* and *R. temporaria*) and bony fishes (*Gadus morrhua*) and from the brains of *R. esculenta* were frozen in melting Freon-22, freeze-dried, vapour-fixed with formaldehyde and embedded in paraffin. Human specimens were obtained at surgery (carcinoma) and animals were killed in slaughterhouses, by mebumal injections or by decapitation. Sections (3 μ m) were deparaffinised and subjected to indirect immunocytochemical staining for the demonstration of (1) NH₂-terminus of human gastrin-17 (antiserum no. 1295, given by Dr J. H. Walsh); (2) mid-sequence of human gastrin-17 (antiserum 4710); (3) COOH-terminus of human gastrin-17 (antisera nos 2604, 2609, 4562)^{13,14}; (4) sequence 25–30 of porcine CCK-33 (antiserum no. 4698); (5) middle region (probably sequence 19–25) of CCK-33 (antiserum no. 4478). Specificity of the antisera was ascertained using differential absorptions with: a, pentagastrin (Peptavlon, ICI); b, synthetic mid-sequence (6–13) of human gastrin-17 (given by Dr Morley); c, synthetic human gastrin-17 (SHG 1, ICI); d, highly purified porcine CCK-33 (given by Professor V. Mutt); e, synthetic caerulein (Caeruletide, given by Farmitalia, Milan). Absorptions were carried out at the optimal dilutions of the various antisera. Usually, addition of 10 μ g peptide per ml antiserum was sufficient. If the addition had no effect, the peptide concentrations were increased 10-fold. For details see refs 13 and 14. The site of antigen-antibody reaction was revealed with the peroxidase-anti-peroxidase (PAP) procedure of Sternberger¹⁵.

we ascertained that mammals (man, pig and dog), birds (chicken and duck) and a reptile (*Testudo graeca*) all contain distinct populations of gastrin and CCK cells. In these species, gastrin cells predominated in the antral mucosa whereas CCK cells occurred in the duodenum and jejunum. In amphibians (*Rana esculenta* and *R. temporaria*), however, no gastrin immunoreactive cells that did not also react with the specific CCK antiserum (no. 4698) were found (Fig. 1). CCK-immunoreactive cells predominated in the antrum but were also scattered in the small intestines. No CCK-immunoreactive cells that did not also react with the COOH-terminus-specific antisera were found. Absorption controls showed that, in all species, staining with the CCK antiserum (no. 4698) was abolished by pretreatment with CCK-33 or with caerulein, but was unaffected by pretreatment with even very large amounts of gastrin-17 (compare Fig. 1). Staining with the COOH-terminus-specific antisera was abolished by pretreatment with either pentagastrin, gastrin-17, CCK-33 or caerulein. Antiserum no. 4478, specific for the middle region (probably sequence 19–25) of CCK-33 and thus devoid of cross-reactivity to caerulein, stained no cells of the frog antral mucosa, whereas it was highly potent in demonstrating, for example, porcine CCK cells. Thus the compound present in the frog antral cells is dissimilar to CCK-33 in its middle region. Similar staining experiments with gastrointestinal mucosa of the teleost fish (*Gadus morrhua*) exactly reproduced the findings with amphibians, revealing a large population of

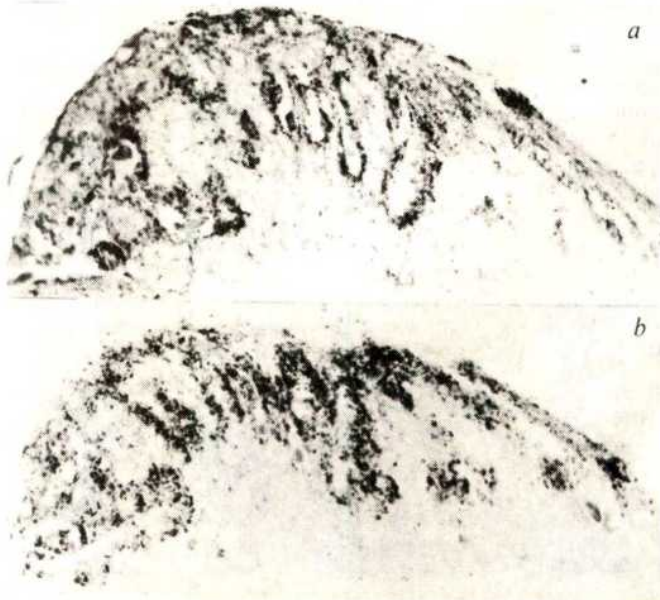
antral cells containing a region similar to sequence 25–33 of CCK-33. In the frog brain, many nerves showed the same pattern of immunoreactivity as the gastrointestinal endocrine cells. These nerves were most numerous in the hypothalamus where they converged towards the median eminence (Fig. 2).

In parallel with the immunocytochemical studies three individual extracts as well as pooled extracts of antrum and brain, respectively, from 30 frogs were subjected to gel chromatography as monitored by three assays: one specific for CCK and caerulein³, one measuring both CCK, caerulein and gastrin⁴ and one specific for gastrin⁴ (Fig. 3). As Fig. 3 shows, antral immunoreactivity eluted in three major peaks. The position of the peak of lowest apparent molecular size corresponded to that of caerulein. The intermediate peak eluted in a position corresponding to CCK-33 and gastrin-34 (big gastrin), whereas the size of the largest component corresponded to gastrin component I⁵ and a large CCK-component previously detected in extracts of porcine jejunum³. The immunoreactivity of the brain extracts also eluted in three major peaks in positions identical to those of the antral extracts. The smallest component, however, predominated in the brain extracts (Fig. 3). Gel chromatography of extracts of teleostean antrum and brain also revealed three immunoreactive peaks of which the quantitatively largest in the antral as well as in the brain extracts eluted in a position corresponding to caerulein.

Our data indicate that the three major components found in frog brain and antrum all contain a sequence similar to region 25–33 of CCK-33 and that the frog has no separate gastrin and CCK components. Gastrin immunoreactivity has been reported in the amphibian gastrointestinal tract⁶. Although no data concerning possible CCK immunoreactivity were given, the gel chromatographic data⁶ suggests that the peaks observed correspond to some of the components presented here.

The decapeptide caerulein and the nonapeptide phyllocaerulein, both isolated from frog skin, have important gastrointestinal actions^{7,8}. In the frog, caerulein is a much more potent stimulator of gastric acid secretion than is gastrin-17 (ref. 9). Studies with synthetic analogues have

Fig. 2 Frog median eminence. a, Staining with CCK antiserum (no. 4698), PAP technique. Numerous dark-stained fibres and dots occur in the vicinity of vessels. b, Adjacent section to (a), for comparison stained with an antiserum against the growth hormone-release inhibiting hormone, somatostatin. The distribution of the somatostatin-containing nerves is similar but not identical to that of the CCK-immunoreactive nerves ($\times 256$).



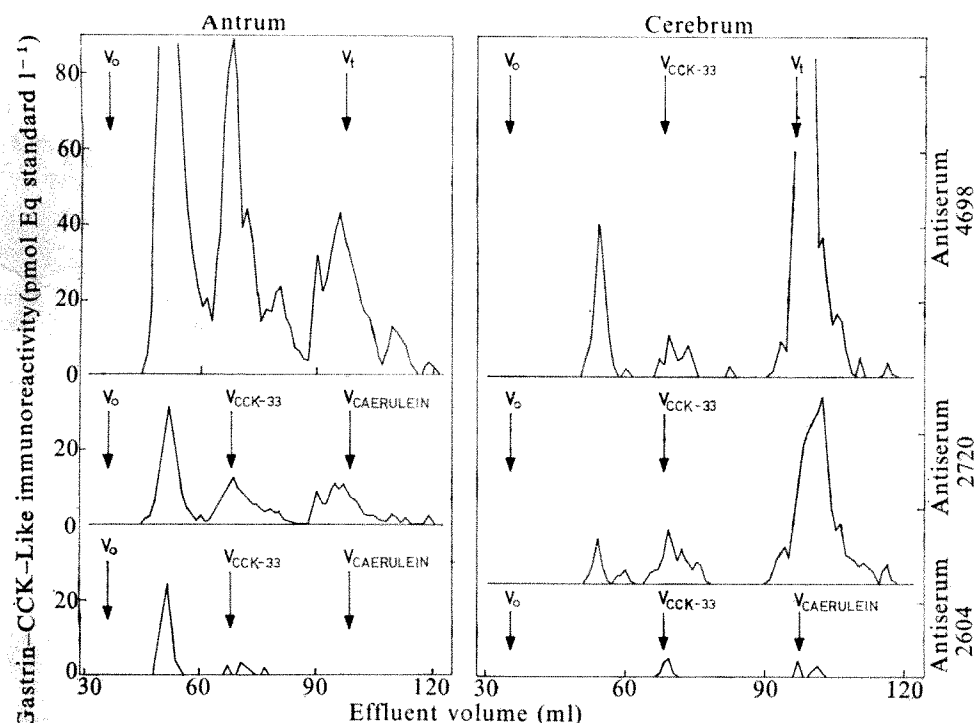


Fig. 3 Elution diagram of gastrin-CCK-like immunoreactivity in extracts of antrum and brain of the frog, *Rana esculenta*. Both individual antra and brains as well as pools of antrum and brains from 30 frogs were frozen immediately in liquid nitrogen and stored at -70°C . Brains and antra were minced while frozen and boiled for 30 min in water (10 ml per g tissue). After homogenisation, the immunoreactivity of the supernatant was measured by three assays. (1) The first was CCK-assay involving antiserum no. 4698 (raised against partially purified porcine CCK-33, final dilution 1:20,000) and ^{125}I -CCK-33. This antiserum does not bind any of the known gastrins (sulphated or nonsulphated). With caerulein as standard, this assay measures 0.3 μg Eq per g cerebrum and 1.3 μg Eq per g antrum (wet weight). The second was an assay involving antiserum no. 2720 (ref. 4) (raised against synthetic human gastrin-17, final dilution 1:150,000) and ^{125}I -gastrin-17. The antiserum binds CCK-33 with a molar potency of 0.2 compared to gastrin-17. With synthetic human gastrin-17 as

standard, this assay measures 0.2 μg Eq per g cerebrum and 0.3 μg Eq per g antrum (wet weight). The third was a gastrin-assay involving antiserum no. 2604⁴ (raised against synthetic human gastrin-17, final dilution 1:200,000) and ^{125}I -gastrin-17. The antiserum binds CCK-33 with a potency of 0.002 compared to gastrin-17. With synthetic human gastrin-17 as standard, this assay measures 9.2 ng Eq per g cerebrum and 4.3 ng Eq per g antrum (wet weight). Antral and cerebral extracts were subsequently applied to Sephadex G-50 superfine columns (11 \times 1000 mm) eluted at 4°C with 0.02 M veronal, pH 8.4, containing 0.1% bovine serum albumin. The columns were eluted at a flow rate of 3.6 ml per h and fractions of 1.0 ml were collected. The immunoreactivity of each fraction was measured by the three assays. The columns were calibrated in separate gel filtrations with ^{125}I -albumin for indication of void volume (V_0), highly purified porcine CCK-33 ($V_{\text{CCK-33}}$), synthetic caerulein ($V_{\text{caerulein}}$) and $^{22}\text{NaCl}$ for indication of the total volume of the column (V_t). The extracts were also applied to Sephadex G-25 fine columns (15 \times 90 mm) eluted at 4°C with 0.25 M NH_4HCO_3 , pH 8.2, calibrated with the same markers as the Sephadex G-50 columns. Fractions of 1.0 ml were measured by the three assays. The data are not shown in the figure because they agreed with results obtained on Sephadex G-50 columns. They revealed also three major peaks in both antral and cerebral extracts. The first peak appeared in the void volume, the second was eluted immediately after the void volume (K_{av} : 0.19) and the third was eluted after the salt peak (K_{av} : 1.21) in a position identical to that of caerulein. Extracts of pools from 15 antra and hypothalami of the teleost *Gadus morrhua* were treated exactly as frog brains and antra. Samples of 0.25 g teleost hypothalamus and 1.89 g teleost antrum were cut in small pieces in the frozen state and extracted as described here. Using the three assays, the following quantities of CCK-gastrin-like immunoreactivity were measured: (1) 108 ng Eq caerulein per g hypothalamus and 106 ng Eq caerulein per g antrum (Ab 4698); (2) 34.5 ng Eq gastrin-17 per g hypothalamus and 25.2 ng Eq gastrin-17 per g antrum (Ab 2720); (3) 1.7 ng Eq gastrin per g hypothalamus and 4.9 ng Eq gastrin-17 per g antrum (Ab 2604). By gel filtration on Sephadex G-50 superfine columns as described here, the immunoreactivity of both the cerebral and the antral extracts was eluted in three major peaks of which the quantitatively largest in both extracts occurred in a position corresponding to that of caerulein.

shown that in amphibians, in contrast to mammals, the occurrence of a sulphated tyrosyl-group in position 7 (as counted from the COOH-terminus), and not in position 6 as in gastrin (Table 1), is very important for acid stimulatory potency⁹. Thus, physiological evidence alone suggests that caerulein-like molecules are frog 'gastrins'. Our demonstration of a molecule with the size and immunological properties of caerulein in frog antrum tends to support this view. It is interesting that examination of the amino acid sequences of caerulein and phyllocaerulein shows that whereas these peptides resemble CCK in most of their sequence, they have similarities to gastrin in both their NH_2 - and COOH-terminal portions (Table 1). Thus, whereas in mammals, birds and reptiles CCK- and gastrin-like molecules occur in separate cell systems, the same cell seems to be responsible for the production of gastrin-CCK-like molecules in amphibians and bony fishes. Although comparisons with hormones of phylogenetic ancestors are impossible, the observations in frog as well as teleost antrum suggest that gastrin and CCK have evolved from a common caerulein-like ancestral molecule. Immunocytochemical studies have revealed cells reactive with antisera against caerulein as well as glucagon in cyclostomes¹⁰. Although the question whether the glucagon and caerulein-like immunoreactivity resides in a single molecule remains to be resolved, it is possible that the two main

families of gut hormones evolved from a single precursor occurring in very primitive vertebrates.

CCK-like peptides have been demonstrated in the brain of mammals^{11,12}. It is interesting that similar molecules exist in the brains of amphibians and fishes and that they have the same characteristics as the peptides found in the

Table 1 Amino acid sequence of caerulein, phyllocaerulein and the COOH-terminal decapeptides of porcine cholecystokinin and sulphated gastrin

Cholecystokinin	-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH ₂
Caerulein	*Glu-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH ₂
Phyllocaerulein	*Glu-Glu-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH ₂
Gastrin	-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH ₂

*Glu, pyroglutamic acid.

gastrointestinal tract and skin. Immunocytochemical studies in frogs show that nerves containing these peptides are concentrated in the median eminence of the hypothalamus in much the same pattern as nerves containing known releasing or inhibiting factors to the pituitary (Fig. 2).

L.-I. LARSSON
J. F. REHFELD

Institute of Medical Biochemistry,
University of Aarhus,
DK-8000 Aarhus C, Denmark

Received 1 February; accepted 21 July 1977.

- ¹ White, A., Handler, P. & Smith, E. L. *Principles of Biochemistry* fifth edn 916 (McGraw-Hill Kogakusha, Tokyo, 1973).
- ² Dockray, G. J. *Gastroenterology* 72, 344-358 (1977).
- ³ Rehfeld, J. F. *First. int. Symp. Gastrointestinal Hormones*, Asilomar, Abstr. no. 127 (1976).
- ⁴ Rehfeld, J. F., Stadil, F. & Rubin, B. *Scand. J. clin. Lab. Invest.* 30, 221-232 (1972).
- ⁵ Rehfeld, J. F. *Biochim. biophys. Acta* 285, 364-372 (1972).
- ⁶ Gibson, R. G., Mihás, A. A., Colvin, H. W. & Hirschowitz, B. I. *Proc. Soc. exp. Biol. Med.* 53, 284-288 (1976).
- ⁷ Anastasi, A., Erspamer, V. & Endean, R. *Arch. Biochem.* 125, 57-68 (1968).
- ⁸ Anastasi, A. et al. *Br. J. Pharmac.* 37, 198-206 (1969).
- ⁹ Negri, L. & Erspamer, V. *Naunyn-Schmiedeberg's Arch. Pharmac.* 277, 401-412 (1973).
- ¹⁰ Van Noorden, S. & Pearce, A. G. E. *Gen. comp. Endocr.* 23, 311-324 (1974).
- ¹¹ Vanderhaeghen, J. J., Signeau, J. C. & Gepts, V. *Nature* 257, 604-605 (1975).
- ¹² Dockray, G. J. *Nature* 264, 568-570 (1976).
- ¹³ Larsson, L.-I., Håkanson, R., Sjöberg, N.-O. & Sundler, F. *Gastroenterology* 68, 1152-1159 (1975).
- ¹⁴ Larsson, L.-I., Rehfeld, J. F., Håkanson, R. & Sundler, F. *Nature* 262, 609-610 (1976).
- ¹⁵ Sternberger, L. A. *Immunocytochemistry* (Prentice-Hall, Englewood Cliffs, New Jersey, 1974).

Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism

FAMILIAL hypogonadism in man, due to an isolated deficiency of gonadotrophin secretion, has been well documented¹⁻⁶, but difficult to investigate because of the lack of a suitable animal model. We report here the genetic and endocrinological background of a mutant strain of mouse in which the testes and ovaries fail to develop postnatally. The primary cause of this seems to be a deficiency in hypothalamic gonadotrophin-releasing hormone (GnRH) with a consequent reduction in pituitary content and circulating levels of luteinising hormone (LH) and follicle-stimulating hormone (FSH). By analogy with the Brattleboro rat (genetic defect in vasopressin synthesis) this mutant should prove useful for studying the synthesis of hypothalamic releasing hormones as well as the role of the hypothalamic-gonadotrophin system in sexual differentiation, puberty, folliculogenesis and spermatogenesis. The mutant has been named hypogonadal, symbol *hpg*.

The mutation was found at Harwell, segregating out from matings between three pairs of full sibs. Each of these was heterozygous for the Robertsonian translocation, Rb(1.3)1Bnr, which is of tobacco mouse (*Mus poschiavinus*) origin; and the matings represented the final step of establishing the translocation on a house mouse genetic background after 10 generations of backcrossing. The house mouse used throughout the crosses was the F₁ hybrid between two inbred strains, C3H/HeH and 101/H. Since the mutation has not been found in either inbred strain, or in the original mixed tobacco mouse/house mouse stock⁷ which carried the translocation, we have concluded that the mutation must have arisen in one of the animals of the latest backcross generations.

Breeding records generally indicated an autosomal recessive inheritance. Thus, *hpg* mice (both male and female) segregated out from matings that produced the mutant in accordance with the 25% expectation for this mode of inheritance (Table 1). In addition, all animals (both male and female) that were capable of producing *hpg* offspring in intercrosses, failed to do so when tested

in outcrosses to unrelated mice. When the progeny of such outcrosses were mated together, 7 out of 22 pairs again produced the mutant. This frequency is not significantly different from the 25% expected if *hpg* is inherited as an autosomal recessive. The only evidence discordant with this form of inheritance was the apparent low frequency of heterozygotes from matings that had been shown to be capable of producing the mutant. Two-thirds of the phenotypically normal progeny of such matings should be heterozygotes and, since they cannot be distinguished from their homozygous normal siblings, the expectation of two heterozygotes being mated together in random intercrosses should be 0.44. In fact, although at

Table 1 Segregation of *hpg* among 17 matings producing the mutant

Normal ♀♀	Mutant ♀♀	Progeny			
		NC ♀♀	Normal ♂♂	Mutant ♂♂	NC ♂♂
219	55	35	197	69	14

NC, Not classified for *hpg*; this includes only pre-weaning loss for males but both pre- and post-weaning loss for females.

least 20 offspring were checked per mating, only 10 out of 47 intercrosses of this origin were capable of producing *hpg* offspring. This is significantly different from the expectation. The reason for this discrepancy is not understood.

The segregation of the Robertsonian translocation in matings producing *hpg* has not been followed, but many of the animals used in the early crosses have proved to be chromosomally normal. There is therefore little possibility that *hpg* is linked to Rb(1.3)1Bnr, and this virtually rules out the proximal regions of chromosomes 1 and 3 as the location of *hpg* in the linkage map. No other mutants similar to *hpg* and, therefore possibly allelic, are yet known in the mouse.

Hypogonadal males can readily be distinguished from their normal male siblings by external examination. The penis is smaller than normal, the scrotum is undeveloped and the anogenital distance is much shorter than in normal males. Internally, all male reproductive organs are present

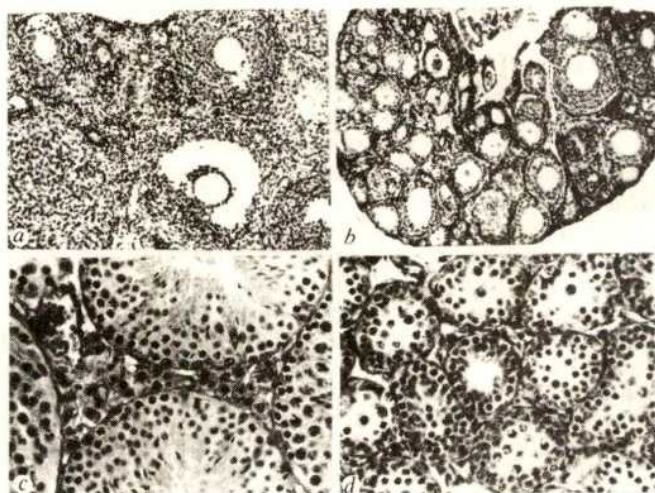


Fig. 1 a, Micrograph of ovary of 71-d-old normal female at same magnification as b. Well developed follicles and a corpus luteum can be seen. b, Micrograph of ovary of 71-d-old *hpg* female. None of the follicles shows any obvious signs of antrum formation. In most cases, the oocyte is surrounded by two layers of granulosa cells. c, Micrograph of testis of 71-d-old normal male at same magnification as in d. Here, spermatogenesis is complete, and the interstitial tissue is abundant and active. d, Micrograph of testis of 71-d-old *hpg* male. The failure of full development of spermatogenesis and the undeveloped state of the interstitial cells can be seen.

Table 2 Change of organ weight (mg) with age in normal and *hpg* mice of both sexes

Males	Age (d)	Testes		Seminal vesicles		Adrenal glands	
		Normal	Mutant	Normal	Mutant	Normal	Mutant
	31	110.7 ± 4.8	5.7 ± 0.4	12.4 ± 2.1	0.7 ± 0.1	3.5 ± 0.4	3.3 ± 0.4
	39	147.7 ± 16.9	—	48.4 ± 8.2	—	4.5 ± 1.2	—
	47	169.9 ± 0.7	4.9 ± 1.2	92.5 ± 6.6	1.0 ± 0.3	5.8 ± 0.5	4.2 ± 1.8
	65	158.7 ± 10.9	5.1 ± 0.4	152.4 ± 16.8	0.7 ± 0.2	4.5 ± 0.1	4.4 ± 1.7
	71	175.0 ± 3.1	4.3 ± 0.4	126.0 ± 42.3	1.4 ± 0.5	6.1 ± 1.0	4.2 ± 0.9
	89	187.6 ± 14.2	4.9 ± 0.5	182.9 ± 13.5	4.2(1)	4.0 ± 0.2	3.6 ± 0.4
	97	152.0 ± 22.3	5.6(2)	94.9 ± 28.7	1.8(2)	4.9 ± 0.4	5.3 ± 1.6
	112	169.9 ± 21.6	3.4 ± 1.3	132.0 ± 12.3	0.5 ± 0.3	5.6 ± 0.9	5.0 ± 0.9
Females		Ovaries		Uteri		Adrenal glands	
		Normal	Mutant	Normal	Mutant	Normal	Mutant
	31-42	4.5 ± 0.6	0.4 ± 0.1	42.3 ± 7.5	3.6 ± 0.7	3.7 ± 0.3	3.2 ± 0.5
	71	7.7 ± 2.4	0.2 ± 0.1	68.8 ± 23.0	3.5 ± 0.9	4.1 ± 1.0	5.2 ± 0.4
	88	8.5 ± 1.1	0.4 ± 0.2	107.0 ± 18.0	2.1 ± 0.4	3.1 ± 0.2	2.5(2)
	100-110	14.5 ± 2.6	0.5(2)	118.4 ± 14.9	2.9 ± 0.4	8.0 ± 0.7	6.5 ± 0.3

Means based on 3-10 animals per group, unless indicated by number in parentheses.

but immature. The testes, located in the abdomen, are very small.

The effect in females is similar. Externally, *hpg* females resemble normal females but can be distinguished with reasonable accuracy by the failure of the vagina to open fully. Internally, a thread-like uterus can be seen, and the ovaries are so small as to be distinguished only with difficulty.

Groups of three to ten normal and *hpg* mice of both sexes were killed at different ages, and body, gonadal, accessory sex organ and adrenal weights were recorded.

showed early stages of antrum formation (Fig. 1b). No luteal tissue was seen and interstitial tissue seemed to be atrophic.

Tables 3 and 4 show that the pituitary content of LH and FSH was markedly reduced in the mutant, although still assayable; the levels of hypothalamic GnRH were exceedingly low or undetectable. That the hypogonadotropism/hypogonadism is due primarily to a lesion involving GnRH synthesis was supported by the fact that plasma LH could be increased in *hpg* mice by injecting synthetic GnRH intravenously, but not by electrically

Table 3 Hypothalamic GnRH, pituitary and plasma gonadotrophic hormone levels during development in normal and *hpg* males

Age (d)	Hypothalamic GnRH (pg)		Pituitary LH (ng per pit.)		Pituitary FSH (ng per pit.)		Plasma FSH (ng per ml)	
	Normal	Mutant	Normal	Mutant	Normal	Mutant	Normal	Mutant
31	499 ± 264	7 ± 2	418 ± 110	25 ± 12	5,380(2)	389(2)	509 ± 159	54 ± 7
39	1,033 ± 330	—	892 ± 155	—	7,745 ± 1462	—	385 ± 92	—
47	1,446 ± 406	18 ± 3	1,304 ± 565	47 ± 9	13,512 ± 4696	218 ± 57	636 ± 355	65 ± 25
61-65	1,278 ± 303	5 ± 0.1	2,126 ± 237	67 ± 10	18,050 ± 1962	296 ± 44	656 ± 80	61 ± 5
71	1,668 ± 343	9 ± 2	1,983 ± 228	124 ± 22	18,286 ± 4273	313 ± 24	—	—
87	1,264 ± 313	10 ± 4	2,403 ± 46	74 ± 15	24,214 ± 2894	297 ± 48	478 ± 63	54 ± 3
97-99	1,814 ± 629	6 ± 1	1,337 ± 602	56 ± 22	18,051 ± 10285	199 ± 46	—	—
112-115	2,230 ± 251	12 ± 2	2,207 ± 549	109 ± 20	26,498 ± 6690	402 ± 76	—	—

GnRH was measured according to the procedures outlined in refs 10 and 11; gonadotrophins were measured according to the procedures outlined in refs 11-13. For each hormone, preliminary studies demonstrated parallelism between inhibition of binding curves produced by serial dilutions of the unknown and the appropriate standard. Standards were synthetic GnRH (ICI), NIH LH-S18, and NIAMD rat FSH-RPI. Means based on 3-10 animals per group unless indicated by number in parentheses.

Table 2 shows that the development of the testes, seminal vesicles, ovaries, and uteri of the normal mice contrasts with the total lack of change with age in the *hpg* animals.

Histological examination of the *hpg* testes revealed that in the majority of seminiferous tubules, spermatogenesis was rarely advanced beyond the diplotene stage. Interstitial tissue was scanty and appeared to be inactive (Fig. 1d). The ovaries contained mainly undeveloped follicles, and the majority of follicles which had undergone growth seemed to be arrested at the pre-antral phase; a few

stimulating the hypothalamus.

A number of parameters have been examined to determine whether other hypothalamo-hypophysial systems are affected in the *hpg* mutant. Body weights (g) of *hpg* females were similar to those of their normal female sibs either at 3 weeks of age (10.54 ± 0.13 , $n=19$ compared with 10.46 ± 0.66 , $n=63$) or at 6 weeks of age (19.51 ± 0.16 , $n=19$ compared with 19.80 ± 0.6 , $n=63$). Although *hpg* males were no smaller than their normal male sibs at 3 weeks of age (10.09 ± 0.16 , $n=26$ compared with

Table 4 Hypothalamic GnRH, pituitary and plasma gonadotrophic hormone levels during development in normal and *hpg* females

Age (d)	Hypothalamic GnRH (pg)		Pituitary LH (ng per pit.)		Pituitary FSH (ng per pit.)		Plasma FSH (ng per ml.)	
	Normal	Mutant	Normal	Mutant	Normal	Mutant	Normal	Mutant
31-36	1,207 ± 364	14(2)	596 ± 41	308(1)	2,207 ± 300	200(1)	—	—
41	831 ± 150	7 ± 2	591 ± 108	154 ± 51	1,098 ± 132	311 ± 89	—	—
71-72	831 ± 289	9 ± 5	514 ± 113	93 ± 5	1,040 ± 464	489 ± 107	211 ± 55	67 ± 6
83	623 ± 198	< 5	601 ± 236	136 ± 39	1,240 ± 358	548 ± 109	220 ± 89	64 ± 5
87-88	1,367 ± 518	9 ± 3	554 ± 129	215 ± 129	1,781 ± 284	687 ± 253	205 ± 34	51 ± 8
100	974 ± 440	4 ± 1	648 ± 163	73 ± 10	1,439 ± 213	378 ± 64	236 ± 87	60 ± 6
110	230 ± 42	9 ± 2	220(2)	—	779(2)	824(2)	—	—

Means based on 3-10 animals per group unless indicated by number in parentheses.

Table 5 Pituitary prolactin and ACTH in adult normal and *hpg* animals of both sexes

	Male		Female	
	Normal	Mutant	Normal	Mutant
Pituitary prolactin (ng/pit.)	141.6±16.0	54.7±9.7	269.8±89.3	92.3±6.6
Pituitary ACTH (ng/pit.)	147.4±24.3	73.6±14.2	94.0±8.7	87.4±4

Prolactin and ACTH were measured using the NIAMD rat assay kit and the method described in ref. 11, respectively. The standards were NIAMD rat prolactin-RP1 and III IWS ACTH (Mill Hill, London, UK). Means based on 5–8 animals per group.

9.85±0.12, *n*=51), by 6 weeks of age, however, they were significantly smaller (17.77±0.22, *n*=23 compared with 22.69±0.10, *n*=51), and their body weights more closely approximated those of their normal female siblings (19.80±0.06, *n*=63). This probably reflects a difference between the two groups in testosterone secretion, if it were due to a defect in growth hormone in the mutant, a difference between body weights would also be expected to occur in the female animals.

Histological studies of the thyroids from *hpg*, and normal mice showed no obvious differences in cell height of the follicular epithelium. This suggests that TSH production may be unaffected in the mutant.

As the foetal adrenal X zone is known to disappear at puberty in the male and during the first pregnancy in the female⁸, it is not surprising that histological examination indicated its presence in *hpg* males, and in both *hpg* and normal virgin females. As there was no significant difference in the width of the zona fasciculata/reticularis between mutant and normal males and females, adrenal function in the mutant would seem to be normal. There were, however, significant intergroup differences in pituitary adrenocorticotrophic hormone (ACTH) and prolactin content in the older animals (Table 5). Although we cannot exclude partial defects in the hypothalamo–ACTH and hypothalamo–prolactin axes, these differences probably reflect the effect which the hypothalamic–pituitary–gonadal system exerts on the other systems⁹.

Gross examination of the *hpg* mice has revealed no associated central nervous or cranio–facial abnormalities. This, together with the genetic findings, suggests that the mutant is akin to Ewer's series of human subjects³ who had familial monotrophic pituitary insufficiency transmitted as an autosomal recessive.

We thank Lesley Clifford, Vivienne Langford and Shirley Lewis for assistance; Drs G. D. Niswender, L. E. Reichert, A. F. Parlow, H. Gregory, J. Gormley and A. L. Walpole and the NIAMDD for radioimmunoassay materials; and the MRC for support. C.A.I. is a MRC scholar.

B. M. CATTANACH

MRC Radiobiology Unit,
Harwell, Didcot, Oxfordshire, UK

CAROL A. IDDON
H. M. CHARLTON
SHARON A. CHIAPPA
G. FINK

Department of Human Anatomy,
South Parks Road,
Oxford, UK

Received 28 June; accepted 4 August 1977.

- Kallman, F. J., Schoenfeld, W. A. & Barrera, S. E. *Am. J. ment. Defic.* **48**, 203–236 (1944).
- Nowakowski, H. & Lenz, W. *Rec. Progr. Horm. Res.* **17**, 53–89 (1961).
- Ewer, R. W. *J. clin. Endocr. Metab.* **28**, 783–788 (1968).
- Naftolin, F., Harris, G. W. & Bobrow, M. *Nature* **232**, 496–497 (1971).
- Santen, R. J. & Paulsen, C. A. *J. clin. Endocr. Metab.* **36**, 47–54 (1973).
- Boyar, R. M., Wu, R. H. K., Kapen, S., Hellman, L., Weitzman, E. D. & Finkelstein, J. W. *J. clin. Endocr. Metab.* **43**, 1268–1275 (1976).
- Cattanach, B. M., Williams, C. E. & Bailey, H. *Cytogenetics* **11**, 412–423 (1972).
- Chester-Jones, I. *Br. med. Bull.* **11**, 156–158 (1955).
- Brown-Grant, K. in *Regulation and Control in Living Systems* (ed. Kalmus, H.) 176–255 (Wiley, London, 1966).

- Nett, T. M., Akbar, A. M., Niswender, G. D., Hedlund, M. J. & White, W. F. *J. clin. Endocr. Metab.* **36**, 880–885 (1973).
- Chiappa, S. A. & Fink, G. J. *Endocr.* **72**, 195–210 (1977).
- Niswender, G. D., Midgley, A. R., Jr, Montoc, S. E. & Reichert, L. E. Jr *Proc. Soc. exp. Biol. Med.* **128**, 807–811 (1968).
- Daane, T. A. & Parlow, A. F. *Endocrinology* **88**, 653–663 (1971).

Nigral and striatal dopamine release under sensory stimuli

SEVERAL groups have demonstrated the presence of a dopamine (DA)-sensitive adenylate cyclase in the substantia nigra. This adenylate cyclase is associated with postsynaptic dopaminergic receptors^{1–3}, supporting the hypothesis of a dendritic release of DA originally suggested by Björklund and Lindvall⁴. Other reports provided further arguments in favour of this hypothesis. Exogenous ³H-DA, taken up in slices of the rat substantia nigra, was shown to be released by potassium through a calcium-dependent process⁵. Furthermore, spontaneous and evoked release of ³H-DA has been demonstrated *in vivo* in the cat substantia nigra⁶. The increase of ³H-DA release in the substantia nigra induced by amphetamine (10^{–6} M) or benztropine (10^{–6} M) is associated with a reduction of the transmitter release from nerve terminals in the ipsilateral caudate nucleus, suggesting a decreased activity of the nigrostriatal dopaminergic⁷ neurones. A similar phenomenon was seen in the caudate nucleus when DA (10^{–7} M) was directly introduced into the substantia nigra⁸. We present here a study of the effects of unilateral sensory stimulations on the release of DA from nerve terminals and dendrites of the two nigrostriatal dopaminergic pathways. Previous studies⁹ revealed an increase in the levels of homovanillic acid in the perfusates of the cat lateral ventricle during electrical stimulation of the paws.

Groups of cats anaesthetised with halothane (2%) were implanted with four push–pull cannulae to simultaneously superfuse both caudate nuclei and both substantia nigrae using techniques detailed elsewhere^{10,11}. Purified L-3,5-³H-tyrosine (40 Ci mmol^{–1}), dissolved in an artificial cerebrospinal fluid (40 μCi ml^{–1}), was introduced in each push–pull cannula at a rate of 300 μl per 10 min using peristaltic pumps. ³H-DA released in successive 10-min fractions of superfusates was isolated from L-3,5-³H-tyrosine and ³H-metabolites by ion-exchange chromatography on Amberlite CG50 and the adsorption on alumina¹⁰. At steady-state level the quantities of ³H-DA released in the caudate nuclei and the substantia nigrae were 0.5 to 0.8 nCi per 10 min and 0.1–0.6 nCi per 10 min, respectively. They represented about 5–40 times the blank value which was identical to the counter background.

In a first experiment, a pair of electrodes was inserted into the paw of the right forelimb and a 10-min stimulation was carried out 3 h after onset of the superfusions using monophasic square pulses delivered by a Grass S 44 stimulator (4–6 V, 0.5 ms, 0.25 Hz) through a stimulus isolation unit. The intensity of the stimulus was adjusted in each experiment just below the threshold of the apparent muscle contraction. The 10-min stimulation of the paw of the right forelimb induced a significant activation (170%) of ³H-DA release in the contralateral substantia nigra when compared with the release of ³H-DA determined in corresponding fractions of control animals. This activation was associated

with a simultaneous reduction (30%) of the ^3H -transmitter release in the corresponding caudate nucleus which lasted for 1 h at least. An opposite pattern was observed in the side ipsilateral to the stimulation—the release of ^3H -DA was reduced in the substantia nigra and enhanced in the caudate nucleus (Fig. 1).

Similar results were obtained in a second experiment in which a visual sensory stimulation was substituted for the somatic electrical stimulation. In this case, the left eye was obturated and the right one was exposed for 10 min to a series of light flashes delivered with a frequency of 0.2 Hz. The two dopaminergic pathways reacted in opposite ways. In the side contralateral to the stimuli, the intense activation of ^3H -DA release in the substantia nigra was concomitant to the reduction of the ^3H -transmitter release from dopaminergic terminals and the reverse effects were seen in the ipsilateral side (Fig. 2).

The release of ^3H -DA into the substantia nigra can easily be modulated by physiological stimuli. As originally postulated⁴⁻⁶, it may originate from dopaminergic dendrites and not from recurrent axonal collaterals; the changes in the nigral release of ^3H -DA were always in the opposite direction to those observed in striatal terminals. This extends our previous data which indicated some differences in the characteristics of the release process of ^3H -DA in the substantia nigra and in the caudate nucleus⁶. Indeed, tetrodotoxin, a neurotoxin which blocks sodium channels, did not reduce the release of ^3H -DA in the substantia nigra in

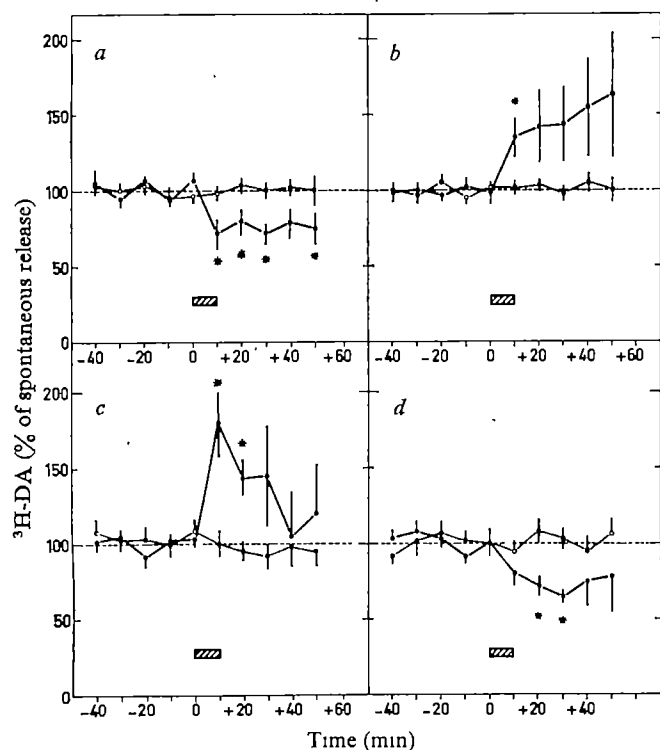


Fig. 1 Effects of unilateral somatic electrical stimulation on the release of ^3H -dopamine from the two caudate nuclei and the two substantia nigrae. Four push-pull cannulae were simultaneously implanted in the left (a) and the right (b) caudate nuclei and in the left (c) and the right (d) substantia nigrae. The four structures were perfused with an artificial cerebrospinal fluid containing L-3,5- ^3H -tyrosine. ^3H -dopamine was estimated in 10-min successive superfuse fractions. The paw of the right forelimb was stimulated (hatched bars) for 10 min using a pair of electrodes (monophasic square pulses, 4–6 V, 0.5 ms, 0.25 Hz). In each animal and for each cannula, ^3H -dopamine in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the stimulation. Data are the mean \pm s.e.m. of results obtained with five animals (a, b) or four animals (c, d). *, $P < 0.05$ when compared with corresponding control values (○) obtained in five non-stimulated animals.

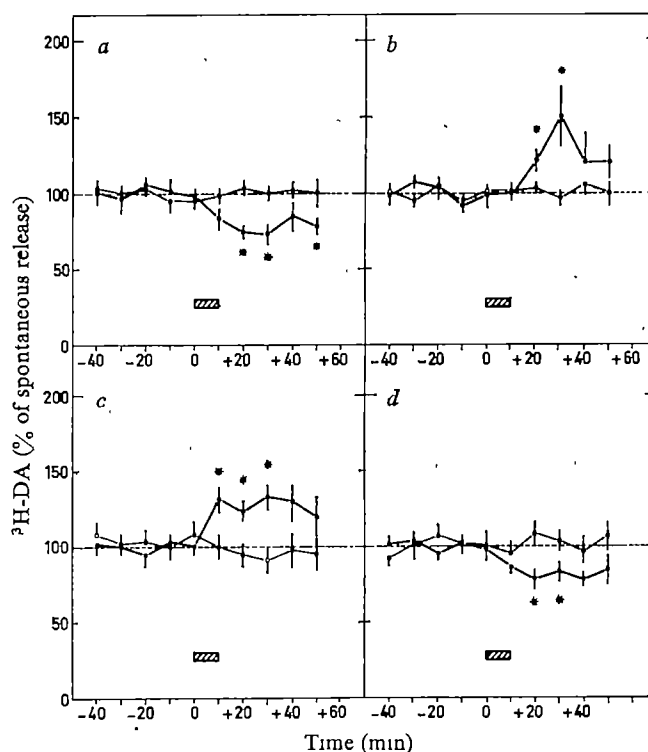


Fig. 2 Effects of unilateral visual stimulation on the release of ^3H -dopamine from the two caudate nuclei and the two substantia nigrae. Animals were treated as described in Fig. 1, except that the peripheral stimulation consisted of a series of light flashes (10 min, 0.2 Hz) directed to the right eye during the obturation of the left one. Data are calculated and expressed as in Fig. 1. *, $P < 0.05$ when compared with corresponding control values (○) obtained in five non-stimulated animals. a, Left, and b, right caudate nuclei. c, Left, and d, right substantia nigrae. Results for a–d are from five animals.

contrast to that observed in the caudate nucleus.

In the side contralateral to the somatic or visual stimulation, the activation of the dendritic release of ^3H -DA was always associated with a decreased activity of the dopaminergic neurones as indicated by the reduction of the transmitter release from nerve terminals. This phenomenon can be compared with the reduction of the activity of these neurones induced by the nigral application of DA⁴ (10^{-7} M), amphetamine⁷ (10^{-6} M) or benztropine⁷ (10^{-6} M), treatments which enhance the synaptic levels of the amine in the substantia nigra⁶. It may be related to the presence of autoreceptors on dopaminergic cells, first suggested on the basis of electrophysiological studies¹². Therefore in physiological states, it can be postulated that the activity of the dopaminergic neurones is inversely correlated to the extent of the dendritic release of the transmitter.

In a previous study¹³, we demonstrated the occurrence of a reciprocal control of the activity of the two nigrostriatal dopaminergic pathways. Briefly, treatments which selectively reduced the activity of one nigrostriatal dopaminergic system activated the release of DA from the nerve terminals of the contralateral dopaminergic neurones. This was observed shortly after the electrocoagulation of the left substantia nigra and during its superfusion with DA (10^{-7} M), amphetamine (10^{-6} M) or benztropine (10^{-6} M). In these experimental conditions, the existence of opposite changes in the release of ^3H -DA from the nerve terminals of the two caudate nuclei was confirmed. If, as already discussed, the activity of the dopaminergic neurones is dependent on the extent of the dendritic release, it can be assumed that the opposite fluctuations in ^3H -DA release from nerve terminals are related to the reverse opposite

changes in the transmitter release occurring at the substantia nigra level.

In our initial demonstration of the reciprocal control of the activity of the two dopaminergic pathways¹³, the opposite changes in activity of these systems were triggered by physical or pharmacological manipulations of the dopaminergic neurones in one substantia nigra. Therefore, the marked opposite variations in the activity of the two systems seen under unilateral sensory stimulation could be initiated by inputs preferentially delivered on one pathway. But, nearly identical evoked potentials have been recorded in the two substantia nigrae during unilateral stimulation of the limbs¹⁴. Similarly, bilateral responses were also found in the caudate nuclei during unilateral somatic stimulation or unilateral visual stimulation¹⁵. The neuronal circuits involved in the opposite changes in the activity of the two dopaminergic pathways are not known, however.

Our results support the concept of the involvement of the nigrostriatal dopaminergic neurones in the integration of multisensory inputs¹⁶. In fact, unilateral degeneration of the ascending dopaminergic neurones is known to produce a pronounced deviation in movements and posture on the side ipsilateral to the lesion and a pronounced deficiency in the ability to orientate toward sensory stimuli which are presented to the contralateral side of the body¹⁶. The demonstration of the dysmetric activity of the two dopaminergic pathways in response to unilateral sensory information should be of heuristic value for the understanding of the role of the extrapyramidal system in sensory motor integration.

This work was supported by grants from DRME (76/329), INSERM (contrat libre 75.5.153.6 and ATP 29.76.61) and la Société des Usines Chimiques Rhône-Poulenc. We thank Mrs M. L. Kemel for technical assistance.

A. NIEOULLON
A. CHÉRAMY
J. GLOWINSKI

Groupe NB, INSERM U.114,
Collège de France,
11, place Marcelin Berthelot,
75231 Paris Cedex 05

Received 9 May; accepted 28 June 1977.

- 1 Gale, K., Guidotti, A. & Costa, E. *Science* **195**, 503–505 (1977).
- 2 Premont, J. et al. *FEBS Lett.* **68**, 99–104 (1976).
- 3 Kébabian, J. W. & Greengard, P. *Science* **174**, 1346–1349 (1971).
- 4 Björklund, A. & Lindvall, O. *Brain Res.* **83**, 531–537 (1975).
- 5 Geffen, L. B., Gessell, T. M., Cuervo, A. C. & Iversen, L. L. *Nature* **260**, 258–260 (1976).
- 6 Nieoullon, A., Chéramy, A. & Glowinski, J. *Nature* **266**, 375–377 (1977).
- 7 Chéramy, A., Nieoullon, A. & Glowinski, J. *Interactions Among Putative Neurotransmitters in the Brain* (eds Garattini, S., Pujol, J. F. & Samanin, R.) (Raven, New York, in the press).
- 8 Chéramy, A., Nieoullon, A., Michelot, R. & Glowinski, J. *Neurosci. Lett.* **4**, 105–109 (1977).
- 9 Portig, P. J. & Vogt, M. J. *Physiol., Lond.* **204**, 687–715 (1969).
- 10 Nieoullon, A., Chéramy, A. & Glowinski, J. *J. Neurochem.* **28**, 918–928 (1977).
- 11 Chéramy, A., Nieoullon, A. & Glowinski, J. *Naunyn-Schmiedeberg Arch. exp. Path. Pharmacol.* **297**, 31–37 (1977).
- 12 Bunney, B. S., Aghajanian, G. K. & Roth, R. H. *Nature new Biol.* **245**, 123–125 (1973).
- 13 Nieoullon, A., Chéramy, A. & Glowinski, J. *Science* (in the press).
- 14 Féger, J., Ohye, C., Jacquemin, J. & Martin, A. J. *Physiol., Paris* **69**, 247 A (1974).
- 15 Albe-Fessard, D., Oswald-Cruz, E. & Rocha-Miranda, C. *Electroencephal. clin. Neurophysiol.* **12**, 405–420 (1960).
- 16 Ljungberg, T. & Ungerstedt, U. *Exp. Neurol.* **53**, 585–600 (1976).

Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures

THE clinical actions of benzodiazepines—relief of anxiety, suppression of convulsions, and relaxation of skeletal muscle—suggest that they enhance inhibition in the central nervous system (CNS) but their mechanism of action is unknown. Indirect evidence^{1,2} indicates that benzodiazepines augment the action of γ -aminobutyric acid (GABA), a putative inhibitory transmitter. For example, diazepam increases presynaptic inhibition in the spinal cord^{3,4}, a type of inhibition that may reflect GABA action on primary afferent terminals⁵.

In addition, chlordiazepoxide mimics GABA in decreasing the firing rate of rat brain neurones⁶. These, and other, actions of benzodiazepines are blocked by the GABA antagonists, picrotoxin and/or bicuculline. Precise interpretation of such indirect evidence is difficult in view of the complexity of the intact brain, and the picture is far from clear. One physiologic study has concluded that benzodiazepines are potent GABA antagonists⁷. Another study based on benzodiazepine displacement of ³H-strychnine binding to CNS membranes suggested that these drugs may interact with glycine receptors rather than GABA receptors⁸. We have analysed the effect of chlordiazepoxide (CDPX) on GABA and glycine responses in spinal cord cell cultures, where individual neurones can be visualised and tested directly. Our results suggest that CDPX selectively facilitates the action of GABA, whether exogenously applied or synaptically released, by a direct action on the postsynaptic membrane.

Cells were dissociated from 4–7-d embryonic chick spinal cords, and plated in collagen-coated 35-mm tissue culture dishes, as previously described⁹. Electrophysiologic experiments were performed on the stage of an inverted microscope equipped with phase-contrast optics. The cells were perfused with oxygenated nutrient medium and temperature (34–36 °C) and pH (7.4) were carefully controlled. Individual neurones were penetrated for intracellular recording with 4 M potassium acetate or 3 M potassium chloride filled microelectrodes ($R = 50$ –100 M Ω). Membrane conductance was monitored by injecting pulses of inward current through the recording electrode (connected in a bridge circuit). Drugs were applied by pressure ejection from blunt tip (3–5 μ m) pipettes; this technique allows application of known drug concentrations. GABA and glycine were also applied by iontophoresis from fine tip (< 0.5 μ m) double barrel pipettes.

Recordings were taken from over 200 cells studied 11–40 d after plating. The cell bodies of neurones selected ranged between approximately 20 μ m and 50 μ m in diameter. All neurones tested were sensitive to GABA and glycine. Both drugs were applied sequentially to 38 cells. With a potassium acetate electrode, and a good penetration (resting potential -70 mV to -80 mV), application of either drug produced a small depolarising response associated with a marked increase in membrane conductance.

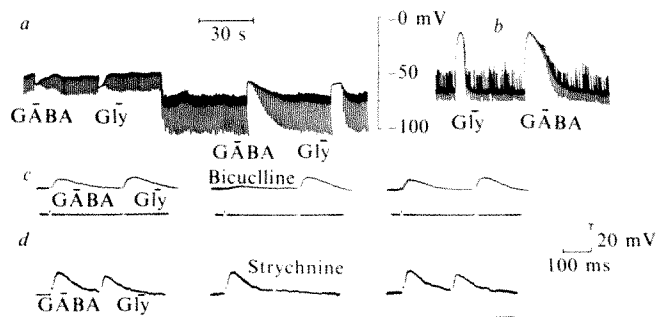


Fig. 1 GABA and glycine (Gly) responses. *a* and *b* are slow-speed tracer records of responses to pressure ejected GABA (1 mM) and glycine (1 mM). Horizontal lines below the records indicate the pressure pulse duration. Vertical lines are electronic potentials produced by inward current pulses (1 nA for 100 ms in *a*, 0.26 nA for 100 ms in *b*): their length is proportional to the cell input resistance. In (*a*) the cell was penetrated with a potassium acetate electrode, and GABA and glycine responses were evoked at two membrane potentials (note the vertical scale). Both responses reversed polarity at about -60 mV. *b* shows responses recorded in another cell with a potassium chloride electrode. After Cl^- loading, both drugs depolarised the cell to about -10 mV. Baseline irregularity in these and other records reflect spontaneous synaptic activity. *c* and *d* are oscilloscope traces showing fast GABA and glycine responses (potassium chloride recording electrode) following iontophoresis from a double barrel pipette. Iontophoretic current pulses are shown below each trace. The paired responses were evoked before (left), during (middle) and 1 min after (right) pressure ejection of the indicated antagonists. The voltage calibration bar also indicates 0.4 μ A in *c* and 1.0 μ A in *d*. Bicuculline was added at 2.2×10^{-4} M and strychnine at 10^{-5} M.

These voltage responses reversed direction when the cell was depolarised above -50 mV to -60 mV (Fig. 1a). As expected from work in other systems (for review see ref. 10), the increase in membrane conductance induced by GABA or glycine reflects primarily an increase in permeability to Cl^- ions. When KCl filled recording electrodes were used, the reversal potential for both drugs was shifted towards 0 mV (Fig. 1b). Pressure ejection of bicuculline or strychnine attenuated GABA and glycine responses, respectively (Fig. 1c, d).

Pressure ejection of CDPX immediately produced a striking potentiation of the GABA response, while having no effect on the glycine response (Fig. 2a). The potentiation was rapidly reversible after termination of the CDPX pulse, but it was maintained for

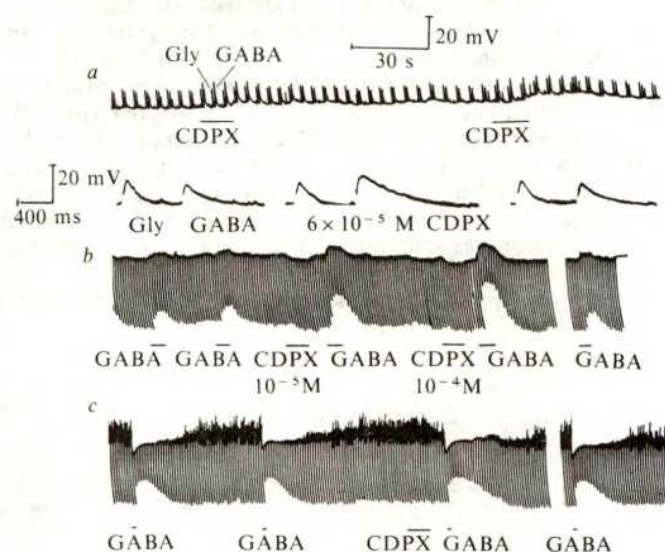


Fig. 2 CDPX potentiation of GABA responses. In *a*, paired iontophoretic glycine and GABA responses are shown on a slow speed tracer (top). Note the selective potentiation of the GABA potential. The same phenomenon is shown in fast oscilloscope sweeps (below) taken before, during and after pressure ejection of CDPX ($60 \mu\text{M}$). Note that different sets of calibration bars apply to top and bottom records. The late depolarisation seen here subsequent to CDPX application (top) probably reflects potentiation of GABA that leaks from the closely applied iontophoretic pipette. It was never observed in the absence of a GABA pipette. *b*, A trace illustrating that potentiation of submaximal GABA responses depends on CDPX concentration. $6 \mu\text{M}$ GABA was applied by pressure ejection at the indicated times. The small hyperpolarisation coincident with CDPX ejection is an artefact. Spontaneous synaptic potentials were suppressed in this experiment with tetrodotoxin ($3.4 \times 10^{-8} \text{ M}$). The break in the record represents a 2-min interval. *c*, Trace illustrating that a maximum (1 mM) GABA response is prolonged by CDPX (10^{-4} M) but the peak potential change and conductance increase are unchanged. Break in baseline, 4 min. Inward current pulses, 0.6 nA for 100 ms in *b*; 0.3 nA for 100 ms in *c*. Calibration bars in *a* (top) apply also to *b* and *c*. Potassium chloride recording electrode was used in *a*; potassium acetate in *b* and *c*.

several hours in the continued presence of CDPX. CDPX alone, at potentiating concentrations (10^{-7} M to 10^{-4} M), had no effect on the resting membrane potential or membrane conductance. Potentiation of GABA responses increased with increasing CDPX concentration between 10^{-7} M and 10^{-4} M (Fig. 2b), with an ED_{50} (50% effective concentration) of approximately $5\text{--}10 \mu\text{M}$ (44 cells). (The conductance caused by GABA was calculated assuming a shunt was inserted in parallel with the resting membrane conductance, and the CDPX potentiation was expressed as a percentage increase in this specific GABA conductance.) The CDPX pulse preceded the GABA pulse (Fig. 2b), so the effective CDPX concentration probably is lower than the pulse concentration, and these estimates of the ED_{50} must be considered upper limits. CDPX increased the magnitude of submaximal GABA responses.

The response to a maximal dose of GABA (1 mM) was prolonged, but the peak response was unchanged (13 cells) (Fig. 2c).

CDPX potentiation of GABA chemosensitivity might be due to: (1) blockade of GABA uptake; (2) induction of GABA release; or (3) a direct effect on the postsynaptic membrane. Blockade of uptake is unlikely. Our transport studies failed to demonstrate an effect of 1 mM CDPX on ^3H -GABA uptake in sister cultures. Also, 1 mM L-2,4-diaminobutyric acid, a known inhibitor of neuronal GABA uptake¹¹, did not alter the potentiating effect of CDPX (five cells). Induction of GABA release cannot account for the potentiation of large GABA responses in the absence of a GABA-mimetic effect. A direct postsynaptic effect on GABA receptors is most likely. Since CDPX at potentiating concentrations is not an agonist, it is probably not acting at the GABA binding site. It may bind to a regulatory site on or near the GABA receptor, and alter either the affinity of the receptor for GABA, or the coupling between GABA binding and opening of membrane chloride channels. This conclusion is consistent with a recent description of saturable, high affinity binding of ^3H -diazepam in rat brain¹². The ^3H -diazepam binding could not be inhibited by GABA or GABA antagonists.

In addition to potentiation of exogenously applied GABA, CDPX augments synaptic potentials that are probably mediated by GABA. Synaptic potentials were evoked by extracellular stimulation of nearby neurones or nerve processes, and KCl recording electrodes were used to convert small inhibitory potentials into large depolarising responses. Large Cl^- dependent synaptic potentials that were attenuated by bicuculline were augmented by CDPX (six cells) (Fig. 3). As expected of a drug which increased GABA-ergic inhibition, CDPX often reduced the overall frequency of spontaneous synaptic potentials.

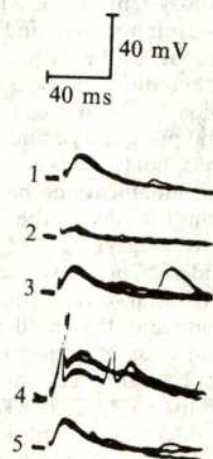


Fig. 3 CDPX potentiation of a bicuculline sensitive synaptic potential. The stimulus evoked synaptic potentials labelled 1–5 were recorded with a potassium chloride electrode and were obtained in sequence. 1, Control; 2, during ejection of 10^{-4} M bicuculline; 3, return to control 1 min later; 4, during ejection of $1.2 \times 10^{-4} \text{ M}$ CDPX; 5, return to control. The stimulus artefact appears as a break in the trace immediately before the evoked response. Spontaneous synaptic potentials appear late in the records. Each record is the superposition of three consecutive oscilloscope sweeps.

This demonstration of potentiation of GABA-ergic synaptic potentials as well as of exogenously applied GABA supports the hypothesis that some central actions of benzodiazepines are associated with enhanced GABA-ergic inhibition. We found no evidence for interaction with glycine receptors. It should be noted that the CDPX ED_{50} for GABA potentiation ($5\text{--}10 \mu\text{M}$) is at least 20 times smaller than the ED_{50} reported⁸ for ^3H -strychnine displacement ($200 \mu\text{M}$). In any case, the selectivity of CDPX, as a complement to the GABA antagonists, should prove useful in identifying and characterising GABA-ergic pathways in the CNS.

This work was supported by the USNIH (grant R01 NS-11160-05).

D. W. CHOI
D. H. FARB
G. D. FISCHBACH*

Harvard Medical School,
Department of Pharmacology,
25 Shattuck Street,
Boston, Massachusetts 02115

Received 23 May; accepted 22 July 1977.

*To whom correspondence should be addressed.

- ¹ Costa, E., Guidotti, A. & Mao, C. C. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. & Tower, D. B.) 413-426 (Raven, New York, 1976).
- ² Haefely, W. et al. in *Mechanism of Action of Benzodiazepines* (eds Costa, E. & Greengard, P.) 131-151 (Raven, New York, 1975).
- ³ Schmidt, R. F. *Ergonomics*, **63**, 20-101 (1971).
- ⁴ Stratten, W. P. & Barnes, C. D. *Neuropharmacology*, **10**, 685-696 (1971).
- ⁵ Barker, J. L. & Nicoll, R. *Science*, **176**, 1043-1045 (1972).
- ⁶ Dray, A. & Straughan, D. W. *J. Pharm. Pharmacol.*, **28**, 314-315 (1976).
- ⁷ Gahwiler, B. H. *Brain Res.*, **107**, 176-179 (1976).
- ⁸ Young, A. B., Zukin, S. R., Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2246-2250 (1974).
- ⁹ Fischbach, G. D. & Dichter, M. A. *Dev. Biol.*, **37**, 100-116 (1974).
- ¹⁰ Krnjevic, K. *Physiol. Rev.*, **54**, 418-540 (1974).
- ¹¹ Iversen, L. L. & Johnston, G. A. R. *J. Neurochem.*, **18**, 1939-1950 (1971).
- ¹² Squires, R. F. & Braestrup, C. *Nature*, **266**, 732-734 (1977).

Phosphorylation of chloroplast membrane polypeptides

ILLUMINATION of chloroplast thylakoids leads to the formation of the so-called high energy state of the membrane¹⁻³. The establishment of this state is accompanied by several structural changes within the membrane, including a conformational change in the coupling factor⁴, increased accessibility of photosystem II to the chemical probe *p*-diazonium benzene sulphonate⁵, and a reduction in the thickness of the partition between stacked thylakoids⁶. I describe here a rather different type of structural change that has not previously been reported for chloroplast membranes—protein phosphorylation. Like the above changes, protein phosphorylation is a reversible, energy-dependent membrane modification, but it differs from the other changes in that it takes the form of a specific chemical reaction involving certain identifiable chloroplast membrane polypeptides. The most conspicuous of these polypeptides is the light-harvesting chlorophyll *a/b* binding protein, the most abundant thylakoid polypeptide⁷.

Isolated class A or class B (ref. 8) chloroplasts of the pea (*Pisum sativum* L. var. Feltham First) incorporated ³²P-orthophosphate into ATP, CTP, GTP, UTP and RNA in the light⁹. In identical conditions of chloroplast isolation and incubation, protein phosphorylation is also readily demonstrated. After a standard incubation period of 10 min, labelled chloroplasts were washed, solubilised in boiling sodium dodecyl sulphate (SDS) solution, and fractionated by SDS-polyacrylamide slab gel electrophoresis¹⁰. Chloroplast proteins were detected by staining the gel with Coomassie Brilliant Blue (Fig. 1a) and radioactivity was located by autoradiography (Fig. 1b). Many polypeptides were visible after staining but three were particularly prominent: (1) the 54,000 molecular weight (MW) large subunit of ribulose 1,5-bis-phosphate (RBP) carboxylase, the most abundant soluble chloroplast protein¹¹, (2) the 12,000 MW subunit of the same enzyme, and (3) the 26,000 MW subunit of the light harvesting chlorophyll *a/b* binding protein complex⁷. There were two major phosphorylated polypeptides: (1) a 26,000 MW polypeptide which co-migrated in every experiment with the chlorophyll *a/b* binding protein, and (2) a 9,000 MW polypeptide whose MW was estimated by comparison with cyanogen bromide cleavage fragments derived from cytochrome *c*¹². Other phosphorylated polypeptides were also evident, their molecular weights falling generally in the

the range 7,000–70,000. Neither subunit of RBP carboxylase was labelled *in vitro*.

In many experiments, it was possible to resolve the 26,000 MW polypeptide into two labelled species (Fig. 2). The MW of the second polypeptide was estimated to be 28,000. These two polypeptides, together with the 9,000 MW polypeptide, were eluted from the gel¹³ and subjected to brief acid hydrolysis. The digestion products were separated by paper electrophoresis and shown to be phosphothreonine and orthophosphate. During acid hydrolysis phosphothreonine breaks down to threonine and orthophosphate. It is therefore likely that the 65% recovery of isotope in phosphothreonine underestimates the percentage of the label in this form in the native protein. It is possible, however, that some of the labelled orthophosphate was derived from other less stable phosphorylated amino acids such as phosphohistidine. At present, we can only say that most of the ³²P-orthophosphate incorporated into the 28,000, 26,000 and 9,000 MW polypeptides was recovered in phosphothreonine.

The 26,000 and 9,000 MW polypeptides were found to be membrane-bound and to be labelled *in vivo* (Fig. 3). The recovery of all but the 70,000 MW polypeptide in the thylakoid and envelope fraction of osmotically shocked chloroplasts indicates that chloroplast protein phosphorylation is essentially a membrane phenomenon and supports the conclusion reached earlier that RBP carboxylase was not phosphorylated *in vitro*.

Leaf pairs were allowed to take up ³²P-orthophosphate

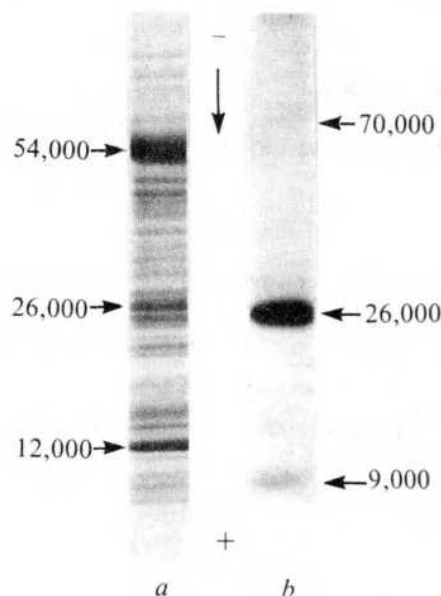


Fig. 1 *In vitro* incorporation of ³²P-orthophosphate into chloroplast phosphoproteins. Chloroplasts from 10-d-old pea seedlings were isolated and resuspended in isotonic KCl medium as described previously^{8,10}. An aliquot (100 µg chlorophyll in 0.5 ml) was incubated at 20 °C for 10 min with carrier-free ³²P-orthophosphate (100 µCi). Illumination was by a single coil 200-W tungsten lamp (Philips). After incubation the chloroplasts were diluted with 10 ml of ice-cold 0.35 M sucrose medium containing 25 mM HEPES-NaOH buffer (pH 7.6), 2 mM EDTA and 2 mM sodium isoascorbate, and centrifuged at 2,800g for 2 min. The chloroplast pellet was washed twice in acetone-water (4:1) and then taken up in 400 µl of 2% SDS solution containing 60 mM Tris-HCl buffer (pH 6.8), 0.5% 2-mercaptoethanol and 10% glycerol. After 3 min at 100 °C, the solution was cooled and an aliquot (40 µl) was electrophoresed through a 10%–30% exponential gradient slab gel of acrylamide which included a 5% stacking gel¹⁰. The gel was stained with Coomassie Brilliant Blue, photographed and autoradiographed. *a*, Photograph of stained gel showing total chloroplast protein. *b*, Autoradiogram showing chloroplast phosphoproteins. Molecular weights were estimated by comparison with known standards, including cyanogen bromide cleavage fragments of cytochrome *c*¹².

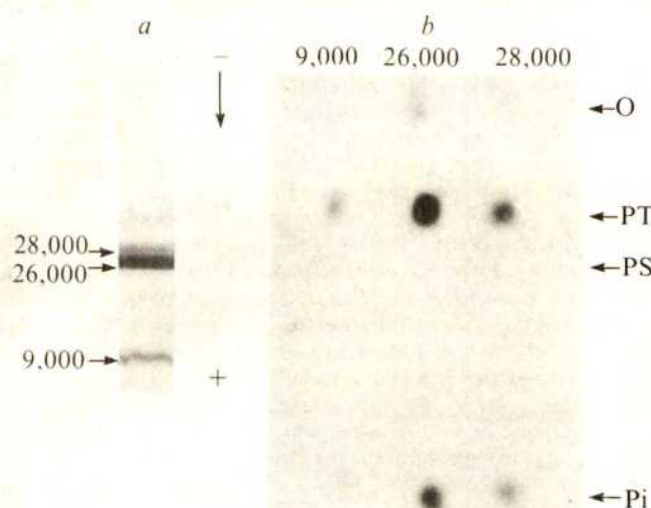


Fig. 2 Identification of threonine as site of phosphorylation in three chloroplast phosphoproteins. *a*, Chloroplast phosphoproteins were labelled *in vitro* for 10 min, fractionated electrophoretically and located by autoradiography. *b*, Bands containing the 28,000, 26,000 and 9,000 phosphoproteins were cut from the gel slab and the polypeptides were extracted as tryptic peptides¹³. After lyophilisation the tryptic peptides were digested under N_2 in 6 M HCl (105 °C, 90 min) and analysed by electrophoresis on Whatman 3 MM paper using formic acid-acetic acid-water (1:2:45) buffer (pH 2.0). Autoradiography showed that radioactive material was located at the origin (O) and also found in phosphothreonine (PT) and orthophosphate (Pi) but not in phosphoserine (PS).

for 6 h under constant illumination and were then homogenised and chloroplasts were isolated. Electrophoresis of the solubilised polypeptides showed that the 26,000 and 9,000 MW polypeptides were again the most heavily labelled chloroplast phosphoproteins and therefore that the *in vitro* labelling of these polypeptides was not an artefact of chloroplast isolation but represented a physiological process. The broad band (X) labelled *in vivo* was phospholipid.

The *in vitro* phosphorylation of chloroplast membrane

Table 1 *In vitro* phosphorylation and dephosphorylation of chlorophyll *a/b* binding protein within isolated chloroplasts

Incubation conditions		³² P-incorporation (10 min light = 100)
Light	0 min	1
	1 min	12
	5 min	48
	10 min	100
	20 min	88
	30 min	72
	10 min + 20 μ M CCCP	1
Dark	10 min	1
Light (10 min) followed by	10 min light	88
	10 min dark	9
	10 min light + 20 μ M CCCP	20
	10 min light + 1 mM KH_2PO_4 (pH 8.3)	37

Replicate aliquots of a suspension of pea chloroplasts (100 μ g chlorophyll in 0.5 ml isotonic KCl medium) were pre-incubated for 10 min in the dark at 20 °C with or without 20 μ M CCCP. Radioisotope (100 μ Ci ³²P-orthophosphate) was then added to each replicate and incubation was continued in the light or the dark as indicated. After the incubations the chloroplasts were washed, solubilised and fractionated electrophoretically as described in Fig. 1. After the position of the chlorophyll *a/b* binding protein (26,000 MW polypeptide) had been determined by staining and autoradiography, the band of gel containing it was excised from each track and ³²P was determined by measuring Cerenkov radiation in a liquid scintillation spectrometer (counting efficiency 45%). Incorporation into the chlorophyll *a/b* binding protein is expressed relative to the incorporation into this protein after 10 min illumination (1.5×10^4 d.p.m. per gel slice, or 1.5×10^5 d.p.m. per incubation).

polypeptides was not linear with time (Table 1). Incorporation of ³²P into the 26,000 MW polypeptide was rapid for 5–10 min and thereafter was reversed. The loss of radioactivity from the 26,000 MW band was greater than could be explained by chloroplast disintegration during incubation or washing. Two processes determined the instantaneous level of protein labelling—phosphorylation and dephosphorylation. Protein phosphorylation was driven by light through photophosphorylation. In this respect it resembles RNA and protein synthesis in isolated pea chloroplasts^{14,15}. Dephosphorylation was readily demonstrated by incubating chloroplasts for 10 min in the light to label the 26,000 MW polypeptide and then subjecting the chloroplasts to various treatments to inhibit or chase protein phosphorylation: (1) dark incubation, (2) illumination in the presence of the uncoupler carbonylcyanide *m*-chlorophenylhydrazone (CCCP), and (3) a chase with 1 mM unlabelled orthophosphate. In each case, rapid loss of radioactivity from the chloroplast proteins was observed. Thus, chloroplast protein phosphorylation is reversible, being sensitive to the ATP levels within the organelle.

Chloroplast protein phosphorylation has not previously been reported, although protein kinase activity has been

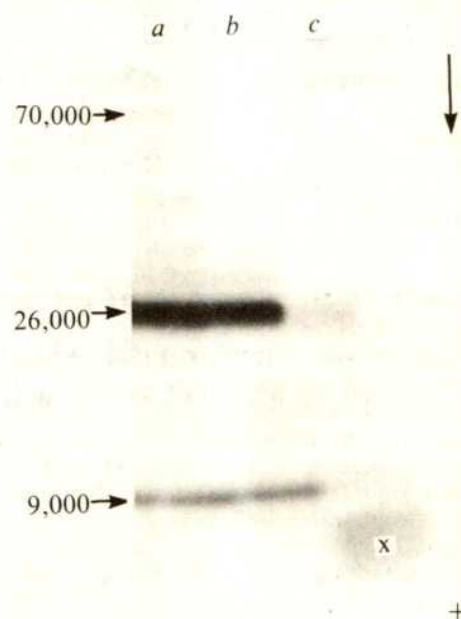


Fig. 3 Location and *in vivo* labelling of chloroplast phosphoproteins. Isolated chloroplasts were incubated *in vitro* with ³²P-orthophosphate for 10 min and then washed in ice-cold sucrose medium. Half of the chloroplasts were solubilised immediately in SDS solution and the remainder were resuspended in hypotonic medium (20 mM Tris-HCl, pH 8.0, 4 mM $MgCl_2$, 2 mM 2-mercaptoethanol) and centrifuged at 30,000g for 30 min. The pellet, containing thylakoids and envelopes, was solubilised in SDS solution. Chloroplasts were also isolated from 10 leaf pairs which had taken up ³²P-orthophosphate (50 μ Ci per leaf pair) for 6 h under continuous illumination. The radioactive chloroplast pellet was solubilised in SDS solution and electrophoresed alongside the two *in vitro* labelled samples. *a*, Total chloroplast proteins, labelled *in vitro*. *b*, Total chloroplast membrane proteins, labelled *in vitro*. *c*, Total chloroplast proteins labelled *in vivo*. The broad band (X) in track *c* represents labelling in phospholipid.

attributed to chloroplasts from *Acetabularia*¹⁶ and to a fraction from *Brassica* containing nuclei and chloroplasts¹⁷. I have shown that two chloroplast membrane polypeptides (with MWs of 26,000 and 9,000) are particularly heavily phosphorylated *in vivo* and *in vitro*, with several other polypeptides also being phosphorylated to a detectable extent *in vitro*. The polypeptide of 26,000 MW co-electrophoreses with the light-harvesting chlorophyll *a/b* binding protein, the most abundant thylakoid polypeptide.

Other evidence that these two polypeptides are identical will be published elsewhere. The function of thylakoid protein phosphorylation is not known but it may be involved in establishing the high energy state of the thylakoids in state 1-state 2 transitions¹⁻³. These transitions are part of the mechanism whereby the chloroplast regulates energy flux from light-harvesting chlorophyll molecules to ensure equal electron flow through the two photosystems. This regulation may involve the binding of divalent metal cations to the chlorophyll *a/b* binding protein^{18,19}. It is likely that phosphorylation of this protein would alter both its cation binding properties and its interaction with the two photosystems.

I thank Mr N. Lengden for technical assistance and Professors W. Ogren and J. Ellis for helpful discussions.

J. BENNETT

Department of Biological Sciences,
University of Warwick,
Coventry, UK

Received 16 May; accepted 27 July 1977.

- 1 Barber, J. *The Intact Chloroplast* (Elsevier, Amsterdam, 1976).
- 2 Govindjee *Bioenergetics of Photosynthesis* (Academic, New York, 1975).
- 3 Anderson, J. M. *Biochim. biophys. Acta* **416**, 191-235 (1975).
- 4 Ryrie, I. J. & Jagendorf, A. T. *J. biol. Chem.* **246**, 582-588 (1971).
- 5 Giacchino, R. T. et al. *Archs Biochem. Biophys.* **162**, 200-209 (1974).
- 6 Murakami, S. & Packer, L. *Biochim. biophys. Acta* **180**, 420-423 (1969).
- 7 Thornber, J. P. A. *Rev. Pl. Physiol.* **26**, 127-158 (1975).
- 8 Hall, D. O. *Nature new Biol.* **235**, 125-126 (1972).
- 9 Bennett, J. & Milewska, Y. in *Genetics and Biogenesis of Chloroplasts and Mitochondria* (eds Bucher, T. et al.) 637-640 (Elsevier/North-Holland, Amsterdam, 1976).
- 10 O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).
- 11 Boulter, D. et al. *Biol. Rev.* **47**, 113-175 (1972).
- 12 Chu, R. C. L. & Yasumobu, K. T. *Biochim. biophys. Acta* **89**, 148-149 (1964).
- 13 Clegg, J. C. S. et al. *J. gen. Virol.* **32**, 413-430 (1976).
- 14 Blair, G. E. & Ellis, R. J. *Biochim. biophys. Acta* **319**, 223-234 (1973).
- 15 Bennett, J. *Phytochemistry* **15**, 263-265 (1976).
- 16 Pai, M. S. et al. *Protoplasma* **85**, 209-218 (1975).
- 17 Ralph, R. K. et al. *Biochem. J.* **130**, 901-911 (1972).
- 18 Armond, P. A. et al. *Archs Biochem. Biophys.* **175**, 54-63 (1976).
- 19 Davis, D. J. et al. *Archs Biochem. Biophys.* **175**, 64-70 (1976).

Identification of a transformation-specific antigen induced by an avian sarcoma virus

GENETIC analyses of avian sarcoma viruses (ASV) have led to the identification of a gene, designated *src*, which encodes a product required for the initiation and maintenance of neoplastic transformation in infected fibroblasts¹⁻³. Because the *src* gene product has not been identified biochemically, this study was initiated to detect a transformation-specific protein, using serum from rabbits bearing ASV-induced tumours. We describe here the identification of a 60,000-MW transformation-specific antigen detectable in ASV-transformed chicken cells and ASV-induced hamster tumour cells by immunoprecipitation of radiolabelled cell extracts with serum from tumour-bearing rabbits. Moreover, the expression of this antigen is temperature dependent in chicken cells transformed by an ASV temperature-sensitive mutant in the *src* gene. The use of this antiserum may lead to the unequivocal identification and characterisation of the ASV *src* gene product and this, in turn, may lead to the elucidation of the mechanism of ASV-induced oncogenesis.

Serum from rabbits whose Schmidt-Ruppin (SR) strain ASV-induced tumours followed two different patterns of growth, labelled A and B and described in the legend of Fig. 1, was tested for the presence of antibodies directed against any non-structural proteins. Cell extracts prepared from radiolabelled normal and SR-ASV-transformed chicken cells were immunoprecipitated with these sera and also with monospecific sera directed against virion structural proteins, and analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (Fig. 1). The proteins precipitated from SR-ASV-transformed chicken cells by serum A and B (tracks 5 and 7) having molecular weights (MW) of 76,000 (Pr76), 66,000, 53,000,

27,000 and 12,000-15,000 are the precursor and intermediate and mature cleavage products of the ASV group-specific (gs) antigens^{6,7}. The 180,000 MW virus-specific protein which precipitated with anti-polymerase IgG and

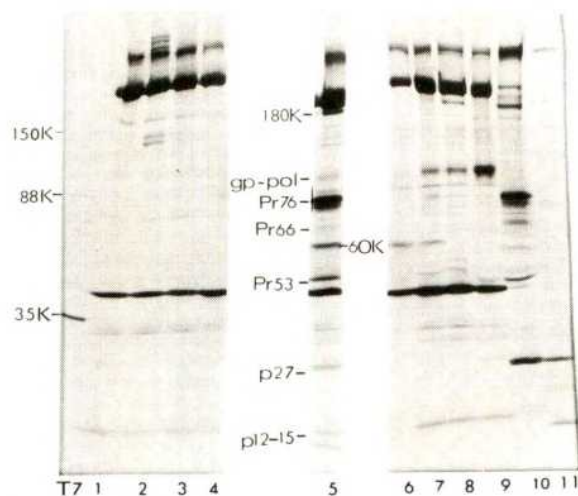


Fig. 1 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ³⁵S-methionine-labelled uninfected and SR-ASV-transformed chicken cells. Tumours were induced in rabbits by the subcutaneous and intramuscular injection of newborn New Zealand rabbits with ~10⁶ focus-forming units of purified SR-ASV (subgroup D). Palpable tumours appeared within 10-14 d in all of the nine rabbits injected. Tumour growth followed two different patterns involving either development of multiple metastases (serum A) or regression of the original tumours (serum B). In the experiments reported here, the serum was obtained 5 weeks after the animals had been inoculated with virus. Sera A and B were both found to contain antibodies to all three virion structural proteins including the viral core proteins or gs antigens, the envelope glycoprotein gp85, and the RNA-dependent DNA polymerase (J.S.B. et al., unpublished observations). These antibodies were detected by immunoprecipitation of ³⁵S-labelled proteins from lysed, purified ASV and by neutralisation of virion polymerase activity and of focus formation. Adult rabbits receiving the same dose of infectious virus did not develop tumours and did not produce antibody to any virus-specific proteins. Uninfected and SR-ASV-transformed chicken embryo fibroblasts (CEF) were seeded at a density of 8 × 10⁶ cells per 100-mm Petri dish and labelled the next day for 4 h with 10 μCi ml⁻¹ ³⁵S-methionine (New England Nuclear, 400 Ci mM⁻¹) in Eagle's medium lacking methionine and supplemented with 5% calf serum (Colorado Serum Co.). The cells were washed three times with STE (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 1 mM EDTA) and lysed in RIPA buffer¹¹ (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM Tris-HCl, pH 7.2) containing 1% Trasylol, a general inhibitor of proteases¹² (FBA Pharmaceuticals, New York) and centrifuged at 90,000g for 30 min. The supernatant was divided into samples of equal volume and incubated with 10 μl of the pertinent antiserum. After 30 min at 4 °C, 200 μl of a 10% suspension of the protein A-containing bacterium, *Staphylococcus aureus*, strain Cowan I were added to adsorb the immune complexes as described by Kessler¹³. After four washes with RIPA buffer, the bacteria were resuspended in sample buffer (0.07 M Tris-HCl, pH 6.8, 11.2% glycerol, 3% SDS, 0.01% bromophenol blue, 5% β-mercaptoethanol), heated at 95 °C for 1 min, pelleted, and the supernatant was electrophoresed through a discontinuous SDS-polyacrylamide slab gel system using the buffer systems described by Laemmli¹⁴. The stacking gel contained 4.2% acrylamide, 0.1% bis-acrylamide (pH 6.8). The separation gel was a 5-15% gradient of polyacrylamide (38:1 acrylamide:bis-acrylamide), 11 cm long. Gels were stained in 0.1% Coomassie blue in 50% trichloroacetic acid for approximately 1 h and destained in several changes of 10% acetic acid, 5% methanol. The dried gels were exposed to Dupont Cronex-4 medical X-ray film. Tracks 1-3 display the polypeptides immunoprecipitated from uninfected CEF with: 1, normal rabbit serum; 2, serum B; 3, serum A. Tracks 4-10 display polypeptides immunoprecipitated from SR-ASV-transformed CEF with: 4, normal rabbit serum; 5, serum B; 6, serum B which was incubated with 125 μg of purified disrupted SR-ASV before addition of the cellular extract; 7, serum A; 8, anti-RAV-0 DNA polymerase IgG; 9, anti-gp85 serum; 10, anti-p27 serum. Track 11 contains ³⁵S-methionine-labelled ASV. T7 virion proteins are included as molecular weight markers.

anti-p27 serum as well as with sera A and B has been identified as the product of genes which encode the gs antigens and DNA polymerase³. The glycoprotein, gp85, migrates on this gel with the same mobility as the viral polymerase. An additional virus-specific protein with a molecular weight of 60,000 is also precipitated by both sera. This protein is not precipitated by antibody against any of the ASV structural proteins, nor is it precipitated from normal chicken cells by any sera tested. Incubation of serum B with disrupted virus before the addition of the labelled cell extract blocks the immunoprecipitation of all the virus-specific proteins except that of the polypeptide of molecular weight 60,000 (track 6). The fact that the intensity of this antigen is identical with and without the block suggests that its immunoprecipitation is not dependent on antibodies against any of the ASV structural proteins.

Figure 2 (tracks 4, 5, and 6) displays the polypeptides immunoprecipitated from a radiolabelled extract of a cloned line of SR-ASV-induced Syrian hamster tumour cells (SR-SHC/C1A). These proteins are co-electrophoresed with immunoprecipitates from SR-ASV-transformed chicken cells (tracks 1, 2, and 3). Serum B precipitated two virus-specific proteins from SR-SHC/C1A cells. One protein co-migrates with the Pr76 precipitated from SR-ASV-transformed chicken cells (track 2). No mature gs antigens are detectable in these hamster tumour cells, which suggests that Pr76 is not processed normally. The only other virus-specific protein precipitated from this hamster tumour cell line by serum B is a protein which co-migrates with the 60K protein immunoprecipitated from chicken cells. This polypeptide is not precipitated by antibody to any structural proteins, nor is it immunoprecipitated from normal Syrian hamster cells by any sera tested. When disrupted virus is incubated with serum B before the addition of the labelled cell extract (track 6), the precipitation of Pr76 is completely blocked, whereas the intensity of the 60K band is identical to

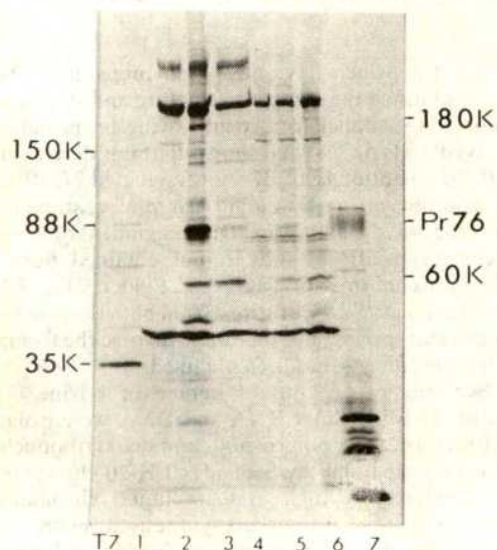


Fig. 2 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from SR-ASV-transformed chicken and hamster cells. SR-SHC/C1A cells were obtained by trypsinisation of a tumour induced by injection of a newborn Syrian hamster with SR-ASV. Cells were labelled with ³⁵S-methionine, immunoprecipitated and electrophoresed as described in the legend to Fig. 1. The cells and serum used are the following: 1, ASV-transformed CEF, normal serum; 2, ASV-transformed CEF, serum B; 3, ASV-transformed CEF, serum B blocked with 125 µg disrupted virus; 4, SR-SHC/C1A, normal serum; 5, SR-SHC/C1A, serum B; 6, SR-SHC/C1A, serum B blocked with 125 µg of disrupted virus. Track 7 contains ³⁵S-methionine-labelled ASV-virion proteins.

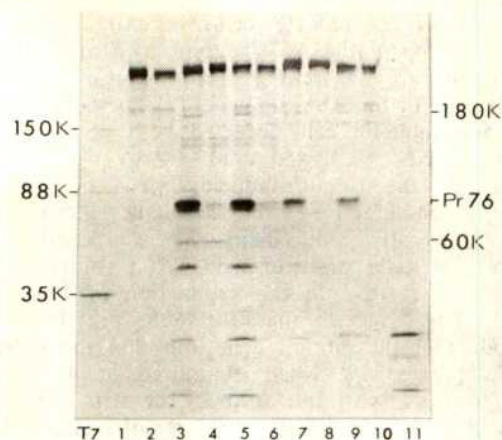


Fig. 3 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ³⁵S-methionine-labelled chicken cells transformed by the SR-ASV mutant NY68 (subgroup A). CEF cells were transformed with NY68 (ref. 4) and maintained at 35 °C. Transformed cells were trypsinised and plated at 35 °C or 41 °C at a density of 8×10^6 cells per 100-mm Petri dish and 16 h later the cultures were labelled for 1 h with 20 µCi ml⁻¹ ³⁵S-methionine, immunoprecipitated and analysed as described in Fig. 1. The temperature of incubation of NY68-transformed cells and the serum used for immunoprecipitation are the following: 1, 35 °C, normal serum; 2, 41 °C, normal serum; 3, 35 °C, serum B; 4, 35 °C, serum B blocked with disrupted virus; 5, 41 °C, serum B; 6, 41 °C, serum B blocked with disrupted virus; 7, 35 °C, anti-p27 serum; 8, 35 °C, anti-p27 serum blocked with disrupted virus; 9, 41 °C, anti-p27 serum; 10, 41 °C, anti-p27 serum blocked with disrupted virus. Track 11 contains ³⁵S-methionine-labelled ASV virion proteins.

that in track 5. The 60K protein is also detectable in another line of hamster tumour cells derived from a tumour induced by SR-ASV in the inbred LSH strain of hamsters.

The presence of an antigen having identical molecular weights in two different species of ASV-transformed cells suggests that this antigen is ASV transformation specific. This conclusion is supported by the finding that cells infected with a SR transformation-defective mutant or with RAV-50 or Carr-Zilber Associated virus (CZAV), both subgroup D viruses, do not contain the 60K protein. The expression of this transformation-specific antigen was also examined in cells infected with the SR-ASV temperature-sensitive (*ts*) mutant, NY68 (ref. 4) (subgroup A), which is defective in the initiation and maintenance of transformation at 41 °C, although virus replication is normal at this temperature. Figure 3 shows that the 60K protein was barely detectable in NY68-transformed cells grown at 41 °C relative to the 60K band from cells cultured at 35 °C. Equivalent amounts of the other virus-specific proteins such as the gs antigens and their precursors (180K, Pr76, Pr53, p27, and p12-15) were immunoprecipitated from cells grown at both temperatures, indicating that overall virus-specific protein synthesis at the two temperatures was the same and suggesting that the presence of the 60K protein is transformation dependent.

The use of serum from rabbits bearing ASV-induced tumours has allowed the detection of a 60,000 MW protein which has the following properties of an ASV transformation-specific antigen. (1) This protein is present in two different species of cells transformed by ASV. It seems unlikely that a virus-induced, cell-coded protein from two such widely divergent species would have identical molecular weights. Tryptic peptide fingerprints of the 60K proteins from transformed hamster and chicken cells are being prepared to characterise these

two proteins further. A 60K polypeptide has been synthesised in reticulocyte cell-free extracts using sub-genomic regions of the ASV genome as a mRNA (ref. 8). Experiments are in progress to ascertain whether it is the product of the ASV *src* gene. (2) The 60K polypeptide is not detectable in cells infected with non-transforming leukemia viruses (RAV-50 and CZAV) of the same subgroup as the tumour-inducing virus or with a SR transformation-defective virus. (3) The amount of 60K protein is greatly reduced at the non-permissive temperature in cells transformed by a mutant, NY68, temperature sensitive in the production of the *src* gene product. This suggests that the 60K protein could be the product of the mutant gene and that the polypeptide loses its antigenicity when denatured at 41 °C and is unable to react with the antibody or that the defective protein may be degraded more rapidly at 41 °C than the normal protein and thus less is detected in the conditions used here. Accelerated degradation of the protein products of *ts* genes at the non-permissive temperature has been demonstrated with mutants of vesicular stomatitis virus and of SV40 (refs. 9, 10). Alternatively, the 60K protein could be a virus-induced cell-coded protein, and its reduced immunoprecipitation at 41 °C may merely reflect host changes secondarily associated with the loss of transformation. All of these possibilities are being investigated.

We thank H. Hanafusa for the mutant ASV NY68, Satoshi Mizutani for anti-DNA polymerase IgG and D. Bolognesi for anti-gp85 serum. This work was supported by grants from the National Institutes of Health. J.S.B. was supported by an NIH Postdoctoral Fellowship.

JOAN S. BRUGGE
R. L. ERIKSON

Department of Pathology,
University of Colorado Medical Center,
Denver, Colorado 80262

Received 13 June; accepted 1 August 1977.

- ¹ Vogt, P. K. *Virology* **46**, 939–946 (1971).
- ² Biggs, P. M., Milne, B. S., Graf, T. & Bauer, H. J. *gen. Virol.* **18**, 399–403 (1973).
- ³ Toyoshima, K. & Vogt, P. K. *Virology* **39**, 930–931 (1969).
- ⁴ Kawai, S. & Hanafusa, H. *Virology* **46**, 475–479 (1971).
- ⁵ Martin, G. S. *Nature* **227**, 1021–1023 (1970).
- ⁶ von der Helm, K. *Proc. natn. Acad. U.S.A.* **74**, 911–915 (1977).
- ⁷ Eisenman, R., Vogt, V. M. & Diggelmann, H. *Cold Spring Harbor Symp. quant. Biol.* **39**, 1067–1076 (1974).
- ⁸ Purchio, A. F., Erikson, E. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁹ Knipe, D., Lodish, H. F. & Baltimore, D. *J. Virol.* **21**, 1140–1148 (1977).
- ¹⁰ Tegtmeyer, P., Schwartz, M., Collins, J. K. & Rundell, K. *J. Virol.* **16**, 168–178 (1975).
- ¹¹ Gilead, Z. *et al. Nature* **264**, 263–266 (1976).
- ¹² Biseid, G. *Acta Pharmac. Toxicol.* **28**, 225–232 (1970).
- ¹³ Kessler, S. W. *J. Immun.* **115**, 1617–1624 (1975).
- ¹⁴ Laemmli, U. K. *Nature* **227**, 680–685 (1970).

Structures of benzo(a)pyrene–nucleic acid adducts formed in human and bovine bronchial explants

PUBLICATION by Sims *et al.* of evidence that the 7,8-dihydrodiol-9,10-oxide of benzo(a)pyrene (BP) is a metabolic intermediate in the covalent binding of this ubiquitous polycyclic aromatic hydrocarbon to DNA in hamster embryo cells¹ was followed by many related publications². Grover *et al.*³ also obtained evidence that the same derivative is involved in the binding of BP to the DNA of human bronchial explants, although details of the specific isomer involved and of the structure of the adduct were not reported. We describe here studies on RNA and DNA adducts formed by human bronchial explants and provide evidence that the structures of the major adducts are similar to those formed in the analogous bovine system.

Two stereoisomers of BP 7,8-dihydrodiol-9, 10-oxide (BPDE) have been synthesised. In isomer I, the 7-hydroxyl and 9,10-oxide groups are on the opposite sides of the plane of the ring system while in isomer II they are on the same side. Each stereoisomer has

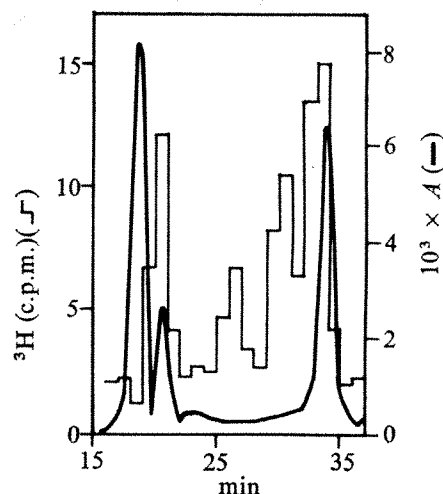


Fig. 1 HPLC profile of RNA adducts formed by human bronchial explants incubated with ³H-BP. Human bronchial explants were incubated for 24 h with ³H-BP (ref. 20). The tissue was then homogenised with a Polytron PT 10-20-350D homogeniser in 3 ml of 10 mM Tris-HCl, 0.4 M NaCl, 2 mM EDTA (pH 7.9). Sodium dodecyl sulphate (SDS) (30 µl 20%) and proteinase K (0.5 ml, 2 mg ml⁻¹) were then added and the mixture incubated overnight to dissolve the tissue. The solution was then extracted several times with water saturated phenol containing 0.1% SDS and 0.1% 8-hydroxyquinoline and once with chloroform-ethyl alcohol (3:1) before the addition of 0.03 volumes of 2 M sodium acetate (pH 5.0) and precipitation with 2 volumes of ethanol. The ethanol precipitation was repeated and followed by the addition of 50 A₂₅₀ units of poly (G) previously modified by reaction with BPDE I (ref. 12) and then hydrolysis of the RNA in 0.3 M NaOH overnight at 37 °C. The samples were neutralised with acetic acid and chromatographed on Sephadex LH 20 columns. The DNA eluted in the void volume and the modified ribonucleotides eluted with 40% methanol. The latter were treated with alkaline phosphatase overnight before separation by Sephadex LH 20 chromatography, this time eluting with 80% methanol. The modified nucleosides were then resolved by HPLC on Zorbax ODS columns operated at 2,500 pounds per square inch at 50 °C, with 30% methanol in water for elution. Adducts resulting from the *in vitro* reaction of BPDE with poly (G) were detected by ultraviolet absorbance at 280 nm and *in vitro* samples by their radioactivity.

two enantiomers which have also been prepared^{4–6}. We have previously published the complete structure and stereochemistry of a major RNA adduct formed in bovine bronchial explants exposed to BP (refs 5, 7, 8) and shown that it is derived from the 7R,8S,9R,10R enantiomer of BPDE (designated 7R-BPDE I) by the *trans* addition of the N² amino group of guanine to the 10 position of the dihydrodiol oxide. Our assignment of the absolute stereochemistry of BP 7,8-dihydrodiol obtained during these studies has been confirmed by Yagi *et al.*⁴. Evidence that RNA (ref. 9) and DNA adducts^{10,11} are formed from both isomers I and II in other tissues and species, and that these involve the formation of similar guanine adducts, has been obtained.

After exposure of explants of human or bovine bronchial segments to ³H-BP, cellular RNA and DNA were isolated and digested to release the modified ribo- and deoxyribonucleosides. The latter were separated by Sephadex LH-20 chromatography and then analysed by high pressure liquid chromatography (HPLC) using appropriate markers synthesised *in vitro*.

Figure 1 shows the HPLC profile obtained when human RNA was digested and cochromatographed with marker nucleoside adducts formed *in vitro* by the reaction of BPDE I with poly G. The profile of the human RNA *in vivo* products is similar to that reported for bovine RNA samples⁵, although the minor products are more pronounced with the human material. The digest of human RNA revealed four distinct radioactive peaks, designated H-RNA 1–4 (Fig. 1 and Table 1). We have previously reported that BPDE I and BPDE II can form adducts *in vitro* with guanosine, cytidine and adenosine¹². Table 1 indicates that during HPLC the radioactive peaks eluted in the same region as some of these guanosine and cytidine adducts. The adenosine adducts formed *in*

in vitro elute much later and very little radioactivity was detected in this region. By comparing the elution positions, before and after acetylation, of the radioactive peaks to the elution positions of the guanosine and cytidine adducts formed *in vitro*, we could conclude that H-RNA peaks 1 and 4 coincided with 7R-BPDE I-guanosine adducts GI-2 and GI-3, respectively; H-RNA peak 3 with a BPDE II-guanosine adduct GII-2; and H-RNA 2 with a BPDE I-cytidine adduct CI-3 (Table 1). A major peak, H-RNA 4, coincides with the major bovine RNA adduct whose structure and stereochemistry has been previously elucidated^{5,7,8}. This compound results from *trans* addition of the 2 amino group of guanine to the 10 position of the 7R enantiomer of BPDE I. H-RNA I also is derived from 7R BPDE I (ref. 8) and represents the analogous compound formed by *cis* addition (unpublished results and ref. 9). The predominance of adducts derived from a specific enantiomer of BPDE I suggests that both bovine^{7,8} and human tissue oxidise BP with a considerable degree of stereospecificity. Separate studies¹³⁻¹⁵ also indicate considerable stereospecificity in the metabolism of BP. Based on elution order and identity of circular dichroism (CD) spectra ($\Delta\epsilon_{250} = -93$), peak GII-2 (Table 1) corresponds to an adduct whose structure has been previously elucidated by Koreeda *et al.*^{9,16}. It results from *trans* addition of the 2 amino group of guanine to the 10 position of 7S BPDE II. Since HRNA-3 is

Table 1 Separation of BP-nucleoside adducts and their corresponding acetates by HPLC

Compound	Retention time		Compound	Retention time	
	Nucleoside	Acetate		Nucleoside	Acetate
HRNA-1	20	21	CII-1	21	18
HRNA-2	26	23	CII-2	29	20
HRNA-3	31	34	CII-3	42	—
HRNA-4	33	37	CII-4	44	—
GI-1	18	27	CII-5	55	—
GI-2	20	21	CI-1	17	19
GI-3	33	37	CI-2	20	13
GII-1	23	23	CI-3	26	23
GII-2	31	34	CI-4	27	14
GII-3	33	28	CI-5	31	15
GII-4	58	—	CI-6	44	12

The nucleoside adducts were prepared and separated on HPLC as described in Fig. 1. Data are retention times of the human bronchial RNA (HRNA) adducts formed *in vivo*, and of guanosine (G) and cytidine (C) adducts formed *in vitro* by reaction with BPDE isomer I or II. Numbers 1, 2, 3, 4 refer to the order of elution of individual peaks from column. After elution individual peaks were acetylated (pyridine, acetic anhydride, 18 h room temperature) and re-run on HPLC using 55% methanol in water as solvent. The underlines indicate *in vitro* products which were cochromatographic with specific human RNA adducts. Further evidence for the chromatographic identity of HRNA-2 and 3 with the *in vitro* markers (CI-3 and GII-2) was obtained when their acetates were run on HPLC with 50% methanol as eluent. In these conditions slight isotopic separations were observed, similar to those previously reported²¹.

cochromatographic with GII-2 this indicates that there is some synthesis of BPDE II by human tissue.

The situation with DNA from both human and bovine samples was much simpler than with RNA. Following enzymatic digestion of the DNA samples, 80% of the modified deoxynucleosides eluted from the HPLC columns essentially as a single component, both before and after acetylation (Fig. 2). (The remaining 20% may be incompletely digested materials, since it eluted in the void volume of the Sephadex LH-20 column, and not been further characterised). With both the human and bovine material, the single radioactive peak cochromatographed with one of the deoxyguanosine-BPDE I derivatives, and not with deoxyguanosine derivatives obtained with isomer II (Fig. 2a). Further evidence is provided in Fig. 2b which indicates that after acetylation the radioactive product was clearly separated from the acetylated isomer II adducts.

The deoxyguanosine adduct formed *in vitro* which corresponded to the major DNA adduct formed in human and bovine tissues was then isolated in a quantity sufficient for CD spectra (Fig. 3). The spectrum of this adduct was identical to that previously reported for the major guanosine adduct^{7,8}. This is consistent with the fact that the presence or absence of the 2'-hydroxyl group of the sugar residue should have little or no influence on the CD spectrum. The CD spectrum is extremely complex since it consists of extrema due to the perturbed pyrene and guanine chromophores as well as the spatial interactions between the two chromophores, the latter being predominant. The identity of the CD spectra of the deoxyguanosine and guanosine adducts and the fact that the absolute stereochemistry of the BPDE I must be 7R (refs 15, 17) indicates that, except for the 2'-hydroxyl group, the structures and absolute stereochemistry of the two adducts are identical (see Fig. 3). As in the case of the RNA adduct^{5,7-9}, the 2-amino group of guanine attacks position 10 of the 7R enantiomer of BPDE I through *trans* opening of the epoxide.

Both human and bovine RNA samples displayed minor adducts, the analogues of which were not detected in the DNA samples. The probable origin of two of these is described above, others were present in insufficient amount for identification. Possible explanations for the greater heterogeneity of adducts of RNA include the following. (1) The double stranded property of native DNA may contribute selectivity to its modification. (2) Both BPDE isomers I and II may be formed in the cytoplasm, but due to the greater instability of isomer II (ref. 18) it may react only with cytoplasmic RNA and not reach the nuclear DNA. (3) There

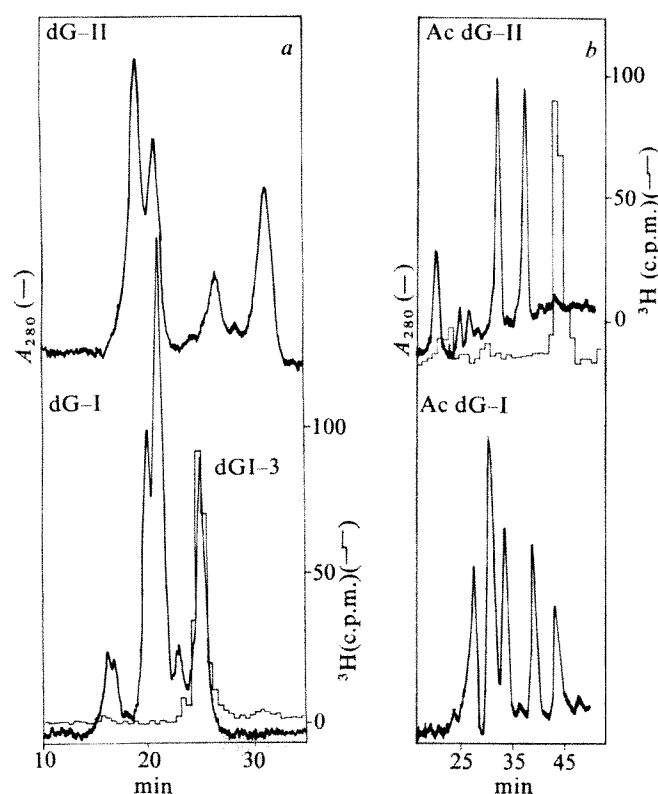


Fig. 2 HPLC profiles of human and bovine DNA adducts formed by bronchial explants incubated with ³H-BP. The radioactive human or bovine DNA which eluted in the void volume of the Sephadex LH 20 column (see Fig. 1 legend) was digested with DNase I, snake and spleen phosphodiesterases and alkaline phosphatase¹². The modified deoxynucleosides were isolated by Sephadex LH 20 chromatography and co-chromatographed on HPLC with ultraviolet markers synthesised *in vitro*. a, Compares the HPLC profile of the human ³H-deoxynucleoside adducts to that of deoxyguanosine adducts synthesised *in vitro* by reaction of 5' dGMP with either BPDE I (dG-I) or BPDE II (dG-II). HPLC conditions: Lichrosorb C18, 5 μ m, 3.2 \times 250 mm column (Rainin), 2,500 pounds per square inch, 45% methanol, 50 $^{\circ}$ C. The major (>90%) *in vivo* DNA adduct eluted with a dG adduct (dGI-3) formed from BPDE I. b, Compares the HPLC profile of the acetates of ³H-bovine deoxynucleoside adducts to those of the acetates of dG-I and dG-II. HPLC conditions: Zorbax ODS, 2.1 \times 250 mm column (Dupont); 2,500 pounds per square inch, linear gradient of 50–65% methanol in 50 min, 50 $^{\circ}$ C. A single radioactive *in vitro* product was obtained (upper panel, b) which, when reanalysed on HPLC with 55% methanol as solvent, was cochromatographic with the acetate of dGI-3. Similar results were obtained with the acetate of the major ³H-human deoxynucleoside adduct.

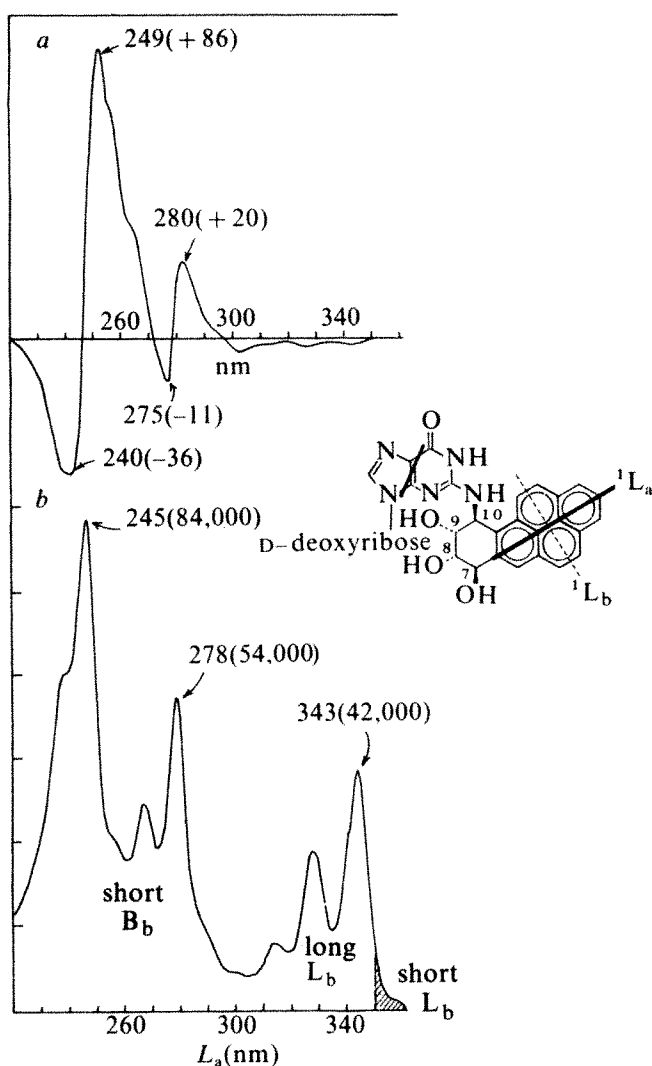


Fig. 3 *a*, CD and *b*, ultraviolet spectra of dG1-3 (the product corresponding to the major *in vitro* DNA adduct from human and bovine bronchial explants). The sample was prepared and purified by HPLC as described in Fig. 2. Spectra in 50% water-methanol. The CD $\Delta\epsilon$ values are 30% lower than previously reported for guanosine adducts⁷ because of a computational error in the previous data. The ultraviolet spectrum of the adduct is dominated by the strong bands of the pyrene chromophore, the transitions of which are: shoulder (shaded area) at 350–360 nm, L_b , short axis; 310–355 nm, L_a , long axis; 260–290 nm, L_b , short axis²². These interact with the guanine transitions at 260–270 nm, as shown by the solid line in the guanine structure, to give rise to the complex CD spectrum.

may exist a separate nuclear monooxygenase system¹⁹ which has greater stereospecificity than the cytoplasmic system in the synthesis of BPDE. Further studies are required to distinguish these possibilities.

Our investigations on the major BP metabolite responsible for covalent binding to cellular DNA in human tissue, and our evidence for the structure of the DNA adduct which is formed may contribute to the understanding of the possible human hazards associated with environmental exposure to BP and other polycyclic aromatic hydrocarbons.

This work was supported by grants and a contract from the US NCI and a grant from the American Cancer Society.

A. M. JEFFREY
I. B. WEINSTEIN
K. W. JENNETTE
K. GRZESKOWIAK
K. NAKANISHI

Institute of Cancer Research and
Departments of Medicine and Chemistry,
Columbia University,
New York City, New York 10032

R. G. HARVEY

Ben May Laboratory for Cancer Research,
University of Chicago,
Chicago, Illinois 60637

H. AUTRUP
C. HARRIS

Human Tissue Studies Section,
Experimental Pathology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 13 May; accepted 11 July 1977.

- ¹ Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. *Nature* **252**, 326–328 (1974).
- ² *Inst. Sci. Infor.*, Citation Index, 1975 and 1976.
- ³ Grover, P. L., Hewer, A., Pal, K. & Sims, P. *Int. J. Cancer* **18**, 1–6 (1976).
- ⁴ Yagi, H. *et al. J. Am. chem. Soc.* **99**, 2358–2359 (1977).
- ⁵ Weinstein, I. B. *et al. Science* **193**, 592–595 (1976).
- ⁶ Harvey, R. G. & Cho, H. *Analyt. Biochem.* **80**, (in the press).
- ⁷ Jeffrey, A. M. *et al. J. Am. chem. Soc.* **98**, 5714–5715 (1976).
- ⁸ Nakanishi, K. *et al. J. Am. chem. Soc.* **99**, 258–260 (1977).
- ⁹ Moore, P. D. *et al. Am. chem. Soc. Symp. Ser.* **44**, 127–154 (1977).
- ¹⁰ King, H., Osborne, M., Beland, F., Harvey, R. & Brookes, P. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2679–2681 (1976).
- ¹¹ Remsen, J., Jerina, D., Yagi, H. & Cerrutti, P. *Biochem. biophys. Res. Commun.* **74**, 934–940 (1977).
- ¹² Jennette, K. W. *et al. Biochemistry* **16**, 932–938 (1977).
- ¹³ Yang, S. K., McCourt, D. W., Leutz, J. C. & Gelboin, H. V. *Science* (in the press).
- ¹⁴ Yang, S. K., McCourt, D. W., Roller, P. P. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2594–2598 (1976).
- ¹⁵ Thakker, D. R. *et al. Chem.-Biol. Interact.* **16**, 281 (1977).
- ¹⁶ Koreeda, M., Moore, P. D., Yagi, H., Yeh, H. J. C. & Jerina, D. M. *J. Am. chem. Soc.* **98**, 6720–6723 (1976).
- ¹⁷ Yang, S. K., Gelboin, H. V., Trump, B. F., Autrup, H. & Harris, C. C. *Cancer Res.* **37**, 1210–1215 (1977).
- ¹⁸ Yagi, H., Hernandez, O. & Jerina, D. M. *J. Am. chem. Soc.* **97**, 6881–6883 (1975).
- ¹⁹ Khandwala, A. S. & Kasper, C. B. *Biochem. biophys. Res. Commun.* **57**, 1241–1246 (1973).
- ²⁰ Harris, C. C. *et al. Cancer Res.* **36**, 1011–1018 (1976).
- ²¹ Jeffrey, A. M. & Fu, P. P. *Analyt. Biochem.* **77**, 298–302 (1977).
- ²² Becker, R. S., Singh, I. S. & Jackson, E. A. *J. chem. Phys.* **38**, 2144–2171 (1963).

Nucleotide sequence of *Bombyx mori* L. tRNA₁^{Gly}

THE functional adaptation of the tRNA population in the posterior silk gland of the silkworm *Bombyx mori* L during growth and secretion of fibroin¹ is the result of changes in the predominant isoaccepting tRNA species to accord with the frequency of various codons in fibroin mRNA (refs 2–4). tRNA adaptation to fibroin biosynthesis can be demonstrated by polyacrylamide gel electrophoresis maps. The main glycine codons GGU and GGA of fibroin mRNA (ref. 3) are translated by means of two prevalent iso-tRNA^{Gly} species: iso-tRNA₁^{Gly} decodes GGU and GGC whereas iso-tRNA₂^{Gly} decodes GGA (refs 4–6). The glycine tRNA₁, the first sequenced tRNA from an insect⁶, has 74 nucleotides and relatively few modified residues⁷: one thymidine, one pseudouridine, one dihydrouridine, one 1-methyl adenine, 1-methyl guanosine, four 5-methyl cytidine and one unusual 2'-O-methyl uridine in the acceptor stem. It carries the expected GCC anticodon⁴. Comparison with three other homodecoding tRNA_{GCC}^{Gly} species reveals a high degree of homology.

The functional adaptation of tRNA to the amino acid composition of proteins being synthesised is best demonstrated by studying the quantitative changes of well-defined iso-tRNA species during development of the silk gland of the lepidopteran *Bombyx mori* L., which produces massive amounts of fibroin and sericin in the posterior and middle part respectively^{1,2}. The observed changes in tRNA species during the last larval stage before cocoon spinning are closely associated with the codon frequencies of glycine, alanine and serine in the rapidly accumulating fibroin mRNA, mainly GGU: GGA in a ratio of 1.4:1, GCU and UCA (refs 2–5). The sequence of the prominent tRNA₁^{Gly} species supports our predictions, based on triplet binding, of the presence of a GCC anticodon able to decode GGU and GGC codons^{4,6}. This remarkable iso-tRNA₁^{Gly} represents $20 \pm 3\%$ of total tRNA at the end of the secretion phase of fibroin in the posterior silk gland whereas iso-tRNA₂^{Gly} able to decode GGA and GGG accounts for $15 \pm 2\%$. Their ratio matches that of the corresponding codons in fibroin mRNA (refs 2, 8).

The posterior silk glands from hybrids of European strains 200 and 300 were dissected at the end of the last larval instar. Transfer

RNA was extracted as previously described⁹ and tRNA₁^{Gly} purified by a 1,500 transfer counter-current distribution to 80% and subjected to a final DEAE-cellulose chromatography at 65 °C 7 M urea^{6,7}. This tRNA species is strongly polar, because of a low ratio of adenine to pyrimidine⁷, and has been identified by two-dimensional polyacrylamide gel electrophoresis as shown in Fig. 1. Co-electrophoresis of tRNA₁^{Gly} from the middle silk gland secreting sericin and from the carcass rich in a chitin-associated protein shows that they have identical mobility. The pancreatic and T₁ RNase digestions, separation of the oligonucleotides on neutral and acidic DEAE-cellulose columns and high voltage electrophoresis, nucleotide analysis by thin-layer chromatography and all subsequent steps for further sequence analysis were carried out as described previously¹⁰. The identity of oligonucleotides has been checked again with ³²P-tRNA₁^{Gly} isolated from two-dimensional gel and subjected to a mini-fingerprint⁸: electrophoresis on cellulose acetate strip at pH 3.5 followed by homochromatography on a PEI-cellulose thin-layer plate for the second dimension. A partial digestion with T₁ RNase, which yields mainly two halves, allows us to reconstruct unambiguously a unique sequence.

The complete sequence of *B. mori* tRNA₁^{Gly} or tRNA_{GCC}^{Gly} is shown in Fig. 2 arranged in the cloverleaf model. It belongs to class I of tRNA characterised by four base pairs in the dihydrouridine stem and a short variable loop with four nucleotides. In addition to

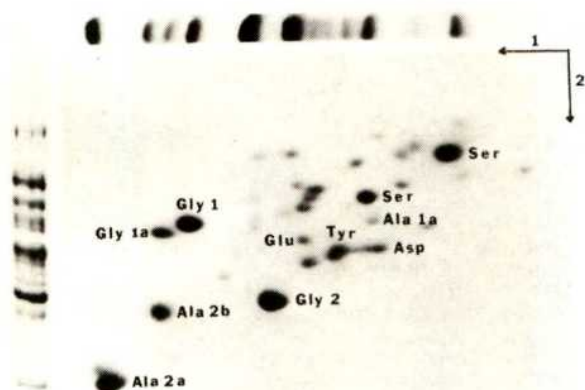


Fig. 1 Two-dimensional polyacrylamide gel electrophoresis of posterior silk gland tRNA. One A₂₆₀ unit of total tRNA from the posterior silk gland of *B. mori* 7-day-old larvae is applied to a 9.6% acrylamide gel, 7 M urea, Tris-borate buffer pH 8.2 and run over 35 cm for 45 h at 450 V (first dimension from right to left). The gel strip is cut out and embedded atop 20% acrylamide, 4 M urea prepolymerised gel slab. The electrophoresis is run in the same buffer for 60 h at 120 V (second dimension from top to bottom). tRNA spots are located after staining with 0.2% methylene blue solution in 2% sodium acetate buffer pH 5.0 or by autoradiography using ³²P-labelled tRNA. Identification is performed either by acylation and deamination with nitrous acid to stabilise the ester bond or by using purified samples of tRNA species from counter-current distribution and/or BD-cellulose chromatography^{8,16}.

the standard 23 invariant and semi-variant residues responsible for the maintenance of the tertiary structure of the macromolecule¹¹ shown in the composite diagram of Fig. 3, the tRNA₁^{Gly} reveals some characteristic features already mentioned⁶. The occurrence of a Um in position 4 of the acceptor stem seems to be a property of eukaryotic tRNA_{GCC}^{Gly}, which have been sequenced, since only tRNA₁^{Gly} species from yeast¹² and from wheat germ¹³ carry a 2'-O-methyl cytidine in the homologous position. The minor spot of tRNA₁^{Gly}, (a in Fig. 1), could be the non-methylated form⁸. The guanosine residue in the wobble position of the predicted GCC anticodon enables tRNA₁^{Gly} species to decode the main GGU codon found in fibroin mRNA (ref. 3). Its exposed position makes an easy partial digestion with T₁ RNase yielding two halves (a pGp 5'-terminal fragment of 33 nucleotides and the 3' end of 41 nucleotides with different compositions) useful in reconstructing

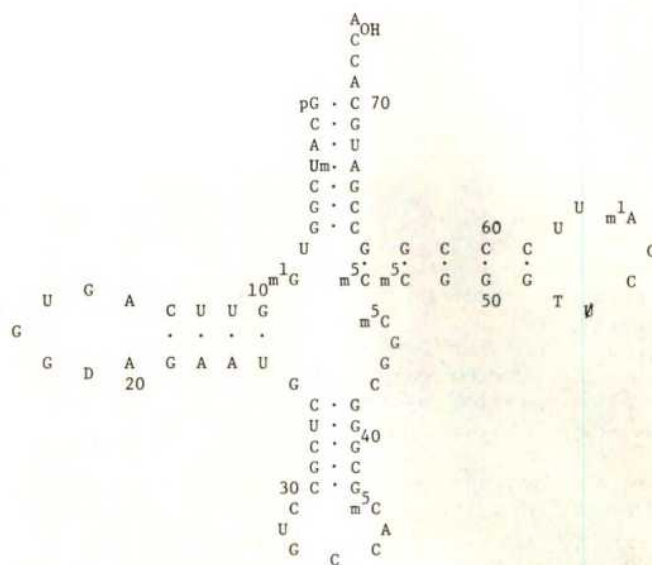


Fig. 2 Nucleotide sequence of *Bombyx mori* L. major tRNA₁^{Gly} or tRNA_{GCC}^{Gly} arranged in the cloverleaf model. Standard abbreviations are used for the nucleotides. Base pairs are represented by a dot. Specific cleavages with pancreatic and T₁ RNases yield 20 and 18 digestion products respectively. Partial T₁ digestion at the exposed guanosine in the wobble position 33 gives two halves used to reconstruct unambiguously the unique sequence. The iso-tRNA₁^{Gly} from posterior silk gland contains the GCC anticodon able to decode the main codon GGU found in fibroin mRNA³.

the total sequence. Like the other tRNA species decoding GNN triplets, the anticodon loop does not contain a hypermodified purine in position 36. The m⁵C residue at position 37 has been found in the homocoding tRNA_{GCC}^{Gly} from wheat germ¹³ as well as in the major cytoplasmic valine tRNA of mouse myeloma cells¹⁴, which carries the homologous tetranucleotide CAm⁵CGp. The m⁵C triplet located at the junction of the short variable loop and the TΨC stem also occurs only in tRNA_{GCC}^{Gly} species of

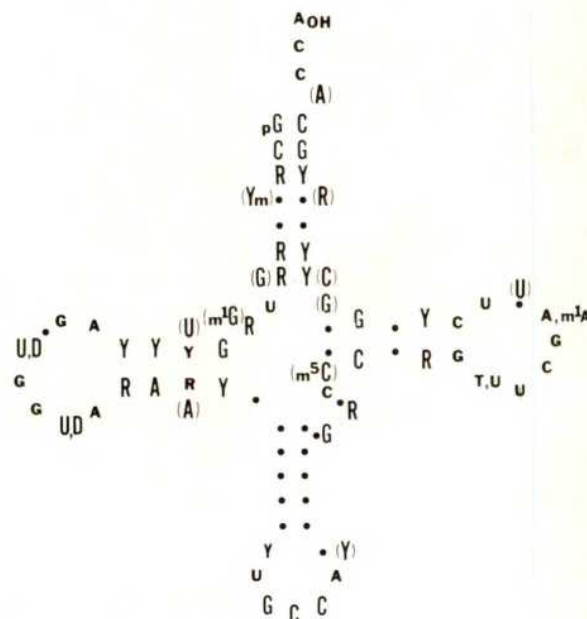


Fig. 3 Composite diagram of homocoding tRNA_{GCC}^{Gly} species. We have included four tRNA_{Gly} species able to decode GGU and GGC: tRNA₃^{Gly} from *E. coli*¹⁷, tRNA₁^{Gly} from yeast¹², wheat germ¹³ and *B. mori*. Nucleotides not common are indicated by dot. The 23 invariant and semi-variant nucleotides are represented in small letters. R stands for purine and Y for pyrimidine. Those homologous to the four tRNA_{GCC}^{Gly} species are noted in large letters. In parentheses we have shown additional similarities between the three eukaryotic species.

higher eukaryotic organisms from wheat germ to human placenta¹⁵. The presence of four m⁵C residues specific for the preponderant iso-tRNA₁^{Gly}, in contrast to the two found in the iso-tRNA₂^{Gly}, can be used as a marker for tRNA biosynthesis studies^{7,8}. We have to point out the presence of the 'invariant' thymidine in the T Ψ C sequence is not ubiquitous with respect to the *B.mori* tRNA species. Our nucleoside and fingerprint analysis demonstrate clearly that both preponderant tRNA_{2a}^{Ala} species (specific for the posterior silk gland) and tRNA_{2b}^{Ala} (common in all silkworm tissue)¹⁶ have no thymidine^{7,8}.

Figure 3 represents the composite diagram of known homodecoding tRNA_{GCC}^{Gly} species: tRNA₃^{Gly} from *E.coli*¹⁷, tRNA₁^{Gly} from yeast¹², wheat germ¹³ and *B.mori*. Considering both purine residues as R and pyrimidine residues as Y, the homodecoding tRNA_{GCC}^{Gly} species so far sequenced have 71.0% homology whereas eukaryotic species alone have 77.0%. There is an increase in sequence homology from *E.coli* tRNA_{GCC}^{Gly} to yeast, wheat germ and *B.mori* corresponding to their phylogenetic relationship. Because of its great variability, however, the anticodon stem could be considered as a target susceptible to evolutionary pressure. This reinforces the presumption that the central part of the tRNA molecule is involved in the recognition sites of cognate ligase and ribosome.

The structural work has been done in Dr G. Dirheimer's laboratory and supported by a grant from INSERM (no 76.10613) and the Fondation pour la Recherche Médicale Française. We thank Dr Dirheimer for his interest and Ms Ch.Fix for her excellent technical assistance. We are also indebted to Dr J. Daillie for his invaluable encouragement and partial support from the DGRST, Interactions Moléculaires en Biologie.

J. P. GAREL

Département de Biologie, Université Claude Bernard,
69621 Villeurbanne, France

G. KEITH

Institut de Biologie Moléculaire et Cellulaire du CNRS,
15 rue R.Descartes, 67000 Strasbourg, France

Received 15 April; accepted 31 May 1977.

- ¹ Garel, J. P. *J. theor. Biol.* **43**, 211–225 (1974).
- ² Garel, J. P. *Nature* **260**, 805–806 (1976).
- ³ Suzuki, Y. & Brown, D. D. *J. molec. Biol.* **63**, 409–429 (1972).
- ⁴ Garel, J. P., Hentzen, D. & Daillie, J. *FEBS Lett.* **39**, 359–363 (1974).
- ⁵ Chen, G. S. & Siddiqui, M. A. Q. *Arch. Biochem. Biophys.* **161**, 109–117 (1974).
- ⁶ Garel, J. P., Dirheimer, G. & Keith, G. *Z. physiol. Chem.* **357**, 293 (1976).
- ⁷ Garel, J. P., Hentzen, D., Schlegel, M. & Dirheimer, G. *Biochimie* **58**, 1089–1100 (1976).
- ⁸ Garel, J. P., Garber, R. & Siddiqui, M. A. Q. *Biochemistry* (in the press).
- ⁹ Chavancy, G., Daillie, J. & Garel, J. P. *Biochimie* **53**, 1187–1194 (1971).
- ¹⁰ Keith, G., Roy, A., Ebel, J. P. & Dirheimer, G. *Biochimie* **54**, 1405–1415 (1972).
- ¹¹ Rich, A. & RajBhandary, U. L. *A. Rev. Biochem.* **45**, 805–860 (1976).
- ¹² Yoshida, M. *Biochem. biophys. Res. Commun.* **50**, 779–784 (1973).
- ¹³ Marcu, K. B., Mignery, R. E. & Dudock, B. S. *Biochemistry* **16**, 797–806 (1977).
- ¹⁴ Piper, P. W. *Eur. J. Biochem.* **51**, 295–304 (1975).
- ¹⁵ Roe, B. A., Chen, E. Y. & Tsien, H. Y. *Biochem. biophys. Res. Commun.* **68**, 1339–1347 (1976).
- ¹⁶ Meza, L., Araya, A., Leon, G., Krauskopf, M., Siddiqui, M. A. Q. & Garel, J. P. *FEBS Lett.* **77**, 255–260 (1977).
- ¹⁷ Squires, C. & Carbon, J. *Nature new biol.* **233**, 274–277 (1971).

Melting fine structure of DNA fragments of known base sequence from ϕ X174

THERE is much experimental evidence of discrete unfolding of double-stranded DNA, revealed as fine structures in the melting profile (refs 1–4 and refs therein). Although this is believed to reflect aspects of the nature of the base sequence through the instability of AT and GC base pairs⁵, detailed quantitative analysis is hindered by factors such as the highly cooperative nature of double-helix stabilisation by base stacking and the long-range correlation of helical states through the entropy effect of the unfolded loop. Furthermore, the lack of appropriate nomenclature for the base sequence has complicated discussion. Fortunately, essentially the whole base sequence of the DNA of bacteriophage ϕ X174 has been determined⁶, and we present

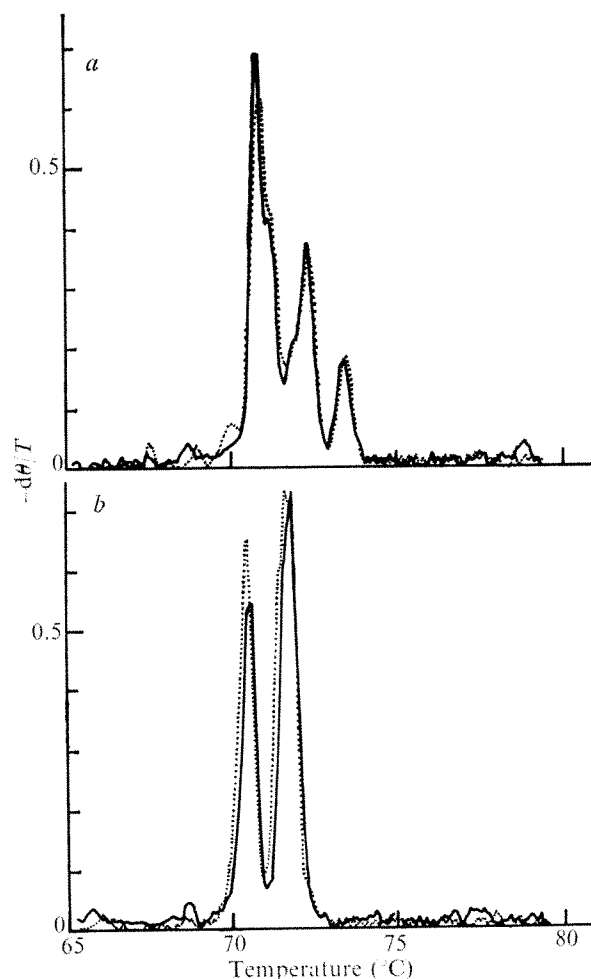


Fig. 1 Melting profiles of Y₁-fragment (a) and Y₂-fragment (b) of DNA of phage ϕ X174. Double-stranded, closed-circular replicative form (RFI) of ϕ X174 phage was prepared from ϕ X174 am3-infected *Escherichia coli* HF4704 cells as before⁷. The RFI molecules were cleaved by restriction enzyme *Hap*II, and the products were separated by polyacrylamide gel electrophoresis⁸. The gels were scanned at 260 nm using a Gilford spectrophotometer 250 to define the regions of the DNA fragments. The gel regions containing the largest (Y₁) and the second largest (Y₂) fragments were excised and these fragments were extracted as in ref. 9. Melting profiles were obtained with a minicomputer-controlled on-line melting plotter¹. The displays are made in differential form as d(1- θ)/dT against T, where θ is the helix content obtained by the optical density measurement at 260 nm, and T is the temperature. The ordinate is not normalised to the fragment length. Two results of independent measurements are shown by the solid and dotted lines in each figure.

here melting profiles of two fragments obtained from the replicative form by means of the restriction enzyme *Hap*II, and examine them with reference to their base sequence.

The differentiated melting profiles of fragments Y₁ (2,745 base pairs: position 3360–5375–729) and Y₂ (1,690 base pairs: position 1104–2793) measured in a 0.1 M SSC aqueous solution are shown in Fig. 1a and b, which have three and two well separated peaks, respectively, with some reproducible hyperfine structure in several of the peaks. Numerical data for the characteristics of the peaks are listed in Table 1.

The base sequences of these fragments are also shown in Fig. 2a and b in the form of the distribution function $P_n(x)$ of the G+C content, x, of sections each containing n base pairs along the DNA fragment, the first section starting with the first base pair, the second with the second base pair and so on until the whole fragment is covered. If the base sequence of the fragment is purely random and sufficiently long compared with the length of the section, n, the distribution function should agree with the binomial distribution

$$P_n(x) = \frac{n!}{\{n(1-x)\}!\{nx\}!} X_0^{nx}(1-X_0)^{n(1-x)}$$

where X_0 is the G+C content of the whole fragment. The binomial distribution for a small value of n is also plotted in the same figure for comparison.

We have speculated that a peak in the melting profile corresponds to a homostability region or regions with a fairly homogeneous base sequence, because computer simulations based on the established molecular thermodynamic parameters for several randomly generated base sequences did not reproduce sharp peaks as obtained in melting experiments^{1,4}. On the other hand, Lyubchenko *et al.* have shown that a sharp fine structure can be simulated by some computer-generated random base sequences³. But, arguments based on a limited number of such sequences may not be meaningful, because a DNA molecule of 1,000 base pairs, for example, has $2^{1,000}$ varieties of base sequence.

We therefore draw the following conclusions. (1) Fragments

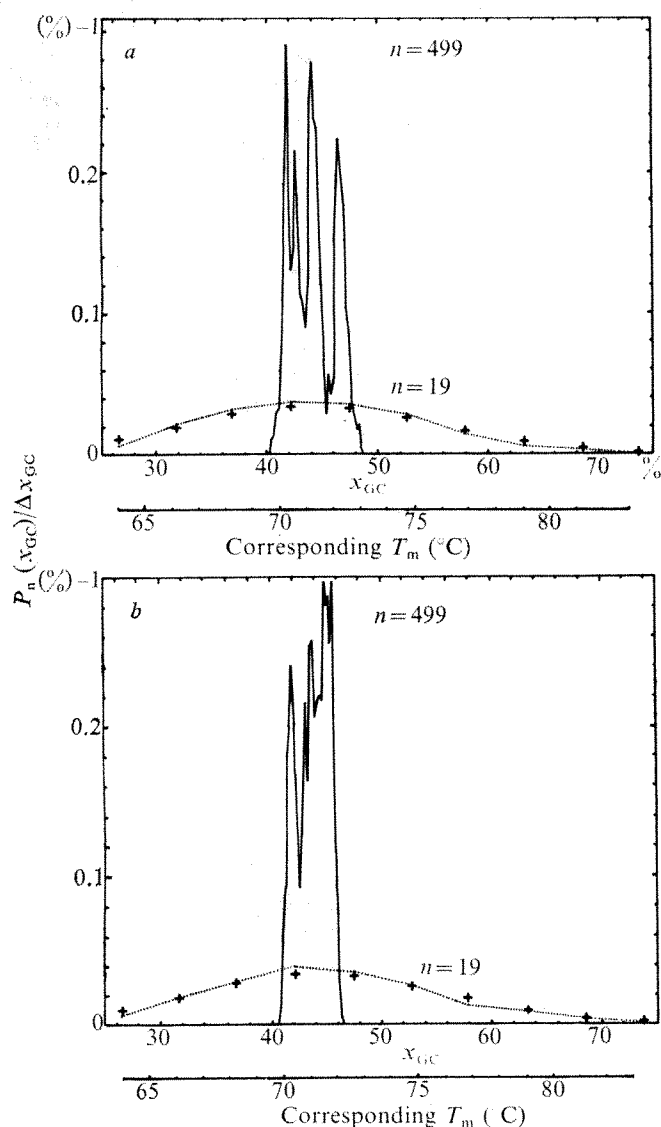


Fig. 2 Local segmental distribution of G+C content in Y_1 -fragment (a) and Y_2 -fragment (b) of DNA of phage $\Phi X174$. The distribution function is expressed in terms of the G+C content, x , of sections each containing n base pairs along the DNA fragment, the first section starting with the first base pair, the second with the second base pair and so on until the whole fragment is covered. The last section, therefore starts with $(N-n+1)$ th pair where N is the total number of base pairs in the fragment. Two distributions with $n=19$ and $n=499$ are shown. The binomial distribution for $n=19$ is shown by crosses.

Table 1 Numerical data for the melting peaks

Fragment	Peak no.	$T_m(^{\circ}\text{C})$	$2\sigma^{\dagger}$	Length (base pairs)
Y_1	1 \ddagger	70.8 ± 0.1	0.54	1,570
	2 \ddagger	72.3 ± 0.1	0.53	850
	3	73.4 ± 0.1	0.42	330
Y_2^*	1	70.6 ± 0.1	0.39	680
	2 \ddagger	71.8 ± 0.1	0.56	1,010

Peaks were measured in 0.1 ssc.

* Mean value of two measurements.

\dagger Full width at $0.61 \times$ maximum height.

\ddagger Peak having hyperfine structure.

Y_1 and Y_2 of $\Phi X174$ DNA have well resolved fine structures corresponding to the cooperative melting of sections containing 300–1,500 base pairs (Fig. 1). (2) The base sequences of these fragments are fairly random when observed in short lengths, 'random' being defined in terms of several criteria, such as the nearest neighbour base frequency, autocorrelation of the base sequence and the binomial distribution of the G+C content (Fig. 2). (3) The distribution of the local segmental G+C content shows some deviation from a smooth distribution when it is averaged over several hundred bases (Fig. 2). Whether this distribution is statistical or has some functional significance cannot be determined because of the small number of samples available. (4) The melting profiles seem to reflect the long-range base distributions when Figs 1 and 2 are compared. (5) Detailed analysis shows that cooperativity in the melting of DNA is much stronger than previously expected, and the value of the cooperativity parameter used by Lyubchenko *et al.*³ seems to be more appropriate than that used earlier.

It is important that ambiguous and unrealistic terminology, such as 'homogeneous' or 'random', should not be used to describe the nature of base sequences with limited length. Instead, because the melting profile of a known sequence unambiguously shows several peaks, we are working on the determination of the molecular thermodynamic parameters for the melting of DNA. A quantitative discussion on the relation between the base sequence and the melting profile will be published elsewhere.

We thank Professor M. Takanami for restriction endonuclease.

AKIYOSHI WADA

HIDEKI TACHIBANA

SHIZUE UENO

Department of Physics,
Faculty of Science,
The University of Tokyo,
Tokyo, Japan

YUZURU HUSIMI

Department of Environmental Chemistry,
Faculty of Engineering,
Saitama University,
Urawa, Japan

YASUNORI MACHIDA

Institute of Molecular Biology,
Faculty of Science,
Nagoya University,
Nagoya, Japan

Received 16 June; accepted 8 July 1977.

- Gotoh, O., Husimi, Y., Yabuki, S. & Wada, A. *Biopolymers* **15**, 655–670 (1976).
- Vizard, D. L. & Ansevin, A. T. *Biochemistry* **15**, 741–750 (1976).
- Lyubchenko, Yu. L., Frank-Kamenetskii, M. D., Vologodskii, A. V., Lazurkin, Yu. S. & Gauze, G. G., Jr *Biopolymers* **15**, 1019–1036 (1976).
- Wada, A., Tachibana, H., Gotoh, O. & Takanami, M. *Nature* **263**, 439–440 (1976).
- Akiyama, C., Gotoh, O. & Wada, A. *Biopolymers* **16**, 427–435 (1977).
- Sanger, F. *et al.* *Nature* **265**, 687–695 (1977).
- Machida, Y., Okazaki, T. & Okazaki, R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Hayashi, M. N. & Hayashi, M. *J. Virol.* **14**, 1142–1152 (1974).
- Takanami, M., Okamoto, T., Sugimoto, K. & Sugisaki, H. *J. molec. Biol.* **95**, 21–31 (1975).

matters arising

Generation length and rates of hominoid molecular evolution

BENVENISTE and Todaro¹ recently presented a large and valuable body of DNA annealing data bearing on Old World monkey, ape, and human evolutionary relationships. For an evolutionary biologist, the basic advantages of such data lie in their potential to provide phylogenetic information with minimal reference to other sources of comparative data on the organisms under study. That is, the molecular data set can, in large part, be tested internally and thus tell its own message. We therefore find it disturbing that Benveniste and Todaro have carried out a phylogenetic interpretation of their data using the assumption that generation length affects rates of nucleic acid evolution—especially when one notes that they present on the same page data from their own work which strongly suggest that it does not. We have specific reference to Fig. 1 and Table 2 of ref. 1.

In Fig. 1 of ref. 1 the authors calculate intrahominoid divergence times assuming an Old World monkey-ape time of 30 Myr and a rate of DNA base substitution inversely proportional to the estimated generation lengths involved. That is, longer generation length forms would undergo less rapid DNA evolutionary change. In this fashion, dates reasonably compatible with current palaeontological views of hominoid evolution² are obtained. Thus Benveniste and Todaro fail to note that the data of their Tables 1 and 2 show no such dependence on the proposed generation lengths.

We can reach this conclusion because the generation length hypothesis is, fortunately, subject to direct test. Whatever the divergence time of, for example, the human and gibbon lineages, both have obviously existed for the same length of time since their separation. If the generation length hypothesis were valid, then the human line would have accumulated fewer nucleotide substitutions in that time than the gibbon line. Therefore our DNA would today remain more similar to that of a monkey than is modern gibbon DNA. Specifically we note the human-gibbon $\Delta T_m R$ of 6.9 °C. In the legend to Fig. 1, Benveniste and Todaro state that they assume for the gibbon line a value of 1 °C $\Delta T_m R$ per 3 Myr of evolution, and for the human line 1 °C $\Delta T_m R$ per 5 Myr. Since the

Table 1 Intraprimate relative molecular distances

Taxa compared	DNA*†	Albumin plus* Transferrin Immunology	Time of divergence (Myr)
<i>Homo-Pan-Gorilla</i>	0.14	0.12	4-5
(H,P,G)- <i>Pongo</i>	0.3	0.25	9-11
(H,P,G)- <i>Hylobates</i>	0.36	0.29	11-13
Apes, man-Old World monkeys	0.60	0.57	20-22
Catarrhini-Platyrrhini	1.0	1.0	35-38
Prosimii-Anthropoidea	—‡	2.0	70-75

*Each number in these columns is the ratio of the distance between those taxa and the catarrhini-platyrrhini distance.

†The first three numbers are means of the relevant data points in refs. 1, 3, and 9.

‡Prosimian-anthropoid differences are apparently too large to give reliable DNA data. But, our immunological studies of albumin and transferrin evolution in primates and other mammals show very similar amounts of change along the various primate lineages along with a prosimian-anthropoid distance almost exactly twice that between New and Old World higher primates (platyrrhines and catarrhines)¹⁰. Placing the beginnings of the adaptive radiation leading to modern primates at 70-75 Myr, this gives a platyrrhini-catarrhini separation at ~ 35 Myr (ref. 11).

human and gibbon lines separated, then, the latter would have accumulated 5/3 as many nucleotide substitutions. That is, of the 6.9 °C, 4.3 °C would have accumulated along the gibbon line, and 2.6 °C along the human. Thus when human and gibbon DNAs were compared to that of an Old World monkey (baboon), that of the gibbon should have given a $\Delta T_m R$ 1.7 °C larger than that given by human DNA, and such a difference is several times greater than the experimental errors implicit in such DNA comparisons. Yet precisely this comparison is presented in Tables 1 and 2 of ref. 1, where we note the baboon-human difference to be 9.1 °C, while the baboon-gibbon difference is 9.05 °C. Indeed, all five hominoids tested are in the range 9.15 ± 0.15 °C, that is identical within experimental error. As the actual data, by direct test, fail to show the assumed generation length effect, why allege its influence in time of divergence calculations? This is, after all, not a new issue. Other authors have argued for generation length effects in hominoid molecular evolution^{3,4}, and had their arguments answered⁵⁻⁸. Indeed, Kohne³, though favouring the generation length hypothesis, had nevertheless allocated the same amounts of change along the human and gibbon DNA lineages as long ago as 1970.

In the absence of any documentation of a generation length effect, and with the direct demonstration that amounts of DNA change along the various primate lineages are a function of the temporal lengths of those lineages, we feel amply justified in using the available data to calculate divergence times as in the accompanying Table 1.

The particular virtue of protein and

nucleic acid data is that we can actually count interspecies differences in constant units and allocate them along lineages with minimal dependence on non-molecular evidence. Unfortunately, it has far too often been the case that the molecular data have been forced into a misguided congruence with what is thought to be hard evidence from a sparse and spotty primate fossil record—thereby long-delaying the day when those data will achieve their proper and invaluable place in providing that framework within which to most usefully view other bodies of evolutionary evidence, including those deriving from the fossil record.

VINCENT M. SARICH
JOHN E. CRONIN

Departments of Anthropology
and Biochemistry,
University of California,
Berkeley, California 94720

- Benveniste, R. E. & Todaro, G. J. *Nature* **261**, 101-108 (1976).
- Simons, E. L. *Primate Evolution* (Macmillan, New York, 1972).
- Kohne, D. E. *Q. Rev. Biophys.* **3**, 327-375 (1970).
- Lovejoy, O., Burstein, H. & Heiple, K. H. *Science* **176**, 803-806 (1972).
- Sarich, V. M. *Biochem. Genet.* **7**, 205-212 (1972).
- Sarich, V. M. & Wilson, A. C. *Science* **171**, 1144-1147 (1973).
- Sarich, V. M. *Yb. Phys. Anthro.* **17**, 98-112 (1973).
- Wilson, A. C., Carlson, S.S. and White, T. J. *A. Rev. Biochem.* **46**, 573-639 (1977).
- Hoyer, B. H., van de Velde, N. W., Goodman, M. & Roberts, R. B. *J. hum. Evol.* **1**, 645-649 (1972).
- Sarich, V. M. & Cronin, J. E. in *Molecular Anthropology* (eds Goodman, M. & Tashian, R. E.) 139-168 (Plenum, New York, 1977).
- Cronin, J. E. & Sarich, V. M. *J. hum. Evol.* **4**, 357-375 (1975).

TODARO AND BENVENISTE REPLY—We are aware of the long-standing discussion in the literature concerning the effect of generation length of a species on the rate of accumulation of base-pair mutations

in cellular DNA (refs 1, 2) and amino acid substitutions in proteins^{3,4}. But, the main conclusions of our paper did not depend on knowing the proper corrections to apply, if any, for the lengthened generation times in the higher apes and man as compared to the Old World monkeys. The conclusions were that (1), nucleic acid sequences related to the genome of the genetically transmitted baboon type C virus can be detected in the cellular DNA of all Old World monkeys and apes; (2), the baboon type C virus specific sequences can be used to discriminate between those Old World monkeys and apes whose habitat is Africa (baboon, mangabey, African green monkey, colobus, chimpanzee, gorilla) and those whose habitat is Asia (various macaques, langurs, gibbon, orangutan); (3), the data obtained with human DNA closely fit the pattern observed with the Asian apes. The suggestion was therefore made that most of man's evolution since his divergence from the other apes had occurred in Asia rather than in Africa.

Sarich and Cronin point out that the hypothesis that animals with longer generation times accumulate fewer mutations per unit of chronological time as suggested by Kohne¹, Lovejoy⁵, and others is subject to a direct test. The accumulation of nucleotide substitutions along the gibbon lineage, where the generation length has changed less, compared to Old World monkeys, should be more than it is along the human lineage where the time of sexual maturity and the maximum lifetime potential⁶ have markedly increased. Although we agree with him that this is experimentally testable, the differences would be small. If man had a common ancestor with the chimpanzee and gorilla as recently as 4 Myr ago as Sarich has proposed^{7,8}, then the effect of the recent prolonged generation time of ancestral man would be even more difficult to detect. We would not conclude that the cellular DNA data as reported in the manuscript was performed in a sufficiently rigorous fashion to 'prove' the lack of effect of a generation time. In our initial studies we did not see a difference along the two lineages, but more refined studies directed specifically at that question might reveal small differences; such a study is currently in progress.

Our data concerning the relationships among the cellular DNA of Old World monkeys and apes agree quite well with the extensive immunological studies of Sarich, Cronin, and others. For example, we state that 'the extent of nucleic acid sequence divergence among chimpanzee, gorilla, and man is three to four times less than that between any of these three Homininae and the Old World monkeys'. But, there is also a considerable body of palaeontological evidence suggesting that apes and hominids had differentiated long before the time Sarich and associates

place the chimp-gorilla-man common ancestor^{6,9}. With methods for dating fossils improving, this is now a substantial body of data we felt should not be ignored. It will be intriguing to see if the traditional palaeontological approach can be correlated to the data being obtained with respect to protein and nucleic acid evolution, or whether the discrepancy in the two methodologies is as large as Sarich suggests.

E. J. TODARO
R. BENVENISTE

NIH Bethesda, M.D.

- ¹ Kohne, D. E. *Q. Rev. Biophys.* 3, 327-375 (1970).
- ² Kohne, D. E., Chiscon, J. A. & Hoyer, B. H. *J. hum. Evol.* 1, 627-644 (1972).
- ³ Goodman, M., Barnabas, J. & Moore, G. W. J. *J. hum. Evol.* 1, 663-686 (1972).
- ⁴ Goodman, M., Moore, G. W. & Matsuda, G. *Nature* 253, 603-608 (1975).
- ⁵ Lovejoy, C. O., Burnstein, A. H. & Heiple, K. G. *Science* 176, 803-805 (1972).
- ⁶ Cutler, R. G. *J. hum. Evol.* 5, 169-202 (1976).
- ⁷ Sarich, V. M. in *Perspectives on Human Evolution* (eds Washburn, S. L. & Jay, P. C.) 94 (Rinehart, Holt and Winston, New York, 1968).
- ⁸ Sarich, V. M. & Wilson, A. C. *Proc. natn. Acad. Sci. U.S.A.* 63, 1088-1091 (1969).
- ⁹ Simons, E. L. *J. hum. Evol.* 5, 511-528 (1976).

Complementation of immune response genes for (T,G)-A-L

Two years ago we reported the results of experiments which demonstrated the *in vivo* complementation of immune response genes required for a response to (T,G)-A-L (ref. 1). Subsequent experiments seemed to confirm and extend this result². Unfortunately others have been unable to repeat our results in as much as they find no evidence for complementation in the response to (T,G)-A-L (ref. 3). Recently in attempts to repeat our original findings with (B10.M×B10.Br)F₁ we also have been unable to do so but now find responses to (T,G)-A-L similar to those reported by others³. We wish, therefore, to express our reservations concerning complementation in the (B10.M×B10.Br)F₁ in the hope that further study of this phenomenon by ourselves and others will clarify the conditions in which genetic complementation in the response to (T,G)-A-L can occur.

A. J. MUNRO
M. J. TAUSSIG

Department of Pathology,
University of Cambridge,
Tennis Court Road,
Cambridge, England

- ¹ Munro, A. J. & Taussig, M. J. *Nature* 256, 103-107 (1975).

- ² Munro, A. J., Taussig, M. J. & Archer, J. in *3rd Ir gene workshop* (ed. McDevitt, H. O.) (Academic, New York, in the press).

- ³ McDevitt, H. O. in *The Role of Products of the Histocompatibility Complex in Immune Responses* (eds Katz, D. H. & Benacerraf, B. A.) 321-326 (Academic, New York, 1976).

High explosive analogue of the Tunguska Event

JONES¹, observes the existence of an important, but little known experiment. The numerical conclusions however are incorrect, using only the

data cited in the letter. Using the ratio 35,000 to 130 as the diameters of symmetric explosion phenomena for the Tunguska event and the 1966 experiment cited by Jones, respectively, and the relevant scaling law, the corresponding equivalent explosive source strength for the Tunguska event would appear to be of the order of $50 \times (35,000/130)^3$, or about 10^9 t, not the 10^3 t cited. Without accurately computing all the necessary corrections for energy partitioning, actual hydrodynamics, etc., this would provide an estimate of source strength about two orders of magnitude higher than ref. 3 cited therein concludes.

B. W. AUGENSTEIN

Rand,
1700 Main Street,
Santa Monica,
California 90406

- ¹ Jones, G. H. S. *Nature* 267, 605 (1977).

JONES replies: Augenstein is correct, and the calculated values should have read 10^5 kt TNT and 200 Mt nuclear equivalent, these being minimum estimates. The Tunguska event devastated an area 35 km in diameter! It is commented, however, that the very low height of burst, almost a surface burst at this yield, should be retained despite the absence of a crater at Tunguska. I have discussed elsewhere (Suffield Reports 217 and 281), experimental evidence that high yield but low energy density explosions on the surface do not cause craters but do produce airblast and surface seismic effects similar to those from high density explosions. Forest blowdown, for example, is insensitive to the initial energy density but depends upon the total energy release. The lack of a crater at Tunguska is thus evidence of the low density, presumably cometary nature of the bolide.

G. H. S. JONES

Box 1404 Station B,
Ottawa, Canada

β -Globin mRNA in Ferrara β^0 -thalassaemia

In their paper on the presence of β -mRNA in Ferrara type β^0 -thalassaemia, Ottolenghi *et al.*¹ stated that our laboratory had obtained "similar findings" to their's with material from one of their patients. Because of possible misinterpretations of that very general statement, we present here the limited data which we did obtain using Ferrara material.

Professor Conconi kindly provided us with ribosomes from one of his patients so that we could attempt to

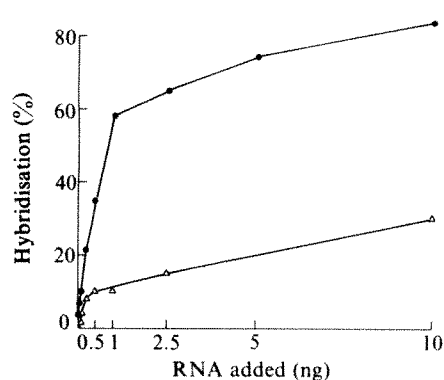


Fig. 1 Hybridisation of Ferrara β^0 -thalassaemia RNA to human α - and β -cDNA. ●, α -DNA; Δ , β -cDNA.

demonstrate β -globin mRNA, in RNA extracted from these ribosomes, by fingerprint analysis of ^{32}P -labelled cRNA synthesised from cDNA²; cDNA was obtained but the cRNA synthesis failed. We did, however, use a small amount of the starting RNA to assay its relative content of α and β mRNA by molecular hybridisation to human α and β globin cDNA as previously described³. A small amount of β or β -like mRNA was detected (Fig. 1). Unfortunately there was insufficient RNA available to obtain a plateau of hybridisation with β -cDNA but the slope of the β -cDNA hybridisation curve predicts a β -mRNA content in the sample of approximately 4% relative to that of the α mRNA in the same sample. Because we lacked sufficient material to demonstrate a plateau or saturation of hybridisation of Ferrara β -like mRNA with β -cDNA, our results do not help to resolve the discrepancy between the results of Ottolenghi *et al.*¹ and those of Ramirez *et al.*⁴. The latter found that the Ferrara β -like mRNA hybridised incompletely with β cDNA (in contrast to complete hybridisation claimed by Ottolenghi *et al.*) and suggested the presence of a substantial abnormality in the structure of Ferrara β mRNA.

Our hybridisation results with Ferrara thalassaemia mRNA are similar to those which we have obtained⁵ with total reticulocyte RNA from a variety of β^0 -thalassaemic patients of different ethnic backgrounds (Southern Italian, Greek, Algerian, Asian and Chinese). In some of these patients there is less than 1% as much β -like mRNA as α mRNA⁶ and this could be due to cross hybridisation of δ -chain mRNA of HbA₂ to β cDNA. In approximately half the patients, however⁵, we have found 4–15% as much β -like mRNA as α mRNA; the latter value is probably too high to be accounted for by δ -chain mRNA alone. Other workers have found substantially more β -like mRNA

in non-Ferrara β^0 thalassaemic reticulocytes^{4,7}. We were unable to identify, by fingerprint analysis of ^{125}I -labelled mRNA, β -chain specific oligonucleotides in RNA from two of our patients with low levels of β -like mRNA detected by hybridisation⁵. But, Temple *et al.*⁸ have been successful in detecting β -chain specific mRNA sequences in one of their patients with β^0 -thalassaemia.

We suggest that until specific structural differences in the β -globin gene of β^0 -thalassaemics are detected by gene mapping or nucleotide sequence studies, one cannot make any definitive conclusions as to the precise molecular basis of the varied forms of β^0 -thalassaemia. The β -globin genes seem to be present in all cases of β^0 -thalassaemia in which they have been sought (refs 4, 7–9 and Tuan, D.G.H. and B.G.F., unpublished). The different results obtained by hybridisation assays of globin mRNA need to be related to specific structural defects in the β -globin gene DNA before one can feel confident about classifying the β^0 -thalassaemias found in different populations into specific molecular subtypes.

BERNARD G. FORGET

Department of Medicine,
Yale University School of Medicine,
New Haven, Connecticut 06510

DIANE G. HILLMAN

Division of Hematology-Oncology,
Children's Hospital Medical Center,
Boston, Massachusetts 02115

- ¹ Ottolenghi, S. *et al.* *Nature* **266**, 231–234 (1977).
- ² Forget, B. G. *et al.* *Ann. N.Y. Acad. Sci.* **241**, 290–309 (1974).
- ³ Forget, B. G. *et al.* *Cell* **7**, 323–329 (1976).
- ⁴ Ramirez, F. *et al.* *Nature* **263**, 471–475 (1976).
- ⁵ Forget, B. G., Hillman, D. G., Cohen-Solal, M. & Prensky, W. *Blood* **48**, 998 (1976).
- ⁶ Forget, B. G., Benz, E. J., Jr., Skoultschi, A., Baglioni, C. & Housman, D. *Nature* **247**, 379–381 (1974).
- ⁷ Kan, Y. W., Holland, J. P., Dozy, A. M. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5140–5144 (1975).
- ⁸ Temple, G. F., Chang, J. & Kan, Y. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3047–3051 (1977).
- ⁹ Tolstoshev, P. *et al.* *Nature* **259**, 95–98 (1976).

OTTOLENGHI ET AL. REPLY—Forget and Hillman conclude that the different results obtained by hybridisation assay of globin mRNA by various groups^{1–5} do not yet allow the classification of β^0 -thalassaemia found in different populations into specific molecular subtypes; this conclusion is based on the wide range of values reported for β -globin-like RNA in non-Ferrara β^0 -thalassaemic patients, and the overlapping of some of these values with those determined by ourselves⁵ and by Forget and Hillman in Ferrara β^0 -patients. As has been pointed out by Benz^{6,7}, however, discrepancies in the level of β -like sequences found in β^0 -thalassaemias may arise as the result of technical differences between laboratories.

In our laboratory we have compared

several different groups of patients. β -like mRNA has been either undetectable in non-Ferrara β^0 -thalassaemic reticulocytes⁸ or present at levels of less than 1% relative to α -sequences (a value that is compatible with cross-hybridisation between cDNA _{β} and mRNA _{δ} sequences), whereas in reticulocytes of Ferrara β^0 -thalassaemics levels of up to 8% can be detected. Moreover, when sufficient RNA has been available, the T_m of the hybrid formed between the low amounts of ' β -like RNA' from non-Ferrara β^0 -thalassaemics (? δ -mRNA) and cDNA _{β} has been 7–8 °C lower than that found for true β -mRNA–cDNA hybrids⁸, again in significant contrast with what we find in Ferrara thalassaemia.

We, therefore, conclude that our results (refs 5, 9 and unpublished) taken together with the translational data of Conconi's group^{10,11} allow us to divide β^0 -thalassaemia into at least two different types, a β -mRNA negative type and a β -mRNA positive type; whether other forms of β^0 -thalassaemia in which significant levels of β -mRNA have been demonstrated have any relationship with the Ferrara type of thalassaemia remains to be investigated, and we agree will require translational experiments, as well as gene mapping and nucleotide sequence studies.

S. OTTOLENGHI

P. COMI

B. GIGLIONI

Institute of General Pathology,
Centro per lo Studio della
Patologia Cellulare del CNR,
Milan, Italy

R. WILLIAMSON

Department of Biochemistry,
St Mary's Hospital Medical School,
London W2, UK

G. VULLO

Divisione Pediatrica,
Arcispedale Sant'Anna,
Ferrara, Italy

L. DEL SENNO

F. CONCONI

Centro di Studi Biochimici
sul Morbo di Cooley,
Cassa di Risparmio di Ferrara,
Institute of Biological Chemistry,
University of Ferrara, Italy

- ¹ Forget, B. G., Benz, E. J., Jr., Skoultschi, A., Baglioni, C. & Housman, D. *Nature* **247**, 379–381 (1974).
- ² Kan, Y. W., Holland, J. P., Dozy, A. M. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5140–5144 (1975).
- ³ Forget, B. G., Hillman, D. G., Cohen-Solal, M. & Prensky, W. *Blood* **48**, 998 (1976).
- ⁴ Ramirez, F. *et al.* *Nature* **263**, 471–475 (1976).
- ⁵ Ottolenghi, S. *et al.* *Nature* **266**, 231–234 (1977).
- ⁶ Benz, E. J., Jr. *Nature* **263**, 635–636 (1976).
- ⁷ Benz, E. J., Jr. *et al.* *Clin. Res.* **23**, 269a (1975).
- ⁸ Comi, P. *et al.* *Eur. J. Biochem.* (in the press).
- ⁹ Tolstoshev, P. *et al.* *Nature* **259**, 95–98 (1976).
- ¹⁰ Conconi, F., Rowley, P. T., del Senno, L., Pontremoli, S. & Volpato, S. *Nature new Biol.* **238**, 83–87 (1972).
- ¹¹ Conconi, F. *et al.* *Nature* **254**, 256–259 (1975).

reviews

Introducing relativity

Paul Davies

Essential Relativity: Special, General and Cosmological. Second edition. By W. Rindler. Pp. xv+284. (Springer: New York and Berlin, 1977.) DM67.70; \$29.80.

AFTER eight years I still find Rindler's book one of the best introductions to the theory of relativity, and I was eager to read his second edition. The title *Essential Relativity* is an apt one, for even though this edition contains some new material, only the bare essentials of the subject are discussed. This is not a drawback, because Rindler manages somehow to get very far with very little investment in detailed calculation or protracted conceptual deliberation. In this respect it makes ideal introductory reading for students of physics, although mathematics students will not find much in the way of modern differential geometry or topology.

In the preface to the second edition the author explains how the emphasis of the book is on the conceptual foundations rather than the mathematical development of relativity theory. Proofs are kept to a minimum and many examples and mathematically-oriented discussion are only punctuated by, rather than rooted in, hard equations. Nevertheless, the reader is taken swiftly and effortlessly (maybe somewhat deceptively) through pre-Einstein ideas of space and time, basic special relativity, Minkowski space and four-vectors, tensors, particle mechanics and electrodynamics. Then we move into general relativity, which starts out with the most 'conceptual' section of the book, in which the idea of curved space-time is explained in just a few pages. The Schwarzschild metric just seems to pop out from this discussion, to be followed by an updated description of black holes and the Kuskal manifold.

When I re-read the section on uniformly accelerated observers in Minkowski space, I was disappointed to find that the author, whose name is famous for his connection with the co-ordination of Minkowski space based on a collection of accelerating observers, had not chosen to expand more on this very useful construction. I have found the ideas of uniform acceleration both useful and elegant exercises for undergraduates, as well as

a very profitable analogy for the more advanced discussions of black holes.

The book concludes with a section on cosmology, and once again I was interested to see if Rindler's discussion of horizons had been elaborated. The earlier edition was one of the first undergraduate textbooks of its time to try and present this subject conceptually. The new edition contains the familiar remarks about crawling bugs and rubber sheets, as well as the usual mathematical conditions for horizons, but also includes some new material and diagrams to help untangle some of the apparent paradoxes associated with them.

Although the organisation of the material is largely the same as before, many sections have been re-written, in the same lucid style. New sections on the plane gravitational wave and linearised general relativity, as well as two appendices on curvature and Maxwell theory, have been added. I was disappointed at the price for a book which should be aiming at the 'mass' student market, to which it is admirably suited. How many undergraduates will pay nearly £20 for a basic course textbook? □

Paul Davies is Lecturer in Mathematics at King's College, University of London, UK.

Immunological series

Comprehensive Immunology. Vol. 1: Immunology and Aging. Edited by T. Makinodan and E. Yunis. Pp. 208. Vol. 2: Biological Amplification Systems in Immunology. Edited by N. K. Day and R. A. Good. Pp. 325. (Plenum: New York and London, 1977.) \$27 each volume.

THE new series *Comprehensive Immunology* is intended eventually to cover all aspects of immunology with "definitive analyses, thoughtful reviews and probing discussions". There already exist several good series on immunological subjects and the launching of another cannot be greeted with un-mixed joy. These first two volumes, however, are well produced and have some valuable features. If their standard can be maintained, the series should prove useful.

The appearance of *Immunology and Aging* is timely, since no comprehensive review has been available and increasing interest is being shown in the field. It is also a good moment to look back over the large amount of data that have accumulated on ageing in relation to the immune system. We are still far from understanding this relationship, and it is certainly complex. The thymus and T lymphocytes, however, are widely believed to be among the central characters in the drama, and several papers emphasise this from various points of view. The known intricacies of the membrane structure and physiological functions of T lymphocytes have

been multiplying so rapidly of late that T-dependent interpretations of gerontological phenomena tend to age rather quickly; but this problem should be resolved within the next few years. Among other good chapters are two dealing with germ-free mice and congenitally athymic (nude) mice and their value in the study of ageing. Overall, the volume can be recommended to anyone seeking an introduction to both biological and clinical aspects of the subject, and the extensive reference lists make it an excellent source book.

I tried the title *Biological Amplification Systems in Immunology* on several immunological colleagues, inviting speculation on the contents of this volume. They produced a range of suggestions, none of which approximated to the truth. I can now reveal exclusively to readers of *Nature* that the book is about complement. Probably an expert on complement would have tumbled to it instantly, but then I don't think that the book is really intended for him. The 15 chapters cover the multiple roles of the complement system, as well as its biosynthesis, phylogeny, ontogeny and genetics. There are also discussions of leukocyte chemotaxis and of pathways activated by Hageman Factor. It seems a pity that this useful collection of reviews should be hidden under such an enigmatic title.

H. S. Micklem

H. S. Micklem is Reader in Zoology at the University of Edinburgh, UK.

Unparalleled textbook of plant anatomy

Anatomy of Seed Plants. Second edition. Pp. 550. By Katherine Esau. (Wiley: London and New York, 1977.) \$21.25; £11.50.

TWENTY FOUR YEARS AGO, Professor Katherine Esau published her first book, *Plant Anatomy*. This book rapidly established itself as a standard by which all others have subsequently been judged. As Professor Esau herself points out, however, there was also a need for a shorter book for students who have relatively limited experience in the study of plants. For that reason *Anatomy of Seed Plants* was first published in 1960 and is now seen in its second edition.

Anatomy of Seed Plants still has to be viewed against the broader, and deeper, backcloth set by *Plant Anatomy* itself, but it remains an attractive, informative and authoritative book in its own right, even if its price will restrict ownership more severely than one might hope. An important feature of this second edition is that it incorporates very significant changes and is very much more than a new edition in name alone.

An immediately apparent change is in the page format which is now enlarged to 23.3 cm × 18.9 cm from the previous 22.8 cm × 14.9 cm—a 30% increase in area. This, together with the fact that the book is extended from 376 to 550 pages, has allowed incorporation of much new material, although the larger page size has often been wasted where micrographs and diagrams from the first edition have been reproduced at the same size. The pleasant format, together with two-column presentation, a clearly legible type-face and a balanced distribution of text and illustrations, has led to the production of a book that demands to be read.

Having attracted the reader's attention, what is to be found inside? A major, and immediately obvious feature is the inclusion of numerous electron micrographs. There were only a couple in the first edition, but the present book is liberally laced with them and they are, as are most of the other illustrations chosen, of good quality. If anything, the half-tones in the present volume are of better contrast than those in the original.

Professor Esau's attention to detail is prodigious and only the closest scrutiny of the book reveals many of the smaller changes that have been

made to take account of advances in knowledge or interpretation over the past 16 years. (The abbreviation for micrometre is now given in its proper form (μm) but it is a pity that the Ångström lingers on.) The enormous task of encompassing new information without completely losing sight of the old has largely been achieved by combining subject matter as much as possible, and by referring only to key items of interest within a particular area of study. Similarly, the references, though strictly limited, serve to acquaint the reader with the most recent work and generally include at least one paper from each main laboratory contributing to a specific field of interest. In this way, *Anatomy of Seed Plants* is able to cover a large subject area while allowing the reader

the opportunity to find his way more deeply into plant anatomy if he so desires.

Anatomy of Seed Plants is unashamedly a textbook of anatomy. As such it is unparalleled. Although the modern trend is, and must be, to combine structure and function in our approach to the way plants work, this can only be accomplished if our basic knowledge of plant anatomy is both broad and sound. Professor Esau has ensured that no botanist—whether student or research worker—will have the excuse that the right information is not readily, and attractively, to hand.

A. W. Robards

A. W. Robards is Senior Lecturer in the Department of Biology at the University of York, UK.

Diatom biology

Biology of Diatoms. Edited by Dietrich Werner. Pp. 498. (Blackwell Scientific: Oxford, 1977.) £17.

FEW algal groups have seen such an expansion of interest over the past two decades as the diatoms, and this volume admirably covers the recent developments. It may come as a surprise to many to read on the first page that it is estimated that diatoms contribute 20–25% of the world's net primary production—a figure derived from experimental studies which almost certainly underestimate true production. Such production at the base of so many food chains ought to stimulate work on grazing, yet this is hardly begun and is mentioned on only a few pages.

Although the title might imply that this book can be read to obtain an overall survey of the diatoms, this is not so, and a student with little knowledge of the group would find it difficult. There is no section on morphology, no simple explanation of cell division, few illustrations of the major groups; and those given are of centric genera leaving a student to guess at pennate morphology.

Growth, nutrition, chemical composition, movement, sexuality, ecology are all discussed; in these chapters, each written by a different author, there is a wealth of data and excellent references. The authors of the chapter on the ecology of marine planktonic diatoms have assembled a mass of fascinating data on distribution in the seas of the world which is a valuable contribution to the much neglected topic of biogeography.

In many places, however, the text is clearly written in review form and

many statements merely stimulate and necessitate consultation of the original papers. The chapter on sexuality will be of great value to many students since much of the excellent original work is very discursive and written in German.

A chapter on ultrastructure reviews the literature but is very poorly illustrated with three very indistinct pictures, all of *Cylindrotheca* which has an unusual morphology. This is a pity since there are now several excellent studies of diatom ultrastructure. Also, apart from a few photographs of centric diatoms, the considerable body of information on the ultrastructure of the siliceous components has not been utilised. The old concept of diatoms as a class of the Chrysophyta again crops up in some chapters although they were clearly recognised as a division, Bacillariophyta, in the system of Husted (1930) and are so distinctive that there can be surely little argument about this in 1977.

Biochemical and physiological studies have blossomed in the past two decades, and the information in the chapters on growth, silicate metabolism, photosynthesis, heterotrophic nutrition and biochemical composition will surprise many who have not followed the field closely. The chapter on silicate metabolism is of great value, since it is a synthesis of chemical and biological studies and provides a valuable introduction to the complexities of silicon chemistry.

The combination of authors has resulted in some repetition of data in several chapters but not in any serious way. The book is well produced, relatively free of errors and will be a source of considerable value for many years to come.

F. E. Round

F. E. Round is Reader in Phycology at the University of Bristol, UK.

Physiological behaviour of cells

Analytical Physiology of Cells and Developing Organisms. By B. C. Goodwin. Pp. 249. (Academic: London and New York, 1977.) £8.50; \$18.50.

THE aim of this book is to present a picture of the physiological behaviour of cells in terms of molecular biology and mathematical models. As the author acknowledges, the choice of material is not comprehensive and reflects his own interests and prejudices. In the first three chapters, Goodwin examines aspects of the behaviour of individual cells. The metabolic network, the adaptation time of which is of the order of seconds, is considered in terms of allosteric enzymes and end-product inhibition. This network is contrasted with the epigenetic one with longer adaptation times (minutes to hours) and in which gene activation and protein synthesis are now major elements. The occurrence of feedback loops in both kinds of systems can lead to complex dynamics such as oscillations. Analogies with statistical thermodynamics are drawn; and this rather formal analysis is extended to the cell cycle.

In considering the various internal networks involved, the important point is made that there may be no single reaction controlling the entry of a cell into the cell cycle: emphasis should be placed instead on a mixture of probabilistic and deterministic processes. The treatment of the cell cycle is, however, unsatisfactory since it provides an inadequate review of current ideas and experiments. For example, although mechanisms based on chalones which are inhibitory are considered, there is little reference to molecules exerting a positive control, for which there is increasing evidence. A nice chapter on biological clocks follows, with an explanation of Winfree's ingenious approach to the eclosion clocks in *Drosophila*. Here, mathematical analysis leads to experiments that could not really be conceived of without that analysis. Unfortunately, most of the other analyses in the book do not seem to have a similar experimental spin-off.

Pattern formation and its relation to gradients and positional information is given quite detailed attention. Emphasis is placed on periodic processes and a membrane wave model is put forward. A formal model to account for the properties of the polar coordinate model of French, Bryant and Bryant is proposed. Goodwin believes in the next

few years we will see the emergence of new dynamical models in which waves and periodicities will feature very strongly. He recognises that gradient-type models of positional information shift the major problem of pattern formation into the area of how the gradients are interpreted by the cells in terms of gene activity, but gives this central theoretical problem inadequate attention.

In the final chapter, he treats the organism as a cognitive and cooperative system. He treats the cell as if it had knowledge about the world which is contained in its control circuits. He also wishes to regard sharp transitions of state at different levels of organisation as being basically similar and analogous to phase transitions.

Goodwin is always worth reading for his independence and insights, but overall this book is disappointing. The mathematics seems to add rather little and too seldom leads to experiments. The approach is not didactic or comprehensive. For theoreticians, however, it is essential.

Lewis Wolpert

Lewis Wolpert is Professor of Biology as Applied to Medicine at the Middlesex Hospital Medical School, London, UK.

Quantum theory

Qualitative Methods in Quantum Theory. By A. B. Migdal. Pp. 435. (W. A. Benjamin: Reading, Massachusetts and London, 1977.) \$21.50.

"THE solution of most problems in theoretical physics begins with the application of the *qualitative methods* which constitute the most attractive and beautiful characteristic of this discipline. By 'qualitative methods' we mean dimensional estimates and estimates made by using simple models, the investigation of limiting cases where one can exploit the smallness of some parameter, the use of the analytic properties of physical quantities, and finally the derivation of consequences from the symmetry properties, that is, the invariance relative to various transformations (eg Lorentz or isotopic invariance). However, as experience in the classroom shows, it is just these aspects of theoretical physics which are most difficult for the beginner." So Academician Migdal introduces the new edition of his book, the first three chapters of which were published in much the same form as

in *Approximation Methods in Quantum Mechanics* (Izdatelstvo Nauka, 1966; English translation, W. A. Benjamin, 1969).

Migdal is a leading exponent of the Landau school of theoretical physics, the members of which have been brought up to know all nine volumes of Landau and Lifshitz. So in this book we have examples from a wide range of subjects. The first chapter covers mainly models in atomic physics. Chapter two is on perturbation theory, and chapter three is on the WKB method and the quasi-classical approximation, a subject which is not normally given much space in textbooks. Chapter four is on analyticity and dispersion relations. Chapter five is on quasi-particle methods in the many-body problem, with applications to quantum fluids and nuclear matter. Chapter six gives a qualitative physical treatment of renormalisation, and a discussion of the Gell-Mann-Low equation and asymptotic freedom.

This book should be required reading for any serious graduate student in theoretical physics.

Norman Dombey

Norman Dombey is Reader in Theoretical Physics at the University of Sussex, UK.

BOOKS

ON PURE
AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £7.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Renaissance in laser spectroscopy

Frontiers in Laser Spectroscopy. (Les Houches 1975 Session XXVII) Vol. 1. Pp. 472. Vol. 2. Pp. 473–907. Edited by Roger Balian, Serge Haroche and Sylvain Liberman. (North-Holland: Amsterdam, New York and Oxford, 1977.) Dfl. 280; \$114.50.

THESE two volumes present the proceedings of the Les Houches Theoretical Physics Summer School held in July 1975 on the use of lasers in atomic and molecular spectroscopy. The stated aim of the school was to "give a general presentation of the theoretical background necessary to understand light-matter interaction problems, and to present a review of the various new methods in which lasers are now being used to improve our knowledge about atoms, molecules and even about nuclei." Underlying these new methods are the recent exploitation of tunability, of saturation and of coherent transients which have contributed so much of late to the renaissance in spectroscopy. The level of presentation of the proceedings tends to assume previous knowledge at about first-year graduate student level of quantum optics and quantum electronics, and the scope of the discussion is very wide with most of the actively-pursued topics in the field at least mentioned.

Cohen-Tannoudji presents a marginally telegraphic overview of the basic phenomena involved in the interaction of atoms with strong resonant fields, and his thoughtful lectures contain many insights into fundamental problems involving the non-linear saturation of fluorescent emission. This is followed in Volume 1 by lectures on laser theory (Sargent); transient and steady-state response of two- and three-level systems, including a very useful discussion of the semiclassical theory of superradiance (Feld); the theory and observation by stark-switching of optical nutation, free-induction decay, photon echoes and two-photon transitions (Brewer); and single-atom and co-operative quantum beats (Stenholm). These lectures form a coherent and well-presented review of a physically well-defined area.

In Volume 2, this coherence is lost to a certain extent by an attempt to cover a very wide range of techniques and specific applications. The use of lasers in very high resolution spectroscopy of molecules is discussed by Lehmann, Oka, Kelley and Javan. Letokhov discusses the coupling be-

tween electronic and nuclear degrees of freedom in laser-driven transitions and the so-far purely theoretical subject of "laser-nuclear spectroscopy."

Finally, one of the topics which ensures adequate funding, namely isotope separation, is admirably discussed by Letokhov. Also included in these volumes are short seminars by Series, Walther, Cagnac, Haroche, Liberman, Toschek, Mukamel and Monchalin, which are useful as illustrations of particular research problems, although a few should have been (and some have been) published in the primary literature.

Some of these lectures are rather experimental in tone; these though no less valuable, do not quite fit the label of "Ecole d'Été de Physique Théorique." Indeed, an interesting feature

of this field is the ability of the best practitioners (some of them contributing to these volumes) to cross the experimental-theoretical divide to the great benefit of both sides. Since there were only 42 "pupils" attending the school, one would hope that the publication of the proceedings would give a wider circulation to these valuable lectures. The price of these volumes seems designed to ensure that this will not be so. I doubt the value of such lavish publication of conference and Summer school proceedings in hard-back textbook format, with its concomitant delay and inflated cost.

Peter Knight ↑

Peter Knight is Jubilee Research Fellow in Physics at Royal Holloway College, University of London, UK.

Explosion in myelin research

Myelin. Edited by P. Morell. Pp. xxii+531. (Plenum: New York and London, 1977.) \$47.40.

DURING the past decade there has been an explosive increase in research on myelin. Now with the publication of a major book on the subject, it is possible to see why. Myelin is one of the most abundant of biological membranes constituting about 50% of the dry weight of the white matter, from which relatively pure fractions can be easily isolated by centrifugation. Such procedures are described in the book together with tabulated data of lipid and protein analysis of myelin fractions of central and peripheral nerve. Extensive biophysical data (for example, X-ray and neutron diffraction) has been correlated with the chemistry and the ingenious use of probe analysis. As a result, we probably know more about the molecular architecture of myelin than many other biological membranes.

The first chapter contains a great deal of impressive and well illustrated information on the morphology of the myelin-formative glial or Schwann cells. Although little is known about the mechanisms controlling the transition stage between glial plasma membrane and the fully mature compact myelin lamellae, the glial cell must, during the period of myelination, have a remarkable synthetic activity, for it seems to be capable of making three times its weight of myelin per day. The myelinating brain therefore provides a unique system for studying membrane biosynthesis. Some of the relevant precursor-product labelling

studies and the so far equivocal attempts at isolation of intermediary 'myelin-like' fractions from developing brain are critically discussed by different contributors.

Study of the biology of myelin is, however, of more than academic interest for in several neurological diseases there is damage to the white matter with accompanying loss of myelin. In the otherwise excellent chapters on neuropathology and neurology of myelin diseases more space could have usefully been devoted to multiple sclerosis, since this is one of the commonest neurological diseases.

Other chapters cover chemical pathology, experimental pathology and animal models of genetic myelin disorder. One of the most important features of myelin pathology relates to the pronounced immunological response to the protein antigens and lipid haptens (for example, cerebroside). This subject is especially relevant following the recent discovery of a relaxing and remitting form of experimental allergic encephalitis.

It will be evident that the whole intriguing subject of the biology of myelin is well covered in this book and it can be recommended as a readable and authoritative account of the subject.

Although the book is on a specialist subject it can be recommended to the general reader who might thereby be encouraged to utilise some of the basic concepts and discoveries on myelin in other fields of biology.

A. N. Davison

A. N. Davison is Professor of Neurochemistry at the Institute of Neurology and Head of the Department of Neurochemistry and Chemical Pathology, National Hospitals for Nervous Diseases, London, UK.

Recent scientific and technical books

Mathematics

- ALCOCK, Donald. Illustrating Basic: (A Simple Programming Language). Pp.ix+134. ISBN-0-521-21703-2. (Cambridge, London and New York: Cambridge University Press, 1977.) Hard cover £4.95; Limp cover £1.90.
- CAMERON, Peter J. (edited by). Combinatorial Surveys: Proceedings of the Sixth British Combinatorial Conference, Merton College, Oxford, England. Pp.vii+226. ISBN-0-12-157150-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £7; \$13.65.
- JACOBSON, David H. Extensions of Linear-Quadratic Control, Optimization and Matrix Theory. (Mathematics in Science and Engineering: A Series of Monographs and Textbooks, Vol. 133.) Pp.x+217. ISBN-0-12-378750-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £6.50; \$12.75.
- LAKSHMIKANTHAM, V. (edited by). Nonlinear Systems and Applications: An International Conference. Pp.xvi+700. ISBN-0-12-434150-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$25; £17.75.
- NAGATA, Masayoshi. Field Theory: (Pure and Applied Mathematics: A Program of Monographs, Textbooks, and Lecture Notes: Vol. 40). Pp.vii+268. ISBN-0-8247-6466-8 (New York and Basel: Marcel Dekker; 1977) \$Fr.78.
- PORTER, Richard D. Introduction to Fibre Bundles (Lecture Notes in Pure and Applied Mathematics, Vol. 31). Pp.v+170. ISBN-0-8247-6626-1. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr.65.

Astronomy

- COLLOQUE DE L'UNION ASTRONOMIQUE INTERNATIONALE. No. 37: Décalages vers le Rouge et Expansion de l'Univers, Paris, 6-7 Septembre 1976, et Colloque International du Centre National de la Recherche Scientifique, No. 263: l'Évolution des Galaxies et ses Implications Cosmologiques, Paris, 8-9 Septembre 1976. Pp.619. ISBN-2-222-02022-0. (Paris: Editions du Centre National de la Recherche Scientifique, 1977.) 180 francs.
- HOYLE, Fred. Ten Faces of the Universe. Pp.ix+207. ISBN-0-435-54427-6. (London: Heinemann Educational Books, Ltd., 1977.) £4.80 net.
- MOORE, Patrick, and COLLINS, Pete. The Astronomy of Southern Africa. Pp.160. ISBN-0-7091-6-76-X. (London: Robert Hale Limited; 1977.) £4.95.
- PACHOLCZYK, A. G. Radio Galaxies: Radiation Transfer, Dynamics, Stability and Evolution of a Synchrotron Plasmon. (International Series in Natural Philosophy, Vol. 89.) Pp.xi+293. ISBN-0-08-021031-7. (Oxford and New York: Pergamon Press, 1977.) £13.95.
- WARNER, Lionel. Astronomy for the Southern Hemisphere: a Practical Guide to the Night Sky. Pp.xiii+159 (67 plates). ISBN-0-589-00864-1. (Wellington, Sydney and London: A. H. and A. W. Reed, Ltd., 1975. U.K. Agents: Bailey Bros. and Swinfen, Ltd., Warner House, Folkestone, Kent.) £10.85.
- WEINBERG, Steven. The First Three Minutes: a Modern View of the Origin, of the Universe. Pp.x+188. ISBN-0-233-96906-3. (London: Andre Deutsch, Ltd. 1977.) £4.50.

Physics

- COOK, A. H. Celestial Masers. (Cambridge Monographs on Physics.) Pp.viii+135. ISBN-0-521-21344-4. (Cambridge, London and New York: Cambridge University Press, 1977.) £7.50.
- MARION, Jerry B. Essential Physics in the World Around Us. Pp.vii+444. ISBN-0-471-56905-4. (New York and London: John Wiley and Sons, 1977.) \$16.75; £9.95.
- MENDELSSOHN, K. The Quest for Absolute Zero: The Meaning of Low Temperature Physics. Second edition with S. I. Units. Pp.281. ISBN-0-85066-119-6. (London: Taylor and Francis, Ltd., 1977.) £5.
- PROCEEDINGS of the International Symposium on Plasma Wall Interaction, Jülich (FRG), 18-22 October 1976. (Institut für Plasmaphysik, Kernforschungsanlage Jülich G.m.b.H., Association EURATOM-KFA.) Pp.xiv+750. ISBN-0-08-021989-6. (Oxford and New York: Pergamon Press, 1977. Published for the Commission of the European Communities.) np.

Chemistry

- ASHWORTH, M. R. F. The Determination of Sulphur-Containing Groups. Vol. 3: Analytical Methods for Sulphides and Disulphides. (The Analysis of Organic Materials: An International Series of Monographs.) Pp.xi+220. ISBN-0-12-065003-7. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £11; \$21.50.
- ASQUITH, R. S. (edited by). Chemistry of Natural Protein Fibers. Pp.xx+417. ISBN-0-471-99518-5. (London and New York: John Wiley and Sons, 1977.) £20; \$38.
- BOND, G. C., WELLS, P. B., and TOMKINS, F. C. (edited by). Proceedings of the Sixth International Congress on Catalysis, Imperial College, London, 12-16 July, 1976. Vol. 1: Pp.xv+1-610+xiii. Vol. 2: Pp.xi+611-1133+xiii. ISBN-0-85186-188-1. (London: The Chemical Society, 1977.) £35; \$70.
- CAZES, Jack (edited by). Liquid Chromatography of Polymers and Related Materials. (Chromatographic Science: A Series of Monographs, Vol. 8.) Pp.viii+189. ISBN-0-8247-6592-3. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr.65.
- ELKS, J. (edited by). Recent Advances in the Chemistry of B-Lactam Antibiotics. (The Proceedings of an International Symposium arranged by the Fine Chemicals and Medicinals Group of the Industrial Division of The Chemical Society, Cambridge, England, 28-30 June, 1976. Special Publication, No. 28.) Pp.vi+313. ISBN-0-85186-198-9. (London: The Chemical Society, 1977.) £14.50; \$29.
- GILBERT, A. (symposium editor). Photochemistry—6. (Plenary lectures presented at the Sixth International Symposium on Photochemistry, Aix-en-Provence, France, 19-23 July, 1976.) (International Union of Pure and Applied Chemistry (Organic Chemistry Division), in conjunction with Centre National de la Recherche Scientifique, Délégation Générale à la Recherche Scientifique et Technique, and National Science Foundation.) Pp.223-398. ISBN-0-08-021201-8. (Oxford and New York: Pergamon Press, 1977.) £18.
- GILL, J. B. (conference editor). Non-Aqueous Solutions—5. (Plenary and Section lectures presented at the Fifth International Conference on Non-Aqueous Solutions, Leeds, England, 5-9 July, 1976.) (International Union of Pure and Applied Chemistry in conjunction with The Chemical Society (London), (Dalton and Faraday Divisions). Pp.124. ISBN-0-08-021202-6. (Oxford and New York: Pergamon Press, 1977.) £13.
- HO, Tse-Lok. Hard and Soft Acids and Bases Principle in Organic Chemistry. Pp.xii+209. ISBN-0-12-350050-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$19.50; £13.85.
- IRVING, H. M. N. H. Dithione. (Analytical Sciences Monographs.) Pp.iv+106. ISBN-0-85186-787-1. (London: The Chemical Society, 1977.) £7.25; \$14.50.
- MAY, R. W., PEARSON, E. F., and SCOTCHER, D. Pyrolysis—Gas Chromatography. (Analytical Sciences Monographs.) Pp.vii+109. ISBN-0-85186-767-7. (London: The Chemical Society, 1977.) £7.20; \$14.40.
- McFARLANE, N. R. Herbicides and Fungicides: Factors Effecting Their Activity. (The Proceedings of a Symposium arranged jointly by the Fine Chemicals and

Medicinals Group of the Industrial Division. The Chemical Society, and the Pesticides Group of the Society of Chemical Industry, Bangor, Wales, 15-17 September, 1976.) Pp.xi+141. ISBN-0-85186-178-4. (London: The Chemical Society, 1977.) £7.50; \$15.

McKILLOP, A. (senior reporter). Aliphatic Chemistry, Vol. 5. (A Review of the Literature published during 1975. A Specialist Periodical Report.) Pp.xi+337. ISBN-0-85186-602-6. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £23; \$47.

MITCHELL, John, and SMITH, Donald Milton. Aquametry. Part 1: A Treatise on Methods for the Determination of Water, Second edition. (Chemical Analysis: A Series of Monographs on Analytical Chemistry and Its Applications, Vol. 5.) Pp.xi+632. ISBN-0-471-02264-0 (v. 1). New York and London: Wiley-Interscience, John Wiley and Sons, 1977.) \$38; £22.45.

PENCZEK, S. (symposium editor). Polymerization of Heterocycles (Ring Opening). (Main lectures presented at the International Symposium on Polymerization of Heterocycles (Ring Opening), Warsaw-Jablonna, Poland, 23-25 June 1975.) (International Union of Pure and Applied Chemistry (Macromolecular Division), in conjunction with Polish Academy of Sciences (Centre of Molecular and Macromolecular Studies, Lodz), and Centre National de la recherche Scientifique (Centre de Recherches sur les Macromolécules, Strasbourg.) Pp.274-371. ISBN-0-08-021367-7. (Oxford and New York: Pergamon Press, 1977.) £10.

SPEAKMAN, J. Clare. Molecular Structure: Its Study by Crystal Diffraction. (The Chemical Society Monographs for Teachers, No. 30.) Pp.iii+53. ISBN-0-85186-689-1. (London: The Chemical Society, 1977. Distributed by The Chemical Society Publications, Blackhorse Road, Letchworth, Herts.) £1.20; \$2.40.

WOLFRAM, E. (conference editor). Colloid and Surface Science. (Plenary and Main lectures presented at the International Conference on Colloid and Surface Science, Budapest, Hungary, 15-20 September 1975. International Union of Pure and Applied Chemistry (Physical Chemistry Division), in conjunction with Hungarian Chemical Society.) Pp.387-494. ISBN-0-08-021570-X. (Oxford and New York: Pergamon Press, 1977.) £11.

WROBEL, J. (symposium editor). Low Molecular Weight Sulphur Containing Natural Products. (Plenary lectures presented at the International Symposium on Low Molecular Weight Sulphur Containing Natural Products, Jablonna, Warsaw, 12-16 July 1976. (International Union of Pure and Applied Chemistry, in conjunction with Polish Committee of Chemistry and University of Warsaw.) Pp.137-210. ISBN-0-08-021204-2. (Oxford and New York: Pergamon Press 1977.) £8.50.

Technology

- CROOME, Derek. Noise, Buildings and People. (International Series in Heating, Ventilation and Refrigeration, Vol. 11.) Pp.xvii+613. ISBN-0-08-019816-3. (Oxford and New York: Pergamon Press, 1977.) Hard cover £25; Flexi cover £15.
- JONES, Martin Hartley. A Practical Introduction to Electronic Circuits. Pp.xii+237. ISBN-0-521-21291-X. (Cambridge, London and New York: Cambridge University Press, 1977.) Hard cover £9.60; Paperback £3.95.
- LEE, Chester M. (edited by). Apollo Soyuz Mission Report. (Advances in the Astronautical Sciences, Vol. 34.) Pp.xiv+322. ISBN-87703-089-8. (San Diego, Calif.: American Astronautical Society, 1977. Distributed by Univelt, Inc., P.O. Box 28130, San Diego, CA.) \$35.
- SCHNEIDER, William C. (edited by). Bicentennial Space Symposium—New Themes for Space: Mankind's Future Needs and Aspirations. (Advances in the Astronautical Sciences, Vol. 35.) Pp.xii+227. ISBN-87703-090-1. (San Diego, Calif.: American Astronautical Society, 1977. Distributed by Univelt, Inc., P.O. Box 28130, San Diego, CA.) \$25.

Earth Sciences

- DAVID, Michel. Geostatistical Ore Reserve Estimation. (Developments in Geomathematics, 2.) Pp.xix+364. ISBN-0-444-41532-7. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl. 110; \$39.75.
- FLEMING, M. C. (edited by). The Undersea. Pp.319. ISBN-0-304-299030-0. (London: Cassell and Co. Ltd., 1977.) £12.50.
- GLASSBY, G. P. (edited by). Marine Manganese Deposits. (Elsevier Oceanography Series, 15.) Pp.xvi+536. ISBN-0-444-41524-6. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.127; \$49.
- LAMB, H. H. Climate: Present, Past and Future. Vol. 2: Climatic History and the Future. Pp.xxx+835. ISBN-0-06-473881-7. (London: Methuen and Co., Ltd.; New York: Barnes and Noble Books, 1977.) £38.
- MEYER, R. F. (edited by). The Future Supply of Nature-made Petroleum and Gas: Technical Reports. (An International Conference Sponsored by The United Nations Institute for Training and Research (UNITAR), and The International Institute for Applied Systems Analysis (IIASA), and The First UNITAR Conference on Energy and the Future, and The Second IIASA Conference on Energy Resources, 5-16 July 1976, Schloss Laxenburg, Laxenburg, Austria. Pp.xxxix+1046. ISBN-0-08-021735-4. (New York and Oxford: Pergamon Press, 1977.) Hard cover £33; Flexi £22.
- PERRY, A. H., and WALKER, J. M. The Ocean-atmosphere system. Pp.xi+160. ISBN-0-582-48559-2 (cased), ISBN-0-582-48560-6 (paper). (London and New York: Longman Group Ltd., 1977.) £5.50 (paper).
- RICE, R. J. Fundamentals of Geomorphology. Pp.vii+387. ISBN-0-582-48429-4 (cased), ISBN-0-582-48430-8 (paper). (London and New York: Longman Group Ltd., 1977.) £6.50 (paper).
- WEST, R. G., Pleistocene Geology and Biology. Pp.xi+440. ISBN-0-582-44620. (London and New York: Longman Group Ltd., 1977.) £6.95. (Pub. 5 September, 1977.)
- WINDLEY, Brian F. The Evolving Continents. Pp.xviii+385. ISBN-0-471-99475-8. (London and New York: John Wiley and Sons, 1977.) £14; \$28.

Biology

- ALEXANDER, R. Mc N., and GOLDSPIK, G. (edited by). Mechanics & Energetics of Animal Locomotion. Pp.xii+346. ISBN-0-412-13630-9. (London: Chapman and Hall (a Halsted Book/John Wiley & Sons, New York, 1977.)
- ANGEL, Martin and HARRIS, Tegwyn. Animals of the Oceans: The Ecology of Marine Life. Pp.156. ISBN-0-85654-612-7. (London: Peter Lowe, 1977.) £4.75 net.
- BARRETT, Roger C., and JACKSON, DAPHNE, F. Nuclear Sizes and Structure. Pp.x+566. ISBN-0-19-851272-4. (London: Oxford and New York: Oxford University Press, 1977.) (Oxford: Clarendon.) £15.
- BECKER, Wayne, M. Energy and the Living Cell: An Introduction to Bioenergetics. Pp.346. ISBN-0-397-47368-0. (New York and Toronto: J. B. Lippincott Company) (distributed by Blackwell Scientific Publications: Oxford, 1977.) £6.40.
- CALLAHAN, Philip S. Tuning in to Nature: Solar Energy, Infrared Radiation, and the Insect Communication System. Pp.xxviii+240. ISBN-0-7100-8694-6. (London and Henley: Routledge and Kegan Paul, Ltd., 1977.) £4.95 net.
- CAMPBELL, R. Microbial Ecology (Basic Microbiology, Vol. 5.) Pp.vii+148. ISBN-0-632-00389-8. (Oxford, London, Edinburgh, Sydney: Blackwell Scientific Publications, 1977.) £4.25.
- CAPPUCINELLI, P., and ASHWORTH, J. M. (edited by). Development and Differentiation in the Cellular Slime Moulds. (Developments in Cell Biology, Vol. 1.) Pp.xx+317. ISBN-0-444-41607-2 (series). ISBN-0-444-41600-0 (Vol. 1). (Amsterdam, New York and Oxford: Elsevier/North-Holland: Assoc. Scientific Publishers, 1977.) \$39.75. Dfl.79.00.
- CATCHESIDE, D. G. The Genetics of Recombination. (Genetics—Principles and Perspectives: A Series of Texts.) Pp.x+172. ISBN-0-7131-2613-2. (London: Edward Arnold (Publishers), Ltd., 1977.) Boards £11.50; Paper £5.75.

COMPORTEMENT DES INSECTES ET MILIEU TROPHIQUE. (Colloques Internationaux du Centre National de la Recherche Scientifique, No. 265, Tours 13-17 Septembre, 1976.) Pp.493. ISBN-2-222-02056-5. (Paris: Editions du Centre National de la Recherche Scientifique, 1977.) np.

CONKLIN, Marue E., and HARTL, Daniel L. Genetics Study Guide. Pp.296. ISBN-0-397-47367-2. (Philadelphia and New York: J. B. Lippincott Company, 1977.) \$5.95.

CUATRECASAS, P., and GREAVES, M. F. (edited by). Receptors and Recognition: Series A, Vol. 4. Pp.x+258. ISBN-0-412-14330 (cased edition), ISBN-0-412-14340-2 (paperback). London: Chapman & Hall (a Halstead Press Book: John Wiley & Sons, New York), 1977. £7.50 (paper), £11.50 (cased).

D'AMATO, F. Nuclear Cytology in Relation to Development. (Developmental and Cell Biology Series.) Pp.viii+283. ISBN-0-521-21508-0. (Cambridge, London and New York: Cambridge University Press, 1977.) £15.

DENNIS, R. L. H. The British Butterflies: Their Origin and Establishment. Pp.xviii+318. ISBN-0-900848-94-4. (Faringdon, Oxon: E. W. Classey, Ltd., 1977.) £10 net.

DINGLE, J. T. (edited by). Lysosomes: A Laboratory Handbook 2nd edition. Pp.xiv+323. ISBN-0-7204-0627-7 (hardback), ISBN-0-7204-0628-5 (paperback). (Amsterdam, New York and Oxford: Elsevier/North-Holland: Assoc. Scientific Publishers, 1977.) \$55.50/Dfl.136.00 (hardback), \$29.95/Dfl.73.00 (paperback).

GOLDSBY, RICHARD A. Cells and Energy (2nd edition). Pp.viii+162. ISBN-0-02-344300-6. (London: Collier Macmillan Publishers, New York: Macmillan Publishing Co., 1977.) £3.75 (paperback).

HANLEY, Wayne. Natural History in America. Pp.xii+339. 16 colour illus. ISBN-0-041148-9. (London: Harper & Row, New York: Quadrangle/New York Times Book Co., 1977.) £8.95, £14.95.

HARTL, Daniel L. Our Uncertain Heritage: Genetics and Human Diversity. Pp.xvi+494. ISBN-0-397-47366-4. (Philadelphia and New York: J. B. Lippincott Company, 1977.) \$13.95.

HARPER, John L. Population Biology of Plants. Pp.xxiv+892. ISBN-0-12-325850-2. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £30; \$58.60.

HERSKOWITZ, IRWIN H. Principles of Genetics: 2nd edition. Pp.xxiv+836. ISBN-0-02-353930-5. (London: Collier Macmillan Publishing Co., New York: Macmillan Publishing Co., Inc., 1977.) £12.75.

HOPPE, Walter, LOHMANN, Wolfgang, MARKL, Hubert and ZIEGLER, Hubert (herausgegeben von). Biophysik: Ein Lehrbuch. Mit Beiträgen von R. D. Bauer, et al. Pp.xvi+720. ISBN-3-540-07474-0. (Berlin and New York: Springer-Verlag, 1977.) DM98; \$43.20.

KREIER, Julius P. (edited by). Parasitic Protozoa, Vol. 4: Babesia, Theileria, Myxosporidia, Microsporidia, Bartonellaceae, Anaplasmatidae, Ehrlichia, and Pneumocystis. Pp.xv+386. ISBN-0-12-426004-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$32.50; £23.05.

KURLAND, J. A. Kin Selection in the Japanese Monkey. (Contributions to Primatology, Vol. 12.) Pp.x+146. ISBN-3-8055-2633-4. (Basel, London and New York: S. Karger, 1977.) SFr/DM 64; \$25.75.

LEATHART, Scott. Trees of the World. Pp.224. ISBN-0-600-38745-3. (London, New York, Sydney and Toronto: Hamlyn, 1977.) £4.95.

NAGL, Walter. Zellkern und Zellzyklen: Molekularbiologie, Organisation und Entwicklungsphysiologie der Desoxyribonucleinsäure und des Chromatins. (Phytophylogie: Klassische und Moderne Botanik in Einzeldarstellungen.) Pp.486. ISBN-3-8001-3417-9. (Stuttgart: Verlag Eugen Ulmer, 1976.) DM 120.

NAGL, Walter. Zellkern und Zellzyklen: Molekularbiologie, Organisation und Entwicklungsphysiologie der Desoxyribonucleinsäure und des Chromatins. Pp.486. ISBN-3-8001-3417-9. (Stuttgart: Verlag Eugen Ulmer, 1976.) DM 120.

PRANCE, G. T., and ELIAS, T. S. (edited by). Extinction is Forever: Threatened and Endangered Species of Plants in the Americas and Their Significance in Ecosystems Today and in the Future. (Proceedings of a symposium held at the New York Botanical Garden, May 11-13, 1976, in commemoration of the Bicentennial of the United States of America.) Pp.vi+437. (Bronx, New York: The New York Botanical Garden, 1977.) \$22.

REES, D. A., Polysaccharide Shapes: Outline Studies in Biology. Pp.80. ISBN-0-412-13030-0. (London: Chapman and Hall (a Halstead Press Book/John Wiley & Sons, New York; 1977).) £1.75.

ROONWAL, M. L., and MOHNOT, S. M. Primates of South Asia: Ecology, Sociobiology and Behaviour. Pp.xviii+421. ISBN-0-674-70485-1. (Cambridge, Mass. and London: Harvard University Press, 1977.) £15.75.

ROST, Thomas L., and GIFFORD, Ernest M. (edited by). Mechanisms and Control of Cell Division. Pp.viii+387. ISBN-0-87933-267-0. (Stroudsburg, Penn. L. Dowden, Hutchinson and Ross, Inc., 1977. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York and Chichester.) \$31.50; £18.75.

SAVORY, Theodore. Arachnida. Second edition. Pp.viii+340. ISBN-0-12-619660-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £10.80; \$21.15.

SELIGSOHN, Daniel. Analysis of Species-Specific Molar Adaptations in Strepsirrhine Primates. (Contributions to Primatology, Vol. 11.) Pp.viii+116. ISBN-3-8055-2634-2. (Basel, London and New York: S. Karger, 1977.) SFr/DM 51; \$19.75.

STLEDYARD STEBBINS, G., Processes of Organic Evolution: 3rd edition. Pp.x+269. ISBN-0-13-723452-X. (London, New York and Sydney: Prentice-Hall; 1977.)

TWEEDIE, Michael. The World of Dinosaurs. Pp.143. ISBN-0-297-77352-6. (London: Weidenfeld and Nicolson, 1977.) £4.95 net.

WADE, Nicholas. The Ultimate Experiment: Man-Made Evolution. Pp.viii+162. ISBN-0-8027-0572-3. (New York: Walker & Co., 1977.) \$8.95.

WARNER, G. F., The Biology of Crabs. Pp.xii+202. ISBN-0-236-40087-8. (London: Paul Elek Ltd., 1977.) £6.95.

WEISSBACH, Herbert, and PESTKA, Sidney (edited by). Molecular Mechanisms of Protein Biosynthesis. (Molecular Biology: an International Series of Monographs and Textbooks.) Pp.xiii+720. ISBN-0-12-744250-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers 1977.) \$55; £39.05.

WHITE, M. J. D. Animal Cytology and Evolution. Third edition. Pp.viii+961. ISBN-0-521-29227-1. (Cambridge, London and New York: Cambridge University Press, 1977.) £8.95.

WRIGHT, Sewall. Evolution and the Genetics of Populations. Vol. 3: Experimental Results and Evolutionary Deductions. Pp.613. ISBN-0-226-91051-2. (Chicago and London: The University of Chicago Press, 1977.) £24.50.

ZUCKERMAN, Professor Lord, and WEIR, Barbara J. (edited by). The Ovary.

Vol. 1: General Aspects. Second edition. Pp.xviii+517. ISBN-0-12-782601-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$35; £24.85.

Applied Biology

BHATIA, Balraj, CHHINA, G. S., and SINGH, Baldev. Selected Topics in Environmental Biology. (Based on the Sessions on Environmental Biology, held at the 26th International Congress on Physiological Sciences, New Delhi, October 20-26, 1974.) Pp.529. ISBN-0-08-021-10-7. (Oxford and New York: Pergamon Press, 1977.) £66.50.

BOREK, Carmia, FENOGLIO, Cecilia M., and KING, Donald West. (edited by). Cancer Biology, IV: Differentiation and Carcinogenesis. (Advances in Pathobiology, Vol. 6) Pp.xvi+323. ISBN-0-913258-48-2. (New York: Stratton Intercontinental Medical Book Corporation, 1977.) \$24.50.

BRAIN, Joseph D., PROCTOR, Donald F., and REID, Lynne M. (edited by). Respiratory Defense Mechanisms, Part I: Lung Biology in Health and Disease. Vol. 5) Pp.xviii+488. ISBN-0-8247-6381-5. (New York and Basel: Marcel Dekker, Inc., 1977.) \$44.50.

BUCKLEY, Joseph P., and FERRARIO, Carlos M. (edited by). In association with LOKHANDWALA, Mustafa, F. Central Actions of Angiotensin and Related Hormones. Pp.xvii+606. ISBN-0-08-020933-5. (Oxford and New York: Pergamon Press, 1977.) £27.

BULLOCK, Theodore Holmes. With the collaboration of ORKAND, Richard, and GRINNELL, Alan. Introduction to Nervous Systems. (A Series of Books in Biology.) Pp.xiv+559. ISBN-0-7167-0577-X. (San Francisco and Reading: W. H. Freeman and Company, 1977.) £21.60.

CHANG, Thomas Ming Swi. (edited by). Biomedical Applications of Immobilized Enzymes and Proteins. Vol. 1: Pp.xx+428. ISBN-0-306-34311-8. \$44.50 Vol. 2: Pp.xx+359. ISBN-0-306-34312-6 (v. 2). \$47.40. (New York and London: Plenum Press, 1977.)

COAKER, T. H. (edited by) Applied Biology, Vol. 2. Pp.x+272. ISBN-0-12-040902-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £9.80; \$19.25. (Pub. 1 Aug. 77).

DAUSETT and SVEJGAARD. (edited by). HLA and Disease. Pp.316. ISBN-X-87-16-02287-4. (Copenhagen: Munksgaard, North and South America: Baltimore: William & Wilkins Co., 1977.) D.kr. 180.00.

DICKINSON, C. H., and LUCAS, J. A., Plant Pathology and Plant Pathogens. Vol. 6 (Basic Microbiology, edited by J. F. Wilkinson). Pp.x+161. ISBN-0-632-00399-5. (Oxford, London, Edinburgh and Melbourne: Blackwell Scientific Publications, 1977.) £4.25.

GARDNER, Howard. The Shattered Mind: The Person After Brain Damage. Pp.xiv+481+viii. ISBN-0-7100-8641-5. (London and Henley: Routledge and Kegan Paul, Ltd., 1977.) £9.75.

GEMMELL, RAYMOND, P., Colonization of Industrial Wasteland. (Studies in Biology no. 00). Pp.75. ISBN-0-7131-2587-X. (London: Edward Arnold (publishers) Ltd, 1977.) £3.20 (hardback) £1.60 (paperback).

GUDZINOWICZ, Benjamin J., and GUDZINOWICZ, Michael J. With the assistance of MARTIN, Horace, and DRISCOLL, James L. Analysis of Drugs and Metabolites by Gas Chromatography-Mass Spectrometry. Vol. 1: Respiratory Gases, Volatile Anesthetics, Ethyl Alcohol, and Related Toxicological Materials. Pp.vii+223. ISBN-0-8247-6576-1. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr. 73.

HILLS, Hilda Cherry. Good Food: Grain-Free, Milk-Free. Pp.123. (London: Roberts Publications, 225 Putney Bridge Road, SW15, 1977.) £2.25.

HUGO, W. B., and RUSSELL, A. D., (edited by). Pharmaceutical Microbiology. Pp.vii+352. ISBN-0-632-00499-1. (Oxford: Blackwell Scientific Publications; 1977.) £8.50.

JARRETT, A. (edited by). The Physiology and Pathophysiology of the Skin. Vol. 4: The Hair Follicle. Pp.xix+1237-1540+xxi. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £18.50; \$36.10.

KELLY, Sally. Biochemical Methods in Medical Genetics. (A Monograph in The Bannerstone Division of American Lectures in Laboratory Medicine.) Pp.xi+345. ISBN-0-398-03630-6. (Springfield, Ill.: Charles C. Thomas, Publisher, 1977.) \$17.50.

LANDSBERG, J. J., and CUTTING, C. V. (edited by). Environmental Effects on Crop Physiology. (Proceedings of a Symposium held at Long Ashton Research Station, University of Bristol, 13-16 April 1975. Fifth Long Ashton Symposium.) Pp.xv+388. ISBN-0-12-435050-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £14.50; \$28.35.

LIPTON, Sampson (edited by). Persistent Pain: Modern Methods of Treatment, Vol. 1. Pp.ix+272. ISBN-0-12-451701-3. (London: Academic Press; New York: Grune and Stratton, 1977.) £10.50; \$20.50.

MEEK, G. A., and ELDER, H. Y. (edited by). Analytical and Quantitative Methods in Microscopy. (Society for Experimental Biology Seminar Series, Vol. 3.) Pp.276. ISBN-0-521-21404-1. (Cambridge, London and New York: Cambridge University Press, 1977.) Hard cover £12; Paperback £4.75.

NEUWIRT, J., and PONKA, P. Regulation of Haemoglobin Synthesis. Pp.206. ISBN-90-247-1999-2. (The Hague: Martinus Nijhoff, Medical Division, 1977.) Dfl.50.

ROSE, A. H. (edited by). Alcoholic Beverages. (Economic Microbiology, Vol. 1.) Pp.xiv+760. ISBN-0-12-596550-8. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £25.

SABINE, John R. Cholesterol. Pp.xii+489. ISBN-0-8247-6516-8. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.82.

SIEGEL, Malcolm R., and SISLER, Hugh D. (edited by). Antifungal Compounds. Vol. 1: Discovery, Development, and Uses. Pp.xvi+600. ISBN-0-8247-6557-5. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.175.

TARG, Russell and PUTHOFF, Harold. Mind-Research: Scientists Look at Psychic Ability. (introduction by Margaret Mead and Foreword by Richard Bach). Pp.xv+230. ISBN-0-244-01424-2. (London: Jonathan Cape; 1977.) £4.95.

WATER PURIFICATION IN THE EEC: a State-of-the-Art Review. (Water Research Centre.) Pp.vii+467. ISBN-0-08-021225-5. (Oxford and New York: Pergamon Press, 1977. Published for the Commission of the European Communities.) £21.

WOLFE, Douglas A. (edited by). Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. (Proceedings of a Symposium, November 10-12, 1976, Olympic Hotel, Seattle, Washington.) Pp.xix+478. ISBN-0-08-021613-7. (Oxford and New York: Pergamon Press, 1977.) £22.25.

A new
Springer
Journal

Physics & Chemistry
of Minerals

Springer-Verlag
Berlin
Heidelberg
New York



Psychology

- BALDESSARINI, Ross J. *Chemotherapy in Psychiatry*. Pp.xiii+201. ISBN-0-674-11380-2. (Cambridge, Mass. and London: Harvard University Press, 1977.) £7.
- EYSENCK, Michael W. *Human Memory: Theory, Research and Individual Differences*. (International Series in Experimental Psychology.) Pp.x+366. ISBN-0-08-020405-8. (Oxford and New York: Pergamon Press, 1977.) £8.
- FOREYT, John Paul. *Behavioural Treatments of Anorexia*. (Pergamon General Psychology Series, Vol. 61.) Pp.xiv+511. ISBN-0-08-019902-X. (Oxford and New York: Pergamon Press, 1977.) £7.50.
- LABOV, William, and FANSHEL, David. *Therapeutic Discourse: Psychotherapy as Conversation*. Pp.x+392. ISBN-0-12-432050-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$15; £10.65.
- NIXON, Mary, and TAFT, Ronald (edited by). *Psychology in Australia: Achievements and Prospects*. Pp.xi+318. ISBN-0-08-020561-5. (Rishcutters Bay, NSW; Oxford and New York: Pergamon Press, 1977.) Hard cover £8.85; Flexi cover £5.25.
- RACHMAN, S. (edited by). *Contributions to Medical Psychology*, Vol. 1. Pp.viii+243. ISBN-0-08-020511-9 (V. 1). (Oxford and New York: Pergamon Press, 1977.) £8.

Sociology

- BLACKSELL, Mark. *Post-War Europe: A Political Geography*. Pp.205. ISBN-0-7129-0789-0. (Kent: William Dawson & Sons Ltd., 1977.) £6.
- DAY, P. R. *Methods of Learning Communication Skills*. (Social Work Series. Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.vii+329. ISBN-0-08-018953-9. (Oxford and New York: Pergamon Press, 1977.) Hard cover £10.50; Flexi cover £7.50.
- FAGENCE, Michael. *Citizen Participation in Planning*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.v+378. ISBN-0-08-020398-1. (Oxford and New York: Pergamon Press, 1977.) Hardcover £8.50; Flexi cover £5.25.
- HALL, Raymond L. (edited by). *Black Separatism and Social Reality: Rhetoric and Reason*. Pp.xvii+280. ISBN-0-08-019509-1. (Oxford and New York: Pergamon Press, 1977.) Hardcover £12.50; Flexi cover £6.
- SHANKS, Michael. *European Social Policy, Today and Tomorrow*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xi+105. ISBN-0-08-021444-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £6.50; Flexi cover £2.75.
- TILBURY, D. E. F. *Casework in Context: a Basis for Practice*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.ix+338. ISBN-0-08-019743-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £8; Flexi cover £5.

Anthropology

- BEALS, Ralph L., HOUER, Harry, and BEALS, Alan R. *An Introduction to Anthropology*. Fifth edition. Pp.xvii+748. ISBN-0-02-307450-7. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £5.85. (b. 1 Aug. '77).
- GUBANE, Harriet. *Body and Mind in Zulu Medicine: An Ethnography of Health and Disease in Nyswala-Zulu Thought and Practice*. (Studies in Anthropology.) Pp.xvi+184. ISBN-0-12-518250-3. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £6.80; \$13.25.
- OAKLEY, Kenneth Page, CAMPBELL, Bernard Grant, and MOLLESON, Thea Ivitsky (edited by). *Catalogue of Fossil Hominids. Part 1: Africa*. Second edition. Pp.210+21 plates. ISBN-0-565-05661-1. (London: British Museum (Natural History), 1977.) £12.

Education

- COLES, Edwin K. Townsend. *Adult Education in Developing Countries*. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xviii+199. ISBN-0-08-021293-X. (Oxford and New York: Pergamon Press, 1977.) £5.
- FARAGO, P. J., FRAZER, M. J., and WALKER, S. D. *Chemical Education in Europe*. Pp.vi+380. ISBN-0-85186-659-X. (London: The Chemical Society, 1976.) £3.50; \$7.
- WARNOCK, Mary. *Schools of Thought*. Pp.176. ISBN-0-571-11161-0. (London: Faber and Faber, Ltd., 1977.) Cloth £5.50; Paper £2.95.

General

- CHEREMISINOFF, Paul N., and MORRESI, Angelo C. *Environmental Assessment and Impact Statement Handbook*. Pp.ix+438. ISBN-0-250-40158-4. (Ann Arbor: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$32.45; £19.75.
- CLARK, Kenneth. *Animals and Men: Their Relationship as Reflected in Western Art from Prehistory to the Present Day*. Pp.240 (219 illustrations, 84 in colour). ISBN-0-500-23257-1. (London: Thames and Hudson, Ltd., 1977.) £10.50 net.
- DESMOND, Ray. *Dictionary of British and Irish Botanists and Horticulturists, including Plant Collectors and Botanical Artists. With historical introduction by William T. Stearn*. Pp.xxvi+747. ISBN-0-85066-089-0. (London: Taylor and Francis, Ltd., 1977.) £40 net. (No pub. date).
- FITZGERALD, Ross (edited by). *Human Needs and Politics*. Pp.xvi+278. ISBN-0-08-21402-9. (Rushcutters Bay, NSW; Oxford and New York: Pergamon Press, 1977.) (No pub. date).
- FOX, James J. *Harvest of the Palm: Ecological Change in Eastern Indonesia*. Pp.xv+290. ISBN-0-674-38111-4. (Cambridge, Mass. and London: Harvard University Press, 1977.) £10.25. (No pub. date).
- HEIKOFF, Joseph M. *Coastal Resources Management: Institutions and Programs*. Pp.xiii+287. ISBN-0-250-40157-6. (Ann Arbor: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$18.15; £11. (Pub. 10 Aug. '77).
- INTERNATIONAL CONFERENCE ON ELECTRICAL VEHICLE DEVELOPMENT, 31 May-1 June. Pp.104. ISBN-0-901223-63-8. (Organised by the Electric Vehicle Development Group.) (Stevenage, Herts: Peter Peregrinus, Ltd., 1977.) UK £6.40; Overseas £7.50 (excl. Americas). (Pub. Aug. '77).

- JOHN, J. A., and QUENOUILLE, M. H. *Experiments: Design and Analysis*. Second edition. Pp.296. ISBN-0-85264-222-9. (London and High Wycombe: Charles Griffin and Company, Ltd., 1977.) £12 net.
- MCINTOSH, I. G., and MARSHALL, C. B. *The Face of Scotland*. Third edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xiv+230. ISBN-0-08-021320-0. (Oxford and New York: Pergamon Press, 1977.) Hard cover £7; Flexi cover £4.
- NEW METHODS AND TECHNIQUES (Scholarly Publishers Guide). Pp.101. ISBN-0-906083-00-1. (Primary Communications Research Centre, University of Leicester).
- MORSE, Philip M. *In at the Beginnings: a Physicist's Life*. Pp.vii+375. ISBN-0-262-13124-2. (Cambridge, Mass. and London: The MIT Press, 1977.) \$14.95.
- PECCEI, Aurelio. *The Human Quality*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xii+214. ISBN-0-084-021480-0. (Oxford and New York: Pergamon Press, 1977.) Flexi cover £3.25; \$6. Hard cover £6.50; \$12.
- PERSINGER, Michael A., and LAFRENIERE, Gyslain F. *Space-Time Transients and Unusual Events*. Pp.xiii+267. ISBN-0-88229-334-6. (Chicago: Nelson-Hall, Publishers, 1977.) Cloth \$9.95; Paper \$5.95.
- PRZELECKI, Marian, SZANIAWSKI, Klemens, and WOJCICKI, Ryszard. *Formal Methods in the Methodology of Empirical Sciences*. (Proceedings of the Conference, Warsaw, June 17-21, 1974.) (Synthese Library, Vol. 103.) Pp.457. ISBN-90-277-0698-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company; Wrocław, Poland: Ossolineum Publishing Company, 1976.) Dfl. 105; \$39.50.
- RABEN, Joseph (edited by). *Computer-Assisted Research in the Humanities*. (A Directory of Scholars Active.) Pp.251. ISBN-0-08-019870-8. (Oxford and New York: Pergamon Press, 1977.) £27.50.
- RAPOPORT, Amos. *Human Aspects of Urban Form: Towards a Man-Environment Approach to Urban Form and Design*. (The Urban and Regional Planning Series.) Pp.viii+438. ISBN-0-08-017974-6. (Oxford and New York: Pergamon Press, 1977.) £8.
- ROZDIENSKI, Walenty. *Officina Ferraria*. (A Polish poem of 1612 describing the noble craft of ironwork.) Translated by Stefan Pluszczewski. Edited by W. Rozanski and Cyril Stanley Smith. Pp.xvi+123. ISBN-0-262-18079-0. (Cambridge, Mass. and London: The MIT Press, 1976. Published jointly with the Society for the History of Technology.) £9.40.
- RUSSELL, Bertrand. *Roads to Freedom*. Pp.158. ISBN-0-04-335033-X. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1918.) £1.50 net.
- RUSSELL, Bertrand. *Education and the Social Order*. Pp.158. ISBN-0-04-370080-2. (London: George Allen and Unwin, Ltd., 1977. First published 1932.) £1.50 net.
- RUSSELL, Bertrand. *Sceptical Essays*. Pp.189. ISBN-0-04-104003-1. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1935.) £1.50 net.
- RUSSELL, Bertrand. *Authority and the Individual*. Pp.96. ISBN-0-04-170030-9. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1949.) £1.25 net.
- RUSSELL, Bertrand. *Political Ideals*. Pp.80. ISBN-0-04-320120-2. (London: George Allen and Unwin (Publishers), Ltd., 1977.) First published in UK 1963.) £1.25 net.
- RUSSELL, Bertrand. *ABC of Relativity*. Third revised edition. Edited by Felix Pirani. Pp.160. ISBN-0-04-521002-0. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1925.) £1.50 net.
- Scientific Glassblowing and Laboratory Supplied Catalogue. Pp.124. (Sawbridge-worth, Hertfordshire: R. B. Radley and Co., Ltd., 1977.)
- SCIENCE YEAR: The World Book Science Annual, 1977. (A Review of Science and Technology During the 1976 School Year.) Pp.432. ISBN-0-7166-0577-5. (Chicago and London: Field Enterprises Educational Corporation, a Subsidiary of Field Enterprises, Inc., 1977.) £6.25.
- SCHUMACHER, E. F. *A Guide for the Perplexed*. Pp.155. ISBN-0-244-01496-X. (London: Jonathan Cape, 1977.) £3.95.
- SELL, R. C., CRAWLEY, J. E., CROCKFORD, G. W., and FOX, J. G. (contributions by). *Human Factors in Work, Design and Production*. Pp.xviii+138. ISBN-0-85066-076-9. (London: Taylor and Francis, Ltd., 1977.) £7.
- SEN, Buddhadev. *To Secure "Certain Unalienable Rights"*. Pp.xiv+203. (Baton Rouge, Louisiana: B. Sen, Louisiana State University and Agricultural and Mechanical College, 1977.) \$7.50.
- SHAFFER, Wade H. (edited by). *Masters Theses in the Pure and Applied Sciences Accepted by Colleges and Universities of the United States and Canada*, Vol. 20. (A Publication of the Center for Information and Numerical Data Analysis and Synthesis, CINDAS.) Pp.xv+291. ISBN-0-306-34120-4. (New York and London: Plenum Press, 1976.) \$30.
- SITCHIN, Zecharia. *The Twelfth Planet*. Pp.384. ISBN-0-04-113001-4. (London, Boston, Mass. and Sydney: George Allen and Unwin, 1977.) £5.50.
- TOMKIES, Mike. *Alone in the Wilderness*. Pp.215. ISBN-0-354-04142-8. (London: Macdonald and Jane's, Ltd., 1977.) £4.95 net.
- TUDGE, Colin. *The Famine Business*. Pp.141. ISBN-0-571-10887-3. (London: Faber and Faber, 1977.) £3.95.
- WALLISER, Bernard. *Systèmes et Modèles: Introduction Critique à l'Analyse de Systèmes*. Pp.248. ISBN-2-02-004638-5. (Paris: Editions du Seuil, 1977.) np.
- WILDEN, Anthony. *System and Structure: Essays in Communication and Exchange*. Pp.xxx+540. ISBN-0-422-76210-5. (London: Tavistock Publications, 1977. Distributed in the USA by Harper and Row Publishers, Inc.; Barnes and Noble (Import) Division. First published 1972.) £5.25.
- WHITE, Gilbert. *The Natural History of Selborne*. Edited with an Introduction and Notes by Richard Mabey. (The Penguin English Library.) Pp.xviii+283. ISBN-0-14-043-112-8. (Harmondsworth, Middx.: Penguin Books, Ltd., 1977. First published 1788-9.) 95p; USA and Canada \$2.95.
- WORLD ARMAMENTS AND DISARMAMENT: SIPRI Yearbook 1977. Pp.xvi+421. ISBN-0-262-19160-1. (Stockholm: Stockholm International Peace Research Institute, in collaboration with Almqvist and Wiksell Internationa, Stockholm and The MIT Press, Cambridge, Mass. and London, 1977.) Sw.kr. 140.
- von WEIZSACKER, C. F. *The History of Nature*. Pp.vi+191. ISBN-0-226-89189-5. (Chicago and London: The University of Chicago Press, 1977.) £4.50.
- YOURGRAU, Wolfgang, and BRECK, Allen D. (edited by). *Cosmology, History, and Theology*. Pp.xvi+416. ISBN-0-306-30940-8. (New York and London: Plenum Press, 1977.) \$54.
- ZIMAN, John. *The Force of Knowledge: The Scientific Dimension of Society*. Pp.ix+374. ISBN-0-521-09917-X. (Cambridge and London: Cambridge University Press, 1977.) £4.75.

A new
Springer
Journal

Irrigation
Science

Springer-Verlag
Berlin
Heidelberg
New York



announcements

On the Move

Dr E. B. Carstens, Department of Microbiology, Centre Hospitalier Universitaire, Sherbrooke, Canada, to Institute of Genetics, University of Cologne, Germany.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Appointments

Dr E. H. Francis, to the Chair of Earth Sciences, University of Leeds, from 1 October 1977.

Professor H. Charnock, Director of the Institute of Oceanographic Sciences, to the Chair of Physical Oceanography, University of Southampton, from 1 February 1978.

Dr P. Crutzen, to Director of the Atmospheric Quality Division of the National Center for Atmospheric Research (NCAR).

Professor R. N. Pryor, to President-Elect of the Institution of Mining and Metallurgy from 20 June 1978.

Awards

Dr G. N. Hounsfield has been awarded the Royal Society Mullard Award for 1977 (£1,000) in recognition of his conception and development of the EMI-Scanner, a computerised three-dimensional X-ray system.

Two Technology Writers' Awards, sponsored by ITT Business Systems under the auspices of British science writers, will be awarded for work published between 1 January and 31 December 1977. One will be for work published in professional periodicals, trade and technical magazines, or national and regional newspapers, the other for material broadcast on radio or television. Both prizes will be £1,000 cash and £500 for travel or equipment. All entries are by submission (to J. Anstiss, MPR Ltd, 293 Gray's Inn Road, London WC1, UK), and it is hoped to announce the winners in February 1978.

Marconi International Fellowship

Nominations, to be received by 15 October 1977, are invited for the 1978 Fellowship (\$25,000), established in 1974 to commemorate Guglielmo Marconi's contributions to scientific discovery, engineering and technology. The recipient, in recognition of out-

standing contribution in these fields, is invited to give a public lecture based on the work during the 12 months following presentation. Address all enquiries to The Marconi International Fellowship Council, Aspen Institute for Humanistic Studies, 1919 Fourteenth Street, No. 811, Boulder, Colorado 80302, USA.

National Academy of Sciences Committee on Aerobiology

A new programme has been outlined by a NAS committee to encourage the advancement of Aerobiology as a discrete science. The NAS wish to compile a register of aerobiologists, giving details of research interests. Please send relevant information to National Research Council, Assembly of Life Sciences, 2101 Constitution Avenue, Washington, DC 20418.

Meetings

30 September, **Fine Structure and Chemical Shifts in Electron Spectroscopy**, London, UK (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

30 September–1 October, **Measurement in Biological Electron Microscopy**, Bristol, (Physiology Departmental Office, The Medical School, University of Bristol, Bristol, UK).

2–8 October, **2nd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Ageing**, Varna, Bulgaria (Secretariat, Colloquium '77, Central Laboratory of Biophysics, Acad. G. Bonchev Str. Bl. 6, 1113 Sofia, Bulgaria).

3–5 October, **5th Biennial Symposium on Turbulence**, Rolla (Turbulence Symposium, Extension Division, University of Missouri-Rolla, Rolla, Missouri 65401).

3–7 October, **Thermodynamics of Magnetic Fluids**, Udine, Italy (B. Berkovsky, Science Sector, UNESCO, 7 place de Fontenoy, 75700 Paris, France).

5 October, **Technology Transfer from the Nuclear Industry**, Harewell (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London, UK).

7–11 October, **VII International Congress of Essential Oils**, Kyoto (Y. Kato, Secretary General, VII International Congress of Essential Oils, c/o Kyoto International Conference Hall, Takaraike, Sakyo-ku Kyoto, 606 Japan).

10–13 October, **XXV International Meeting on Transportation and Com-**

munications, Genoa (International Institute of Communication, Villa Piaggio, Via Pertinace, 16125 Genova, Italy).

11–13 October, **4th Annual UMR-DNR Conference on Energy**, Rolla (N. Fleming, Conference Coordinator, Extension Division, University of Missouri-Rolla, Rolla, Missouri 65401).

11–13 October, **AGU Conference on Oceanic Fronts**, New Orleans (American Geophysical Union, 1909 K St, NW, Washington, DC 20006).

17–18 October, **Conference on Genetic Epidemiology**, Hawaii (N. E. Morton, School of Public Health, University of Hawaii, Honolulu 96822).

17–21 October, **1st International Congress on Phosphorus Compounds and their Non-fertiliser Uses**, Rabat, Morocco (IMPHOS (World Phosphate Rock Institute), 8 rue de Penthievre, 75008 Paris, France).

19–21 October, **Symposium on Current Topics in Drug Research**, Uppsala (R. Dahlbom, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden).

24–26 October, **ASTM Symposium on Erosion: Prevention and Useful Applications**, Vail, Colo (Miss J. B. Wheeler, ASTM, 1916 Race Street, Philadelphia, Pa. 19103).

24–28 October, **International Conference on Water Chemistry of Nuclear Reactor Systems**, Bournemouth (The Conference Office, Institution of Civil Engineers, 1–7 Great George Street, London SW1, UK).

25–28 October, **International Conference—Radar 77**, London, UK (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

31 October–3 November, **Advancing Energy Technology**, Eastbourne (The Institute of Fuel, 18 Devonshire Street, Portland Place, London W1, UK).

31 October–4 November, **Symposium on Radioimmunoassay, and Related Procedures in Medicine**, Berlin (International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

31 October–4 November, **Conference on Water Chlorination: Environmental Impact and Health Effects**, Gatlinburg (R. L. Jolley, Chemical Technology Division, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

2–3 November, **International Symposium on Polarography in Action**, London, UK (The Secretary, Scientific Symposia Ltd, 42–43 Gerrard Street, London W1, UK).

nature

29 September 1977

Political will now the issue

THE Commons Select Committee on Science and Technology has recently reported on the development of alternative sources of energy for the United Kingdom (House of Commons Paper 534). With painstaking care the committee has heard evidence on tidal power, solar energy, nuclear fusion, geothermal resources, wind and waves. And yet at the end of it all one is left wondering whether the United Kingdom is going to think itself into an energy policy and, if so, whether we will be prepared to bite the bullet on the subject of alternative energy sources.

The report itself is on the whole unobjectionable. Various sources are reviewed and commented on; solar energy for space and water heating is seen as having the greatest immediate potential, and the committee recommends its vigorous exploitation. Solar electricity is given a less cheerful assessment, although the rate of development of amorphous semiconductors might well render this opinion obsolete in the near future. Biomass, which is one of the pillars of Amory Lovins' *Soft Energy Paths* (offering according to Mr Lovins "practical economically interesting technologies sufficient to run an efficient US transport sector"), is barely mentioned. Wave power for electricity generation is seen, somewhat delphically, to have considerable potential "if the technical difficulties can be overcome, and economic viability established". Wind and geothermal energy are not highly rated. And so on. A disappointment is that there is no parallel assessment of energy storage techniques which, as Martin Ryle recently argued, could make a lot of difference to the viability of some alternative-energy projects.

Technical analyses of the sort that the committee provides are now common; there are plenty of excellent books, reports and magazine articles to inform the public, and this latest document can add relatively little to what has already been put about—and what fairly quickly becomes outdated. Where those in the political arena should now be more exercised is in asking just

what alternative energy sources are for. And before such a question can be answered some distinctions need to be made between different types of alternative energy source. To include nuclear fusion in the same general category as, say wind power is clearly wrong. The former has the potential at some time in the future to take over a massive proportion of the country's energy requirements if the political will is there, the latter does not, and it seems foolish to talk about such divergent technologies under the same heading.

Some clear thinking is also needed in the near future on what will be politically acceptable in terms of import and export. It has been customary to discuss some alternative-energy technologies as worth pursuing mainly for their export potential. But the opposite side of the coin is the possibility of importing energy. The question of biomass again springs to mind. Undoubtedly the United Kingdom is not favourably situated compared with equatorial countries. Many of these countries fall into the developing category. The policy questions of independence *versus* support for the developing world *versus* fear of a future OPEC are certainly worth pursuing at this early stage.

Finally, what is the purpose of research into alternative energy sources? If in future years the source could be expected to provide, say, 5% of the UK's energy needs, does it make sense to pursue the subject? Small indentations into a major problem may be attractive scientifically and emotionally but could be trivial in the context of resource conservation or reduction in expenditure on foreign currency. Somewhere along the line this far-from-easy problem will have to be faced; on the assumption that the United Kingdom will continue to place its energy reliance on the major sources in view at the present, for how long will it make sense to encourage vigorous research in the small alternative sources? The question would, of course, be very different if we were to move to a 'softer' path, but on that there seems as yet to be no political discussion. □

BA address: Huxley responds

Sir Andrew Huxley, Royal Society Research Professor in the Department of Physiology at University College London, responds to Nature's editorial on the presidential address he delivered to the recent British Association meeting

SIR,—Your editorial (8 September) about my presidential address to the British Association misses the point of what I said on the relations between human values and scientific research. I discussed this matter in several contexts—evolution; paranormal phenomena; the Lysenko controversy; and the inheritance of human ability. You mention only the last, and you write as though I had simply called for more research in this field. You have misread me. What I was urging was that the conclusions of whatever research is done should not be biased by preconceptions or by external pressures. In recent years, this issue has been bedevilled by strong pressures, which in Europe and in most of the USA have come almost exclusively from those who insist that the genetic component is negligibly small. These pressures have taken forms which I hope you agree are improper—the baseless insinuation that there is necessarily something 'racist' in admitting the possibility of a genetic component; denial of freedom of speech; and physical attacks. It is easy to imagine similar pressures being exerted by others in the opposite direction; if this occurs I shall resist them just as forcibly, but as far as I know this has not been happening, at least in Europe, since the defeat of Hitler.

I did not say much about the good that might come from an increase of knowledge on the causes of differences in ability, partly because this was not the subject of my address, partly because I am no expert in these matters but also because I did not wish to buy off opposition to my main thesis by playing down the risk of uncomfortable passages ahead if, in any field, we allow evidence to be collected and published. In my view the independence of Science is something that should be accepted irrespective of the consequences that may appear to us to be likely. Human affairs are so unpredictable that it is more probable that good will be done by letting our beliefs follow from the evidence, than by deciding without the evidence what beliefs will lead to the best actions. Since you challenge me, however, I will set out some of the ways in which increased knowledge could do good.

You say "Questions are bound to be asked about motives for doing more research, especially as there is little doubt that inheritance does play at least some and maybe a very significant part in the acquisition of ability". One answer—already given in my address—is as follows. If inheritance plays no significant part, long-term policy need only aim to provide equal opportunity and to eliminate prejudice based on class and race. But if its role is "very significant", we must also build into our system compensatory advantages for groups who would otherwise feel excluded from a fair share of well-rewarded and respected occupations: 'affirmative action' as in the USA would be a step in this direction but other measures might be needed as well—perhaps for example up-grading those jobs which have the lowest requirements in scholastic attainment. Such policies would naturally be resented by the groups whom they do not favour (witness the case of Allan Bakke now before the US Supreme Court) and probably ought not to be vigorously implemented unless it is clear that a substantial genetic

component exists.

A second way in which further investigation could be valuable is the following: if there are no appreciable genetic differences, the best way to establish the fact will be to carry out genetic investigations. An example is that Shockley's approaches to the National Academy of Sciences in the United States led indirectly to a review of the position being undertaken under the auspices of a committee of the US Social Science Research Council, and the resulting book (J. C. Loehlin, G. Lindzey & J. N. Spuhler, *Race differences in intelligence*, W. H. Freeman & Co., 1975) gives prominence to two interesting genetic investigations, one based on correlation of IQ with blood groups in persons of mixed ancestry, and the other on the performance of illegitimate offspring of Negro and White US servicemen with German women during the occupation of Germany after World War II. Neither of these gave any support for a simple genetic difference between Negro and White.

Yet another reason to wish for greater knowledge is that, as understanding improves, so emotional reactions will become less. Suppose, for example, that a gene were indentified which, like the sickle-cell gene, confers some resistance against a tropical disease but whose compensatory disadvantage in the homozygote is a moderate drop in scholastic ability instead of a haemolytic disease: I believe this would be regarded purely as a piece of misfortune to those affected and in no way as a matter for shame.

It would be idle to suggest more specifically the way in which improved knowledge and understanding might be valuable. The chief characteristic of research is that its outcome is not known in advance. A large part of my address was devoted to examples where the progress of science was held up by a false assumption that a result could be deduced by extrapolation from what was already known. This part of my address you dismiss as "a lengthy aside", but it is highly relevant to the question of the causation of differences of ability. Our understanding in this field is still in a primitive state, and I would guess that there will be several unforeseen changes of view on both the environmental and the genetic side before an agreed position is reached. Meanwhile, what is needed is, on the one hand, for policy-makers to be conscious of this uncertainty, to be guided largely by humane considerations and by common sense rather than by predictions based on particular theories, and to avoid decisions liable to be harmful if unsuspected factors turn out to be at work. On the other hand, research should continue, with as little bias as can be attained and without premature claims on either side to have reached a final solution.

I retain a conviction—difficult no doubt to justify rigorously in each individual case—that an improvement in our understanding of a problem will in the long run improve our attempts to solve it. I find it deeply depressing that you, the Editor of the leading general scientific journal of the whole world, should in your final sentences tell us in effect that, in any field which is important enough to generate emotion, we ought not to undertake research until we are sure that its conclusion will be to our liking.

ANDREW HUXLEY, FRS

The full text of Sir Andrew Huxley's address appeared in The Times Higher Education Supplement of 2 September

Desertification: another mirage?

Robin Sharp reports on the UN Conference which ended in Nairobi earlier this month

DESERT rash is an ugly dermatitis which spreads in blotches and peels off bits of the world's skin. Having diagnosed it, traced it to no fewer than 45 sources and called it desertification, the United Nations has this month successfully patented a selection of medicines to control and cure the disease.

The UN Conference on Desertification (UNCOD) was very much a synthetic event, in the best sense of the term. Neither a scientific nor essentially a political gathering, it sought to bring together a range of disciplines and socio-political interests to create a new holistic approach to the problems of desert rash in the world's dry lands. The conference did not pretend to be inventing a new branch of science but rather a new conceptual framework for research and policy-making. Judging by the high degree of consensus among the 90 countries present on the ailment and its cure, this seems to have been achieved.

One danger with a newly-identified problem is that people too readily assume it needs new-fangled solutions. In the case of desertification, the teams of experts who produced the basic conference documents were cautious enough to recognise modern technology as one of the hazards as well as the hopes. Because of their fragility, the principal document noted, dryland ecosystems are particularly vulnerable to misapplied technology; and in the developing countries where acceptance by the local community is a critical factor, modifications of existing technology and practice are more likely to be effective than radical innovation.

In their official conference paper, 'Ecological change and desertification', Andrew Warren and Judith Maizels of University College, London, also warn that stories of technological success in dryland agriculture "should not be accepted complaisantly". Fifteen million hectares of land have been irrigated along the Indus River in Pakistan, for example, but an estimated two-thirds of this vast area is now affected by consequent waterlogging and salinisation.

The conference was presented with three other 'component reviews' covering technology, climate and human and social factors. All four were then summarised in an overview report, which

provided the basis for the first part of the conference debate. In fact, apart from various nuances and some disagreement over whether and how to define the neologism coined for the conference title, there was little dispute over the secretariat's analysis of the causes and effects of desertification.

Bone of contention

The only real bone of contention was whether or not, as averred by Dr Mostafa Tolba, the conference Secretary-General, "man now has in his possession both the wealth of knowledge and adequate technical means to bring desertification to a halt and, in many instances, to reverse the process". The overview paper emphasised the same point, asserting that the knowledge and experience to tackle most aspects of the problem was "available right now".

This, however, was not good enough for the six national and regional science associations which held a seminar in Nairobi immediately before the conference to feed in their views. In their opinion it reflected an implicit overconfidence in the adequacy of existing technology, not to mention an underestimation of the human and economic costs which in the short-term could well rival the costs of the problem itself.

Both concerns drew attention in the debate, a number of countries pointing out that more basic and applied research was still needed in areas such as water harvesting, rainfall prediction, water desalination, alternative energy sources and water-crop relationships. Several delegates stressed the need for intensified efforts in the field of climatology and a closer working relationship between climatologists and agriculturalists at various levels.

One experiment which may eventually enable climatological events to be predicted in the world's dry regions is to be launched next year by the World Meteorological Organisation (WMO). Under its Global Atmospheric Research Programme (GARP), run jointly with the International Council of Scientific Unions, the WMO will make detailed worldwide observations of the atmosphere over a 12-month period. It is described as the largest scientific experiment ever undertaken internationally for peaceful purposes, using satellites, aircraft, ships, balloons and Press handouts, so they'd better come up with something.

Plan of action

After agreeing that desertification has many causes and that most are man-made, the conference then moved on to consider what could be done about them. Though most of it may be no more than a set of guidelines for any government caring to take note, the UN conference formula these days makes it *de rigueur* to produce a 'plan of action'. And as these things go, the desertification action plan went pretty well. It recommended careful assessment and monitoring of the extent of desertification, proper management of water resources, rangelands, livestock and wildlife, urgent measures to overcome the loss of water and land caused by irrigation, special steps to improve soil conservation, national land-use planning, and—just to make sure that nothing was left out—"national systems for monitoring the human condition".

Focusing on the developing countries, the 'plan of action' called for measures to strengthen indigenous capabilities in science and technology, paying special attention to the rational use of dryland resources. This might include setting up or strengthening national scientific institutions concerned with desertification. The plan further urged that research be vigorously pursued on cheap alternative energy sources which could help to halt the alarming rate of deforestation caused by the pressure of demand for firewood in many dry countries.

Pilot project experiments could be set up to test the use of wind energy for generators and water pumps, and solar energy for water heaters and distillers, cookers, food dryers and refrigerators. On all these points the conference anticipated further action at the second UN Conference on Science and Technology to be held in two years' time.

The need for public awareness campaigns to educate people in general and dryland populations in particular about the sustainable use of their environment was emphasised by a number of countries. As the Australian delegation put it, the people contributing to the problem must be made aware of its existence and causes. On another tack, the Australians were scathing about the special desertification map produced for the conference. Not only did it contain serious errors, but at a scale of 1:25,000,000 it wasn't going to be much help and in any case the validity of mapping a set of dynamic processes was questionable.

Less than enthusiastic

For similar reasons, they were less than enthusiastic about the use of information from remote-sensing satellites as an anti-desertification tech-

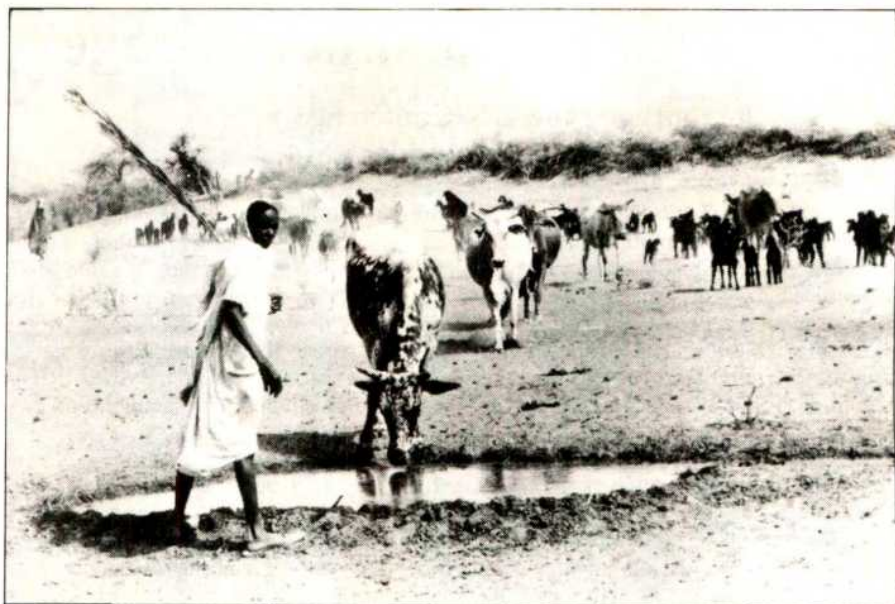
nique. Out of six transnational feasibility studies drawn up for the conference, two were devoted to the monitoring of desertification in south-west Asia and South America by Landsat imagery, using the Earth Resources Technology Satellite (ERTS). The use of satellite imagery thus came in for quite a bit of notice, but the Australians and others thought it had aroused "excessive expectations" and reckoned that for most purposes 'ground truth' observations were a much better bet.

The transnational feasibility studies were commissioned as the basis for possible inter-governmental agreements on large-scale regional projects to combat desertification. Apart from the two remote-sensing schemes, the studies provide for the planting of 'green belts' north and south of the Sahara, the management of livestock and rangelands in seven countries of the Sudan-Sahelian region, and management of the major regional aquifers in north-east Africa and the Arabian Peninsula. Though challenged on grounds both of cost and effectiveness, the Secretariat was able to claim that several governments had already signified their readiness to go ahead.

Inevitably in the volume of research amassed for this meeting there were a number of inconsistencies, plus a few worrying omissions. At some points in the documents and the debate, the need of developing countries for simple, low-cost technologies was carefully stressed, but elsewhere sophisticated remedies were advanced without much evidence whether those to whom they were addressed could or should afford them. Likewise, it was acknowledged that desirable changes in traditional farming and other forms of land use were subject to important economic, social and cultural restraints. Yet when it came to calculating how readily the threatened drylands would respond to the conference's action package, the implications of this seem to have been overlooked in favour of more measurable financial, scientific and administrative criteria.

Two omissions

Two significant factors in the desertification process were glossed over in Nairobi, intentionally or otherwise. Firstly, though the UN Food and Agriculture Organisation (FAO) has a conference on land reform scheduled for next year, the degree to which feudal or other land tenure systems still contribute to desertification in several countries—for example erosion caused by peasants forced out of the valleys to farm marginal slopes—was studiously ignored. And again, no one asked aloud whether those governments determined to get their nomads settled



Marginal living on the Saharan fringe

down had analysed the long-term consequences of demobilising the people best equipped to balance the economic potential and the limitations of life on the desert margin.

Perhaps the secretariat was a bit too anxious not to ruffle any political feathers. In any event, for better or worse, this was one of the least politicised UN meetings in recent times. Apart from an Arab move obliging the conference to pretend that Israel's impressive record of desert reclamation did not exist, the proceedings were remarkably free of ideological entanglements.

Of course there had to be a bit of a crunch at the end, and, not surprisingly, it centred on cash and administrative machinery for the post-conference follow-through. True to form, the industrialised countries came out against the creation of any new international body for desertification which they might have to pay for. They were having nothing to do with the notion of an international tax to help finance the 'plan of action', and Dr Tolba knew the odds when he conceded at the start that there might be "some problems" in attempting to set up another Special Fund for the purpose.

In the end, as everyone expected, the follow-through responsibility was handed over from Dr Tolba, Secretary-General of the conference, to Dr Tolba, Executive Director of the UN Environment Programme. For whatever apprehensions there might be in various capitals about UNEP, it was the cheapest, neatest, most logical answer—and Dr Tolba was generally acknowledged to have done a masterly job in getting the show on the road.

So from its barrack-like HQ on the outskirts of Nairobi, enlivened with

outdoor murals of wallowing hippos and the like, UNEP will now have the task of pulling all the other UN agencies together to work out uniform methodologies, to coordinate and expand their research efforts on desertification and to build a solid organisational structure inside the conference's conceptual scaffolding. A mammoth task, one might say, but there's no doubt much has been accomplished in the two years leading up to the conference.

Not too disappointing

By the time UNDP, UNIDO, FAO, UNESCO, WMO, WHO, the World Bank and others have reshuffled their budget headings to make room for this new arrival, even the present level of spending to combat desertification will probably not look too disappointing, given the many competing development priorities. And it should be worth much more if and when all the bits are linked into a concerted programme. Added to this, according to one estimate, are 15,000 or more researchers and 300 institutions around the world already involved in arid-land research.

With resources on this scale available to be mustered in a global campaign, the prospect of halting and pushing back the deserts might appear no longer a mirage. But there's still one more snag the conference tended to forget. By the year 2000, when the 'plan of action' is due to be fully implemented, the population of the world's drylands, now around 600 million, will be up to 1,000 million or more, with all the concomitant pressures this will bring. Those charged with the problem may therefore find themselves having to run very hard just to stand still. And come to think of it, that is a bit like chasing a mirage, after all. □

BRITAIN

Discomfiting prognoses

The UK Science Research Council (SRC) published its latest annual report last week. Chris Sherwell outlines some of the difficulties

BRITAIN'S Science Research Council sees itself as the country's only supporter of 'big' science. Research in high energy physics, astronomy, space and geophysics, it feels, would collapse if it withdrew its funding. The SRC is not about to pull out of these fields. But the pressure over the past few years to cut back has now reached such a pitch that the SRC believes it may not be able to discharge its responsibilities adequately by the early 1980s. Much now rests on whether some sort of turn-round materialises in the next couple of years.

Last week's report*, covering the twelve months to March this year, continues to promote the image of unrelieved gloom that has become familiar in recent years, and in some respects offers even more discomfiting prognoses. Some idea of the worrying trend appears in the accompanying table, showing how the distribution of SRC expenditure will change. But the report also spells out in cold terms what is called the "best disposition" for big science over the coming years.

Nuclear physics expenditure, for example, will run down more steeply, at 6½% a year instead of the 5% planned last year. The £43.5 million to be spent this year will fall to £33 million by 1981-82, of which fully £20 million will go on the CERN subscription. This level of support, says the SRC, is inadequate to continue the full exploitation of existing facilities, and will not permit the full use of the nuclear structure facility at Daresbury or allow British participation in any future international high energy facility.

The more important question for now, however, is whether this level is below the 'minimum viable level' of support which became common parlance by last year but which went largely unspecified. That it is lower than forecast is certain; but different views will abound on whether in the process the minimum level has actually been breached or simply redefined. The high energy physics community's argument for the former would presumably be dampened by a more efficacious use of the CERN facilities.

Astronomy and geophysics fare

even worse than nuclear physics. Expenditure of £28 million this year, which the report says is 14% down on last year, will fall further still to £23 million in 1981-82. Space expenditure outside the ESA subscription (which remains at £8 million in all years) will fall from something like double that subscription to two-thirds next year and one-half by 1981-82. The report says this means proper advantage cannot be taken of opportunities through ESA and the use of the Space Shuttle and Spacelab.

The corollary, however, is that the domestic programme suffers. Such are the consequences of tight money that this obviously threatens to raise questions about the value of organised international efforts in space research. The alternative would be *ad hoc* use of US facilities. No one is saying publicly that Britain is even close to this position. But the signs are that such a state of affairs could loom in the future.

One comparatively bright spot focuses on the special programmes in the areas which the SRC has selected for support because of their national importance. Three in particular—the Teaching Company Scheme, Marine Technology and Polymer Engineering—collect a 25% increase from £14.5 million in 1977-78 to about £18 million in 1981-82.

A reminder of the sort of problems that big science projects pose is contained in the report's review of the Science Board's work. The Board is experiencing them for the first time with the conversion of Nimrod, the proton synchrotron at Rutherford, to a spallation neutron source—one of the SRC's now famous attempts at cannibalising existing facilities to save money. The Board chairman, John Jinks, says control of year-by-year expenditure was simple and policy was quickly effective when research grants were its main activity; with central facilities, on the other hand, long-term financial commitments, quasi-permanent operations and established staffs mean that delays are the only

way to economise, and this can vitiate the usefulness of the research.

How far the SRC has gone along the immensely fruitful line of cannibalising existing facilities is not certain. Plainly, though, the need to do it depends on financial exigencies. International subscriptions, which form some 30% of the SRC's total expenditure, are fixed; with the international value of sterling beyond SRC influence, and expenditure subject to the Treasury's cash limits regime, the SRC lacks control over a sizeable fraction of its outgoings. Couple this with inadequate compensation for increased student grants and for subscriptions, mix in the effect of public expenditure cuts last year and those planned for coming years, and the SRC has a recipe for lower expectations. What makes the fare unsavoury is the higher number of qualified candidates for studentships for whom lack of support means lost opportunities for advanced training.

A pick-up in the British economy is one way the outlook might theoretically improve. Other more logical and practical ways, at least from the SRC's point of view, include a greater commitment to the country's research effort on the part of the government, and a greater commitment to the SRC rather than the other research councils on the part of the ABRC. If there is cause for hope on the latter two of these fronts it may indicate an incipient 'backlash' effect following the SRC's past acceptance of negative growth rates (now -1.7% until 1981-82). The SRC wants a constant budget to do a competent job, and to achieve that between now and 1981-82 it says it needs £25 million. But as yet this would demand a turn-round in policy as well as a turn-round for the economy.

● In a display of acute deadline consciousness, the appointment of Professor Geoffrey Allen as chairman of the Science Research Council has, after an unusually lengthy delay, been confirmed by Shirley Williams, the Secretary of State for Education and Science. The four-year appointment, which takes effect this coming week-end, first became known in March this year, and followed press reports that included names of certain individuals who had allegedly been approached for the job and turned it down. The usual need for clearances contributed to the delay, but administrative hold-ups and the publicity that surrounded the selection did not help.

Professor Allen takes over from Sir Sam Edwards, who takes on a part-time appointment as chairman of the Defence Science Advisory Committee.

Changes in approximate distribution of SRC expenditure under present programme

	%1976-77	%1981-82
Astronomy,		
Space and Radio	22	18
Nuclear Physics	36	26
Science	24	30
Engineering ¹	13	19
Central Programmes	1	3
Administration	4	4

¹ including radio propagation research

*SRC Report of the Council for the year 1976-77 (HMSO, £2.50)

COMECON

● A team from the Warsaw Institute of Plasma Physics and Laser Fusion, headed by Professor Sylwester Kaliski, Poland's Minister for Education and Science, has achieved the generation of neutrons by thermonuclear fusion using a concentric explosion with an exceptionally high degree of symmetry. The result was announced at an international conference in Prague last week and received considerable eve-of-conference publicity on Polish radio and television. According to the broadcast, this is the first published result of neutrons from thermonuclear fusion being obtained by pure explosion. The broadcast spoke optimistically of "extending the process to a commercial scale", but the figures indicate the production of only 3×10^7 neutrons from 10^{-7} g deuterium, and it is estimated that a level of some 10^{17} neutrons would be required for commercially viable thermonuclear power generation.

● It is reported from Poland that, in spite of the amnesty of last July, repressive measures are still being taken against supporters of the Workers' Defence Committee. The WDC was set up a year ago to defend the rights of the Radom and Ursus workers suffering reprisals for their part in protests against soaring food prices. During the next few months, a number of WDC members, including several scientists, were themselves subjected to repression, but support for the WDC grew. At the beginning of June, a group in Wrocław, mostly from higher academic institutions, sent a letter to the government criticising the press campaign against the WDC and urging that sanctions against WDC members be withdrawn.

A letter to the WDC now claims that shortly afterwards reprisals began against the signatories. Three lecturers, including Dr Eugeniusz Porada, a mathematician, lost their jobs, ostensibly for trivial administrative reasons. The letter was signed by Professor Stanisław Hartman of the Mathematics Institute of the Polish Academy of Sciences; he and Professor A. Duda have been relieved of their posts as consultants to the special mathematics courses for gifted young people, founded in 1973 in several university cities.

● Last month, the supervisors of the Comecon Departments of Inventions met in Ulan-Bator, Mongolia, to discuss the problems of patent protection within the Comecon bloc. Until the late 1950s, the Comecon countries somewhat neglected the protec-

tion of inventions and innovations; since then, the position has changed so much that now even minor factory modifications are granted a patent or its equivalent. However, a survey of Comecon patent law, published in Warsaw in 1970, revealed considerable discrepancies in practice between the various countries. Thus, in the



USSR and Bulgaria, an 'author's certificate' gives unlimited protection; in most Comecon countries, the patent protection is for 15 years, in East Germany 18 years and in Hungary 20 years. The invention must be used within two years of its being reported in Hungary, and within three years of the granting of the protection in Poland; in the USSR there is no obligation to use an economic patent at all. In 1971, the Conference of Supervisors of Departments of Inventions was established to promote cooperation in the field of patents and to eliminate such anomalies.

The twelfth meeting of the conference, which was opened recently by the Mongolian Deputy Premier, S. Luvsangombo, and chaired by the Deputy Chairman of the Mongolian State Committee for Science and Technology, presented a promising picture of work in hand. A progress report was presented on the co-ordination of draft agreements on mutual legal protection of instructions of origin and designation of origins of commodities. A model bilateral agreement on conducting patent investigations was approved. Special attention was paid to problems of "rationalising" the economy, including the work of "rationalisers" within the framework of international economic organisations and a model system for handing over rationalisation proposals as part of scientific and technical cooperation agreements.

● One important element in Comecon integration plans is computerisation of both production and planning. As far as the production of components is concerned, the computerisation plans seem to be going forward well. Bulgaria has recently built up with Soviet aid a flourishing electronics industry, and last year earned more than 480 million foreign-currency leva from exports.

When it comes to the introduction of computers into the Comecon economies, however, the picture is less rosy. In Czechoslovakia, for example, the predominant trend so far has been the construction of data-processing systems for individual factories. These facilities, however, have been grossly under-used, the factory computers often simply recording data which is not applied in subsequent management and planning decisions. Recent figures from the Czechoslovak Federal Statistical Office indicate that on occasion factories have not used as much as two-thirds of the available machine-time, but either sold it to other consumers, or else simply wasted it. Accordingly, greater emphasis is now being placed on the construction of combined data-processing centres.

● Anti-pollution measures have recently been commanding a great deal of attention throughout the Comecon media. Thus Bulgaria, which recently introduced a set of "Basic Guidelines for the Conservation of the Environment", is to spend 78 million leva in Sofia *okrug* (county) alone, mainly on water-treatment plants on the Iskar, Maritsa and Topolnitsa rivers, and on equipment to remove toxic gases from the air. This is clearly a matter of some urgency—last year in the Sofia *okrug* 106 industrial, economic and commercial enterprises had to be closed down, 25 of them permanently, for violating public health requirements.

In Hungary, air pollution surrounding the Metallokema works at Nagytetyen has been causing concern since the middle of April. Complaints from local residents indicate that household pets were dying and fruit, vegetables and window-sills were covered with a greyish dust. In addition, three small children had to be treated in hospital for lead poisoning: an investigation by the local health authorities showed that the lead content of the atmosphere was 30–40 times the permitted level, and on 30 June the factory was ordered to close. The order was ignored.

Vera Rich

IN BRIEF

JET: Costly hold-up?

Confirming the loss of members of his group to the United States, Dr Paul Rebut, the French scientist who heads the design team based at Culham working on the European fusion project JET, was quoted as saying last week that even if a decision on the site of the next stage of the project was taken now, it could take up to a year to re-form the team.

Dr Rebut's comments, in an interview with the *Oxford Mail*, followed the news that the EEC Council of Foreign Ministers had not even discussed the subject, let alone come to a decision, when it met last week. The subject did not appear on the agenda because meetings between representatives from

Britain and Germany, the countries with the strongest candidates for the siting of JET, had not taken place as planned since the last Council meeting in July. A high level Anglo-German meeting in Bonn set for 9-10 September was cancelled because of the kidnapping of Hans-Martin Schleyer. The ministers will meet informally on 8-9 October, and formally on 17-18 October.

NERC report, appointment

Like the UK SRC, the Natural Environment Research Council (NERC) last week published its report for the year to March 1977 and acquired a new chairman. A feature of the report is a

6-page description of the contribution NERC research can make to solving problems of energy and the environment; in particular, on the controversial issue of disposal of high-level radioactive waste, the report says the funds needed are "many times greater than the very modest sums" with which NERC is undertaking current studies.

The report also shows that the main bulk of the £26.675 million spent on NERC's scientific programme went on studies of the earth (42.2%) and the sea (32.9%). Commissioned research totalled nearly £15 million.

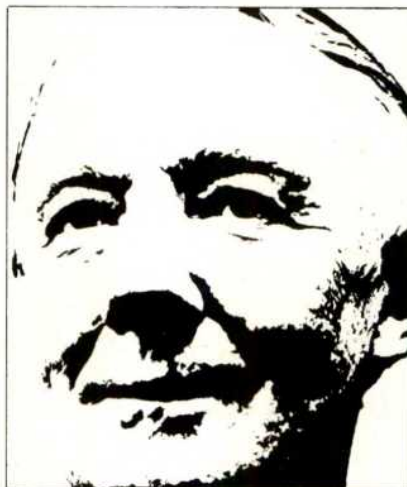
The man who will succeed Sir Peter Kent as chairman of NERC is Professor J. W. L. Beament, the insect physiologist.

MANY observers thought that the 1972 United Nations Conference on the Environment, in Stockholm, Sweden, was marred by the evident differences of opinion between the rich and poor nations on their priorities. The delegates from the so-called developed countries in Europe and North America were enthusiastic about controlling pollution (even when their governments were not always effective in carrying out these intentions). Many speakers from the poorer countries, now optimistically called 'developing', said that increasing their productivity and raising the standard of living of their citizens was more important. They pointed out that Britain and other comparatively wealthy countries had done little to control pollution during their industrial revolution, in the nineteenth century, and to impose unrealistic and expensive standards on those who were just setting out on the same road would hold them back unnecessarily.

It is encouraging to find that this view is not universally held. I am just back from Mauritius, where I was invited by its government to advise on environmental problems and their control. Mauritius, known to many only as the former home of the extinct dodo, and the place where the most valuable postage stamp was printed, is a volcanic island in the middle of the Indian Ocean. It covers some 760 square miles and has over 800,000 inhabitants. It was first a Dutch and then a French colony, until captured by the British in 1810. It remained a British colony until 1968, when it became an independent state, a member of the Commonwealth. Its main income is from sugar, and some 54% of the island's surface is covered by sugar cane. It

is a net food importer, and attempts to reach greater self-sufficiency by the diversification of agriculture are being made, though little good land remains uncultivated. Industrial development, particularly of light in-

Dirty development



KENNETH MELLANBY

dustries, has recently made some progress, and is said to produce nearly a quarter of the island's income.

As yet Mauritius has no really damaging pollution problems. It would be hard to produce serious air pollution in a small oceanic island in the region of the trade winds. There is some smoke from factories, dust from stone crushers, and road safety is often impaired by the smoke screens from badly maintained diesel lorries and buses. The beaches of white coral sand, the main tourist attraction, are clean except near settlements and sewage outfalls. How-

ever, there are many at present trivial nuisances which could soon grow into environmental dangers. Refuse, likely to increase with population and a growing GNP, is seldom collected and never dealt with hygienically. Much is thrown into streams, the one place where the maximum damage will occur. Factories are surrounded by noisome piles of uncollected debris. Some people exhibit a genius for doing the most damage with the smallest amount of a pollutant. Thus a small tea factory in a sparsely inhabited hilly area has a leaky pipe from its oil tank. A few gallons seep out each year into the adjoining stream, half a mile above the intake for the local water supply. Garages always seem to empty their used sump oil into the nearest river.

Thus with a growing population, rising incomes and more industry the future for Mauritius could be a dirty one, unless immediate steps are taken to preserve the environment. Some encouragement can be obtained from the sugar industry, which could be a major polluter. The largest and best-run factories have remarkably little harmful impact on the environment, and the others are improving. The industry clearly knows what needs to be done. None of the other problems is insoluble—and success will be easier the sooner vigorous action is taken. In all developing countries prospects are better than they were some years ago. The most valuable aid that the developed countries can give is knowledge of how they have got over the abuses of the nineteenth century. They must show that with modern industrial development, pollution control need not be unduly expensive, and that early action can be an excellent investment.

correspondence

Europe versus itself

SIR,—With reference to the editorial "Million-dollar problem—billion dollar solution?" (14 July, page 89), the problem of sulphur pollution is not a problem of Norway versus the United Kingdom, but rather of Europe versus itself.

The OECD study, to which the article refers, has shown that there is a more or less continuous export and import of pollutants between European countries and that the problem is not confined to any two countries. Nor can it be solved by individual national control programmes. It should also be emphasised that the problem is not predominantly confined to the effects of acid rainfall on fish populations. The effects of sulphur pollution fall into four main areas: human health, vegetation, the fresh water ecology, and corrosion of metals, painted surfaces and other materials.

It is established that several categories of human disease are linked to the occurrence of sulphur dioxide and associated pollutants in the atmosphere. Governments' efforts to improve the quality of the air so as to provide better health protection are likely to have the reduction of sulphur emissions as one of their main targets.

With regard to corrosion we know that material damage due to sulphur pollution in Sweden and the United States cost between \$5 and \$10 per capita per year in the early 1970s. We know that damage is inflicted on vegetation in areas polluted with high concentrations of sulphur. It is also suspected that the dispersion of sulphur will result in decreased tree growth in areas with relatively low average concentrations of pollutant. The economic consequences of such effects can be highly significant.

We must also expect synergetic or combined effects in wide areas within Europe where various harmful pollutants are dispersed through the atmosphere. Efforts should now be directed at developing a coordinated European policy for reducing emissions. The costs involved will, it is true, be formidable. Only gradual improvement can therefore be expected. The result, however, will be beneficial to health, to materials susceptible to corrosion and to the quality of the natural environ-

ment all over Europe, with particular benefits accruing to people and areas closest to emission sources.

Yours faithfully,

ERIK LYKKE

Ministry of Environment,
Norway

Nutrition in food policies

SIR,—Blythe and Rush (4 August, page 386) outlined the state of the cautious dialectics of a UK food policy which incorporates nutritional criteria. The arguments are complex and controversial, not least because of the range of disciplinary interests involved. Three of the arguments referred to by the authors warrant further comment.

First, is the search for 'absolute proof' of causal linkages between dietary constituents and diseases necessary before recommendations commensurate with formulating food policy can be made? Is 'absolute proof' actually attainable? The scientific method sets out to falsify theories through experimentation, never to prove their validity, it is neither the intent of the method nor logically possible to prove the validity of theories, irrespective of the number of experiments. Thus, the argument should be concerned with the degree of confidence that can be attached to the corroboration between theories and observations in scientific experiments. Thus, for example, where there exists a sustainable conjuncture that a particular level of dietary fat (or saturated fat) is detrimental to health, this should be sufficient basis for a recommendation.

Second, the lack of an explicit food policy which links nutrition and health to food supply is tantamount to accepting, by default, that the fragmentary components of existing policies which affect food consumption adequately accommodate nutrition and health goals. Equally, the nutrition and medical professions, by failing to make recommendations, are by default condoning the present dietary trends.

Concerns that the introduction of nutritional goals into agriculture and food supply policy would require significant structural changes are exaggerated. The food supply industry is not static: at present there is a programme

for expansion of UK agriculture, and for further structural change within the EEC. There is no reason to believe that the changes needed to include nutritional goals would be any greater than those already under discussion or being implemented for sectional economic interests alone.

Thirdly, the controversy surrounding state intervention in consumers' freedom of choice through a food and nutrition policy is a red herring. The extent to which a real freedom of choice exists is academic, but it is evident that consumers' choice is directed by a wide range of measures from diverse sources. Effectively, consumers are free to choose from numerous, but pre-determined, alternatives, and that choice is further constrained by such factors as their income, access to information, and susceptibility to advertising. A food and nutrition policy would not necessarily impose further restrictions on choice, but could provide the consumer with a more rational and coherent basis for his choice. This implies that all consumers have access to a nutritionally adequate diet with established health safeguards, while maintaining the widest feasible variety and availability of foods. As a wide access to relevant information on nutrition, health and foods is a component of most conceptions of a food and nutrition policy, it can be argued that such a possibility would increase the consumer's capacity to exercise a free choice, rather than reduce it.

Yours faithfully,

C. J. ROBBINS

Reading,
Berkshire

Crater good

SIR,—I shall say an Ave Mare for the soul of David W. Hughes (15 September, page 197).

Yours faithfully,

L. ROSE

Leicestershire, UK

Correction

In T. R. C. Boyde's quoted derivation of the word 'enzyme' (15 September, page 194), the Greek for 'in yeast' should have read *ενζυμη*, not *ενζυμη*. The modern Greek would read *ενζυμοσ*.

news and views

The ultraviolet sense in flies

from Jonathan Ashmore

MANY insects can detect light in the near ultraviolet. So also can humans but there are problems in explaining the much greater insect ultraviolet sensitivity in terms of what is known about the visual pigments. Rhodopsin is probably one of the most intensively studied membrane proteins, yet great gaps exist in the description of the chain of events starting with the photoisomerisation of the chromophore 11-*cis* retinal and ending with the signal appearing in the nervous system. But in both vertebrate and invertebrate systems the chromophore has always turned out to be retinal, the aldehyde of vitamin A. The peak absorption wavelength λ_{\max} for those rhodopsins which have been studied either in the membrane or in solution lies in the range 460 nm to 535 nm. Free retinal has a λ_{\max} of 378 nm, and its association with the protein by way of the usual protonated Schiff base would tend to move λ_{\max} to longer wavelengths only.

However, the insect ultraviolet peak measured in the photoreceptors of a number of species both by electrophysiological and microspectrophotometric methods occurs around 360 nm. A pigment from the moth *Ascalaphus* has been isolated by Hamdorf and co-workers (*Nature* **231**, 438; 1971) with a λ_{\max} at 350 nm and retinal as the presumed chromophore, but the matter is not settled there. In rhabdomeres R1-6 of the dark adapted dipteran ommatidium, spectral absorption peaks not only at 500 nm but also in the ultraviolet. The precise wavelengths are species dependent, but the ultraviolet absorption has peculiarities: the phenomenon appears to be fairly labile, varying from cell to cell and may even change seasonally (Horridge & Mimura *Proc. R. Soc. B* **190**, 211; 1975). Since its first description 15 years ago it is still not clear whether one or two pigments are present. The sharp angular sensitivity of the receptors seems to rule out the possibility

that there is coupling from the specifically ultraviolet receptors R7 and R8.

On page 386 of this week's issue of *Nature* Kirschfeld *et al.* sidestep the one/two pigment issue by suggesting that the ultraviolet sensitivity of dipteran photoreceptors may be due to a mechanism similar to that used to extend the spectral sensitivity of photographic emulsions. Performing the microspectrophotometry on rhabdomeres R1-6 of the housefly *Musca*, they extend the earlier work on *Calliphora* by Langer and Thorell (*Expl. Cell. Res.* **41**, 673; 1966) to show that although the ultraviolet peak occurs in absolute absorption spectrum there is no conspicuous change in the ultraviolet extinction following adaptation by ultraviolet light. Such a change is virtually a prerequisite for the presence of a rhodopsin-like pigment, and special mechanisms need to be invoked to explain its absence.

The first relatively stable reaction product of rhodopsin following the absorption of light is metarhodopsin. Insect metarhodopsins are distinguished by the large redshifts of the absorption peak relative to rhodopsin. In *Musca* the effect of absorption of ultraviolet light seems to be the production of only the metarhodopsin with λ_{\max} of 580 nm which is that formed from the blue-absorbing rhodopsin with a λ_{\max} of 490 nm.

Where is the ultraviolet light being absorbed? The new technique which has been used to study the mechanism further is electrophysiological. Pak and Lidington (*J. gen. Physiol.* **63**, 740; 1974) reported that the fast potential elicited by short intense flashes of light with external recording from the photoreceptors is proportional to the metarhodopsin concentration. This provides a direct assessment of the pigment state, whereas earlier work relied on the slower receptor potentials where several stages of transduction are involved before the electrical signal can be measured. Kirschfeld *et al.* find in *Drosophila* that those flies fed a vitamin A deficient diet have a lower ultraviolet quantum efficiency com-

pared to blue light than those flies raised on a vitamin A enriched medium. The method used can now leave little doubt that the vitamin A deficiency alters in some way the efficiency of ultraviolet light absorption at the level of the visual pigment itself. Species differences in visual performance between various dipteran species have been noted as a result of vitamin A deprivation (reviewed by Stark *et al.* *Naturwissenschaften* **63**, 513; 1976), but the differences between *Musca* and *Drosophila* are probably not significant here.

The novel explanation offered for these observations is that a photostable pigment is present in normal flies which absorbs in the ultraviolet and transfers energy to the chromophore on the rhodopsin molecule. Although vitamin A deficiency may be working by reducing the rhodopsin concentration (see for example Harris *et al.* *Nature* **266**, 648; 1977), the primary effect must be the reduction in retinal and its derivatives. A vitamin A derivative is thus a likely candidate for the photostable pigment postulated, presumably membrane-bound in view of the sensitivity of these receptors to the plane of polarisation of ultraviolet light (Horridge & Mimura *op. cit.*). Cattle rhodopsin has in fact been prepared artificially with two retinals bound and has a double absorption peak, one in the ultraviolet (Rotmans *et al.* *Biochim. biophys. Acta* **357**, 151; 1974). Functional studies of such a system have not been made but it is known that fluorescent probes can be attached to sites on the rhodopsin molecule (Wu & Stryer *Proc. natn. Acad. Sci. U.S.A.* **69**, 1104; 1972) whose behaviour suggests that the kind of energy transfer required to explain the insect data may be possible given a suitably close pair of sites.

Although not based on radically new data, the suggestion of a 'sensitising pigment' may be the solution to old problems. Further developments including a more direct attack on the protein chemistry involved should tell whether it is right. □

Interferon action revisited

from Paula M. Pitha

INTERFERONS are species-specific cellular glycoproteins synthesised by a wide variety of animal cells in response to viral infection (or various non-viral stimuli) which induce antiviral resistance in homologous cells. In interferon-treated cells, a wide variety of different viruses do not replicate well. It was shown with a majority of the viruses studied that the primary effect of interferon is a general inhibition of viral protein synthesis. In addition to this block it has also been found that with some viruses such as VSV and SV40 the transcription of viral mRNA is decreased in interferon-treated cells. Oncornavirus replication seems to be inhibited by interferon at the level of virus maturation and assembly.

The nature of the molecular mechanism by which interferon induces the antiviral state in the cell has not yet been clarified. Interferon by itself is not an antiviral agent. The antiviral state does not develop in enucleated cells or in cells treated with actinomycin D or cycloheximide. This indicates that the antiviral effect of interferon requires both transcription and translation of the cellular genome and leads to the proposal that there is an interferon-induced antiviral protein (Taylor *Biochem. biophys. Res. Commun.* **14**, 447; 1964). The situation, however, is not that simple; recent results from the laboratories of I. Kerr (MRC, Mill Hill, London), P. Lengyel (Yale University, Connecticut) and M. Revel (Weizmann Institute of Science, Rehovot) with cell-free protein synthesising systems, indicate that interferon treatment induces the synthesis of several translational inhibitors.

The first step in the induction of the antiviral effect is the interaction of interferon with the cellular membrane of a sensitive cell, and it has been observed that the interferon binding involves both cellular gangliosides and glycoproteins (Besancon & Ankel *Nature* **252**, 478; 1974; Vengris *et al. Virology* **72**, 486; 1976; Kohn *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 3695; 1976). The cellular uptake of interferon is not required for the antiviral effect (Ankel *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 2360; 1973); the sensitivity to interferon seems to be determined by a receptor located at the plasma membrane. Using antiserum to interferon, which neutralised all free inter-

feron in the medium, Vengris *et al. (Virology* **64**, 410; 1975) demonstrated that for the development of the antiviral state interferon had to interact with the external cellular membrane of the cell producing interferon.

The results obtained by several groups (for example F. Ruddle at Yale University, Connecticut, and F. C. Chany at INSERM, Paris) using mouse-human cell hybrids demonstrated that the sensitivity to human interferon is governed by human chromosome 21. Trisomy 21 cells were markedly more sensitive to human interferon than normal diploid cells or monosomy 21 cells (Tan *et al. Science* **186**, 61; 1974; Chany *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3129; 1975). Antibodies to hybrid cells containing human chromosome 21 were found to inhibit the antiviral effect of human interferon in human cells. Revel *et al. (Nature* **260**, 139; 1976) suggested that chromosome 21 codes for the receptor for human interferon on the cell surface.

The species specificity of interferons seems to be also determined at the receptor level. Recent data indicate that in primate-mouse hybrid cells containing only one or a few human chromosomes interferon specificity is due to the interaction with the receptor but the antiviral mechanism induced with both mouse and human interferon is of mouse origin (Chany, *Biomedicine Express* **24**, 148; 1976). This indicates that at least some components of the antiviral effect do not have a requirement for species specificity. Samuel and Joklik (*Virology* **75**, 166; 1976) came to a similar conclusion when they showed that interferon treatment induced a ribosome-associated inhibitor of viral mRNA translation in cell-free systems that could be dissociated from ribosomes and inhibit the translation of viral mRNA in a heterologous cell-free protein synthesising system.

In this issue of *Nature* (page 422) Blalock and Baron from the University of Texas at Galveston present another type of evidence that the interferon-induced antiviral 'machinery' may not be species specific. The authors also suggest that the antiviral compound induced by interferon treatment of the homologous cells can be transferred to the cell of the heterologous species and elicit in it resistance to viral infection. The experiments are based on cocultivation of mouse cells (sensitive to mouse interferon) with interferon-insensitive human cells in the presence of mouse interferon, infection with

VSV or vaccinia virus, and measurement of virus yield. To achieve the effect close contact between the two cell populations was required; the effect was not observed in cultures plated at low cell density. Actinomycin D treatment of the mouse cells blocked both the antiviral effect in the mouse cells and their ability to transfer resistance to the heterologous cells. It seems, therefore, that the transferred compound may be a product of interferon induction rather than interferon itself.

This conclusion, however, is based on experiments done in mass cultures and the challenging viruses replicated both in homologous and heterologous cells. In the future much clearer data would be obtained if the infecting virus could not replicate in the cells homologous to the interferon but only in the cells to which the antiviral compound was transferred. It has been shown previously that the effect of interferon is an all-or-none phenomenon in L cells and in chick embryo cells (Chang *et al. J. gen. Virol.* **20**, 139; 1973; Radke *et al. J. Virol.* **13**, 623; 1974), and thus it should be possible to measure the transfer of viral resistance from the cell in the antiviral state to the adjacent neighbour. Fluorescent antibody or infectious centre techniques could be used to confirm the requirement for cell-cell contact.

One of the remarkable properties of the interferon system is its high biological activity. The specific activity of both human and mouse interferon is estimated to be close to 10^9 units per mg protein (one unit is the amount of interferon which inhibits virus replication by 50%). It is probable that such a system involves mechanisms which amplify the initial response. Both the above described transfer of antiviral resistance and the mobility of the interferon receptors on the cell surface (Lebon *et al. Proc. Soc. exp. Biol. Med.* **149**, 108; 1975) may be amplification factors in the interferon system.

Currently it seems that the action of interferon at the cellular surface resembles in many respects the action of the polypeptide hormones. The major task for the future is to answer the question of how the impulse from the cell membrane is transferred to the nucleus. One possibility which remains to be determined is that the antiviral effect of interferon is mediated through a second message such as cyclic AMP (Weber & Stewart *J. gen. Virol.* **28**, 363; 1975). □

High-energy physics and nuclear structure

from C. J. Batty

A conference on high-energy physics and nuclear structure was held in Zurich on 29 August–2 September and organised by the Swiss Institute for Nuclear Research. This was the seventh in an international series of conferences held every two years. The theme of this conference series is the use of high-energy particles and accelerators to study the properties and interactions of nuclei and the use of low energy techniques and instrumentation to study the fundamental properties and interactions of elementary particles.

OF recent years this field has become increasingly dominated by the three 'pion factories' at LAMPF (Los Alamos), SIN (Zurich) and TRIUMF (Vancouver) and this conference was no exception, with all three accelerators producing a flood of papers on a very wide range of topics but with an

emphasis on proton and pion interactions with nuclei.

Of particular interest on the last day of the conference was a presentation of experiments from all three laboratories on the rare decays of muons. In an introductory talk L. Wolfenstein (Carnegie-Mellon University) briefly reviewed the gauge theories of the weak and electromagnetic interaction which have evolved from the work of S. Weinberg (who also spoke earlier in the meeting on this topic) and A. Salam. Wolfenstein pointed out that recent suggestions of the existence of a third lepton could lead to a coupling between the muon and electron such that decays of the type $\mu \rightarrow e + \gamma$ could occur, although at a very low rate compared with the normal decay $\mu \rightarrow e + \nu_e + \bar{\nu}_\mu$. The question now is, do these rare decays occur?

All three laboratories presented status reports on experiments to measure the relative rate of the decay $\mu \rightarrow e + \gamma$. The work is technically very difficult and much of the discussion was concerned with presenting the many checks and detailed arrangements required to reduce the background to satisfactory levels and to ensure that any events seen which satisfied the

many selection criteria were true rare decays. The TRIUMF team have obtained an upper limit of 3.6×10^{-9} at 90% confidence for the relative rate. In a similar experiment at SIN in which 6.3×10^{11} muons were observed the upper limit obtained was 1.6×10^{-9} again with 90% confidence. In an attempt to reduce these levels even further a group at LAMPF led by H. L. Anderson are using a very high intensity muon beam with a magnetic spectrometer to detect the decay electrons. Running on the experiment has now started and the results are eagerly awaited.

In a related experiment a group at SIN have been looking for interactions of the type $\mu^- + S \rightarrow e^+ + X$ using a sulphur target and a streamer chamber to detect the electrons. Again no true events have been seen and with the very low backgrounds achieved the group are able to set limits of 4×10^{-10} and 1×10^{-9} for reactions leading to electrons or positrons respectively.

However it would be wrong to give the impression that work from the three pion factories entirely dominated the conference. Measurements at Saclay of the scattering of 600 MeV electrons by ^{208}Pb with the cross sec-

It is difficult to test experimentally the effectiveness of r and K selective pressures in the environment. Most of the evidence which has accrued in support of this useful concept has been circumstantial. For example, plant ecotypes of disturbed habitats often produce more seeds per plant and have a lower mean seed weight than their equivalents in stable situations (see *Nature* **262**, 351; 1976) and it is reasonable that one should account for this by reference to the selective pressures imposed upon populations. In a situation where disturbance, or calamity is frequent, the individual with a capacity for rapid and abundant seeding is more likely to have its genes represented in subsequent generations.

One of the clearest demonstrations of this in plants has been the work of Solbrig and Simpson on dandelions (*J. Ecol.* **62**, 473; 1974). In the triploid, apomictic species *Taraxacum officinale*, it has been possible to identify biotypes cytologically which are associated with different grassland conditions. Biotype A is found to predominate in disturbed sites and produce a larger number of smaller seeds than biotype D, which is more common in stable, undisturbed grass-

land. Experimental work has shown that although the two biotypes have similar growth rates in pure cultures, when grown together biotype D is more productive and has a lower mortality (see *Nature* **252**, 191; 1974). In other words, biotype D is more competitive in undisturbed experimental conditions.

Solbrig and Simpson have now (*J. Ecol.* **65**, 427; 1977) developed and extended this experimental approach to the investigation of r and K selection by introducing a disturbance factor

Disturbed environments

from Peter D. Moore

into their experimental conditions. Seven plots of 2 m by 2 m were established and each was divided into four. Of the four subplots, two were planted with a mixture of A and D biotypes of dandelion and the other two with pure populations of each of the biotypes (four plants in each subplot). Screens were erected to prevent seeding from one subplot to another. In two of the seven plots the entire above-ground vegetation was removed (from all four subplots) in the middle of the second and the third growing season. In two more

plots, the above and below ground biomass was removed after tilling the soil (leaving only the residual seed in the soil). The remaining three plots were left undisturbed until the completion of the experiment at the end of the fifth growing season. All dandelions were then dug up, typed cytologically and weighed.

The results after final harvest showed that biotype D dominated the undisturbed plots; 85% of the plants were D type and constituted 91% of the biomass. In the plots where aerial biomass had been removed on two occasions, the situation was reversed and type A was dominant (93% of the plants occupying 93% of the biomass). A similar predominance of type A was found in the plots where all dandelion material (excepting seeds) had been removed. Type A was responsible for 92% of the plants and 97% of the biomass.

It seems therefore that the high competitive ability shown by biotype D in previous experiments indeed depends on no disturbance during the experiment. The balance between the two biotypes in a grassland habitat is evidently controlled by the degree of disturbance experienced. These experimental data therefore fully support the theoretical framework which is currently being assembled around the concept of r and K selection.

Peter D. Moore is a Lecturer in the Department of Plant Sciences, King's College, London.

tions spanning 11 decades have given charge distributions, and hence proton density distributions which significantly disagree with some of the best theoretical nuclear structure calculations. Measurements of electron scattering at high momentum transfer for non-zero spin nuclei give information on magnetic scattering which arises essentially from a single nucleon. Recent measurements of the radii of the orbits of these nucleons are again found to be in significant disagreement with theoretical calculations. It seems to be once again back to the computers for the nuclear structure theorists.

Studies of exotic atoms in which an electron is replaced by a heavier particle such as a muon, pion, kaon, sigma hyperon or antiproton are in progress at several laboratories. A collaboration of groups from the Universities of Birmingham and Surrey and the Rutherford Laboratory using the kaon beam from the Nimrod accelerator (now unhappily due to be closed down next June) reported the first measurements of the effects of the strong nuclear interaction on the line-shapes and energies of X rays from sigma-hyperonic atoms. Much of the discussion in this field however was focused on attempts to observe X rays from \bar{p} -p atoms where the usual electron in the hydrogen atom has been replaced by an antiproton. Teams working at CERN and Brookhaven have so far failed to see any evidence for X rays corresponding to 2p-1S atomic transitions. However a second team composed of physicists from CERN, Daresbury, TRIUMF and the University of Mainz also working at CERN claimed preliminary evidence for the observation of X rays leading to the 2p state. Theoretical calculations also presented at the meeting now suggest that the nuclear interaction in the 2p state may be such as to prevent the observation of 2p-1S transitions.

Although the evidence for X rays from the \bar{p} -p system is still controversial one of the groups at CERN presented some very direct evidence for the observation of high energy γ rays from quasi-stationary bound states of the \bar{p} -p system below threshold. Three narrow γ -ray lines were observed using a very large NaI crystal for detection. The existence of states of this type was predicted several years ago by a group of Russian physicists and their discovery, together with that of narrow resonance in \bar{p} -p interactions at energies just above threshold will give an added impetus to attempts at both CERN and at the Fermi National Accelerator Laboratory in the USA to

produce intense beams of low energy antiprotons for experiments of this type.

From these brief glimpses of just a few of the topics covered at the conference it can be seen that the discussions ranged over a wide variety of subjects. At a time when the two disciplines of high-energy (elementary particle) and nuclear (structure) physics seem to be growing even further apart the conference showed that the two topics have not entirely lost contact and that a very fruitful and productive liaison still exists. \square

The truth about the lower crust

from Peter J. Smith

WHAT is the physical and mineralogical structure of the lower continental crust? Several models have been proposed in the past, ranging from a fairly homogeneous layer of gabbro or basalt (based primarily on geophysical data) to a much more complex zone in which granulitic metamorphic rocks predominate (from geological evidence). Yet, local variations apart, both of these cannot be correct. Ultimately, all geochemical observations must be reconciled and integrated into a single consistent picture, which is what Smithson and Brown (*Earth planet. sci. Lett.* **35**, 134; 1977) have now attempted to do.

The 'simplest and most important conclusion' to be drawn from the new analysis is that the lower crust is certainly not homogeneous. Evidence for heterogeneity has been available for some time in the velocity variations and anomalies revealed by seismic refraction studies. But what really clinches the matter, according to Smithson and Brown, is the existence of numerous seismic reflectors in the lower crust. As they put it themselves: 'With no speculative interpretation at all, these reflections demonstrate the presence of numerous interfaces and a much more complicated lower crustal structure than has previously been supposed.'

Indeed, things are more complicated than even the mere presence of reflectors would suggest, for individual reflectors usually lack continuity. Moreover, the lower crust must on average be 'distinctly less mafic (and less dense) than gabbro', partly

because most seismic velocities found there correspond to granulitic facies rocks from granitic to intermediate composition and partly because if the most mafic rock type to be expected there in general is gabbro, more felsic rocks must also be present in abundance to provide the contrasts giving rise to the reflections.

What Smithson and Brown concluded from all this and more (for example, from direct observation of lower crustal sections now exposed) is that the lower crust can only be a complex series of metamorphic rocks interspersed with igneous bodies. Specifically, it probably comprises such rock types as granite gneiss, syenite gneiss, anorthosite, pyroxene granulite and amphibolite, all deformed, interlayered with both sharp and gradational contacts, and intruded by granite and gabbro. In such a predominantly metamorphic environment, isoclinal folding, abrupt changes in dip, distortion of layers, changes in layer thickness and igneous intrusion are only to be expected, offering an obvious explanation for the discontinuous reflectors observed.

The age of innocence is over as far as the lower continental crust is concerned. As a few people have consistently maintained, the geophysical simplicity has always been merely an illusion based on insufficient data. Not that geophysical data will ever be sufficient. For as Smithson and Brown point out, 'if the lower crust is metamorphic, not even high-resolution seismic reflection studies will ever reveal the detailed structure. Complex fold patterns... are unlikely to be resolved by any geophysical method.' \square

The nucleolus at Salamanca

from E. G. Jordan and U. E. Loening

The Fifth European Nucleolar Workshop of the European Cell Biology Organisation (ECBO) was held at the University of Salamanca on 27 June-1 July 1977, and was organised by Professor Giménez-Martín and others from the Consejo Superior de Investigaciones Científicas (CSIC), Madrid.

W. BERNHARD (Institute for Cancer Research, Villejuif) opened the workshop with the hope that the many specialities represented might be integrated, even if it was too early to speak of a synthesis, through a mutual respect and understanding between scientists.

The nucleolar rRNA genes provide an excellent system for studying transcription and gene organisation. The 'spreading technique' for visualising transcription in the electron microscope was first developed using nucleolar genes by Miller and Beatty. It has now been combined with autoradiography by N. Angelier *et al.* (Centre de Recherche, Ivry Seine) and S. Fakan *et al.* (Cancer Research Institute, Lausanne) to study transcription in nucleolar genes from amphibia and non-nucleolar genes from mammalian cells respectively. Autoradiographs of the typical nucleolar 'Christmas tree' transcriptional unit gave two surprising quantitative results. First, genes which were morphologically identical nevertheless showed different incorporation rates, indicating separate, single cistron control of the rate of transcription. Second, the latter part of the 'Christmas tree' covering the last third or so of the transcribed area showed no increase in the specific activity of the RNA. This may suggest that processing, the trimming of the transcript by nucleases, begins before termination.

One outstanding question is whether there are different sets of ribosomal genes. U. Scheer (Cancer Research Centre, Heidelberg) presented evidence for several size classes of transcription unit within the same organism although the apparent length of consecutive units on the same DNA axis seemed very uniform. M. Buongiorno-Nardelli (Embryology Institute, Rome) showed, in *Drosophila*, that the ribosomal genes of the same size class occur adjacent to each other in blocks and only selected size classes are amplified. Not only may the spacers and transcription units be different in length, but further confirmation that the rRNA precursor may be heterogeneous was again forthcoming. Scheer confirmed that the molecular weight of the pre-rRNA did not always agree with the transcript length and was sometimes heterogeneous. Supporting Scheer's electron micrographs D. Rungger and M. Crippa (University of Geneva) provided evidence that it is possible to detect RNA which hybridises to cloned ribosomal spacer DNA and that longer transcripts than the 40S pre-rRNA in *Xenopus* can be detected when processing is inhibited by fluorouridine. This suggests that transcription sometimes runs into the spacer.

A lively round-table discussion tried to put these and other results together: it is still not possible to define the primary transcript exactly since the pre-

rRNA may already be partially processed; no triphosphates have been found at the 5' start of the precursor; there may be some variability or multiplicity of initiation sites and read-through into the spacer, and perhaps a sliding or non-transcriptional movement or relocation of the polymerase carrying the RNP fibril. The mechanism and control of transcription of the ribosomal genes is clearly far from being understood despite the large amount of established data. The failure to find initiation *in vitro* with isolated nucleoli does not at present clarify anything.

However, as on every previous occasion, possible candidates for enzymes which process the precursor were described. M. Muramatsu (Tokyo University) had one; I. Grummt (Max-Planck-Institute, Munich) in collaboration with R. Crouch and S. Hall (National Institutes of Health, Bethesda) described a purified enzyme from the nucleolus which cleaves double-stranded RNA and converts the 45S precursor into distinct cleavage products; it is inactive under the conditions usually used for *in vitro* transcription by nucleoli. Perhaps this time a real processing enzyme has been identified. The spreading technique also provides evidence for the absence of nucleosomes in the nucleolar transcription complexes, which have the same length as measured on purified DNA (Scheer). This can be reconciled with the biochemical evidence for the presence of histones in the transcription complex by the idea that the nucleosome is a dynamic structure (reviewed by T. Tsanev (Bulgarian Academy of Sciences)) whose components may be rearranged in certain conditions. This would allow the conclusion that all the histones are present and arranged in units which can readily associate to form nucleosomes but which are sensitive to detergent and may only appear as a 3-nm fibril in spread preparations.

A clue to the way histones and nucleosomes might regulate transcription was presented by H. Busch (Baylor College of Medicine, Texas). By comparing the protein patterns from chromatin from active and inactive nucleoli he has discovered four proteins which markedly decreased in active chromatin. One of these proved to be a branched molecule containing a complete H2A sequence linked to the protein ubiquitin. One-fifth of the nuclear H2A could be accounted for in such molecules. He postulated that such a molecule in this proportion might easily have a key role in transcriptional regulation through the linking of nucleosomes in the stabilisation of the supercoils in condensed chromatin.

Various reports showed that both

HnRNA and nucleolar RNA synthesis is located at a cytological boundary. For HnRNA this is between dense and diffuse chromatin; for rRNA it is at the surface of the nucleolus organising region within the nucleolus.

Several speakers addressed the problem of how pre-messenger RNA is transported to the cytoplasm. It appears that it is wrapped in a wide variety of proteins, transported to the cytoplasm through the nuclear pore and there stored as protein-bound RNA or used immediately on polysomes. S. Penman (Massachusetts Institute of Technology) described a cytoskeleton (See *Cell* 10, 67; 1977) on which all cellular RNP is apparently translocated within the cell. Penman believes that this cytoskeleton provides a morphological explanation for the rapid interference with processing and translocation when transcription is inhibited and also accounts for the fact that RNA is never 'free' in the cell.

Support for the idea of such a cytoskeleton was provided by U. Lönn (Karolinska Institute, Stockholm) who reported microdissection experiments on the cytoplasm of polytene cells



A hundred years ago

SOME of our readers may like to know that, as might have been expected, the three rhinoceroses now exhibited in the Alexandra Park are specimens of the African Black Rhinoceros (*Rhinoceros bicornis*). This species is extremely uncommon in menageries, and we have heard of no other in this country except the fine adult male now living in the Zoological Society's Gardens in Regent's Park.

It is perhaps a fortunate thing that our politicians, like the Chancellor of the Exchequer and Mr. John Bright, are beginning to concern themselves in their public addresses with science as well as art. With reference to Mr. Bright's recent address, as the *Times* remarks, if his hearers complain that they have not been told much about either science or art, we can only say that we agree with them, and that we deplore our common loss. In the coming time it is to be hoped that public speakers, like Mr. Bright, will know better what science really is than they seem to do now.

from *Nature*, 14, 27 September; 1876.

E. G. Jordan is in the Department of Biology at Queen Elizabeth College, London and U. E. Loening is in the Department of Zoology, University of Edinburgh.

which showed a gradient of ribosomes from nuclear envelope to plasma membrane, with the oldest ribosomes furthest from the nuclear membrane. The discovery of a very specific 28S rRNA nicking enzyme in erythrocyte cell membranes (M. Herzberg, Bar-Ilan University, Israel) fits with the idea that the lifetime of a ribosome is the time it takes to traverse this gradient.

The old idea of a link between the transport of messenger and ribosomal RNA which implies a role for the nucleolus in all RNA transport was supported by a report from S. Ghosh (Cancer Research Centre, Heidelberg) who showed that RNA transport was much lower in the absence of a nucleolus in artificially produced multi-nucleate cells.

It might be that the dogma that RNA polymerase A is confined to the nucleolus and polymerase B to the nucleoplasm does not hold any more. Penman questioned the former and a report from M. Laval (Cell Pathology Laboratory, Paris) using high resolution autoradiography, found evidence for polymerase B activity within the nucleolus. Such observations and a possible function in the transport of RNA make us cautious in limiting the role of the nucleolus to ribosome

manufacture.

Several speakers provided evidence that the so-called 'fibrillar centres' in animal cells and the lacunae of nucleoli in plant cells are in fact nucleolar organising regions. These reports precipitated a special session to discuss nucleolar nomenclature and agreement was reached that evidence for the presence of DNA in the fibrils of the pale staining regions, especially that from cytochemistry and autoradiography, and the presence of a synaptonemal complex at meiosis, justified abandoning the various vague and misleading terms 'fibrillar centre' 'pars amorpha' or 'lacunae' in favour of the nucleolar organising region. Bernhard observed that this represented an important advance since the last nucleolar workshop in 1975 in our progress towards an understanding of the nucleolus. It clarifies much of the earlier work and suggests a unifying concept for the interpretation of nucleolar ultrastructure.

Certainly this meeting will be remembered for the atmosphere of quiet scholarship in the midst of this beautiful old University city, and for considerable success in correlating structural with biochemical findings on the nucleolus and nucleus.

were reported in its diagnosis at an early stage. The test consists of the examination of oesophageal cell scrapings which are collected by a single-lumen tube or a double-lumen tube with an abrasive balloon (both of Chinese improved design). These on-the-spot techniques have made possible mass screening of patients, and the probability of detecting carcinoma in its early stages is now better than 90%. Its incidence in various areas of central and southern China has been correlated with possible carcinogenic factors such as local climatic conditions, hydrogeology, soil types and trace elements, diet and particular life-style. In some areas, which now serve as bases for experiments, the populace are voluntarily attempting to reduce their intake of nitrosamines and their precursors (suspected of contributing also to cancers of the bladder, kidney, liver, stomach and nasal sinuses), to minimise fungal contamination of food and to apply ammonium molybdate as fertiliser (nitrates and nitrites in food are suspected of being precursors to the nitrosamines), and the effects of such measures are being monitored. The principle 'put prevention first', it was emphasised, was paramount in the running of the Chinese health care system.

Another main topic of discussion was liver cancer. The method for its detection most widely used in China is the serum α -fetoprotein assay, which requires only a drop of blood from the individual's ear or finger. Great success has been achieved in its early detection; specimens of it have been obtained which have not grown larger than 0.5 mm. In areas where it is common, efforts have been made to prevent mould contamination of food and improve drinking water, and to ensure early treatment of hepatitis by medical education. Other topics covered included the viral aetiology of nasopharyngeal carcinoma, to which southern Chinese people are particularly prone. Some medical workers reported that they have prepared several lymphoblastoid cell lines and found Epstein-Barr virus, and have successfully established an epithelioid cell line of the carcinoma.

Part of the conference was also devoted to cancer therapy and pharmacology. Over the past few years the Chinese claim many new anti-tumour drugs have been discovered. With the help of traditional medical knowledge some effective anti-cancer drugs, especially N-formylsarcosine and cephalotaxine ester, an alkaloid extracted from the plant *Cephalotaxis fortunei*, have been identified and their effectiveness is now being assessed in clinical practice. □

Cancer conference in China

from T. B. Tang

A NATIONAL retrospective survey of cancer deaths is being compiled in China, on a scale which is probably unique hitherto. The investigation began in 1971 and was started first as a pilot project in Lian-axian in the north of Honan Province. That county is inhabited by some 110,000 people and has a high incidence of oesophageal cancer. The survey established that over a 30-year period the toll taken by the disease had been constant each year, at an annual average of between 1 and 1.5 per thousand. This is despite significant emigration and immigration into the district and improvement in living conditions since 1949, suggesting that the main contributory factors to this cancer are environmental.

The work has been subsequently expanded to cover the 50 million people living in Peking and three provinces of North China. Then, on the basis of experience from this extended survey, the incidence of a number of impor-

tant cancers was investigated nationally by the Academia Sinica. This programme involved eight of the Academia's research institutes and the 1.8 million barefoot doctors in the countryside. Up to now 500 million people in 16 provinces, municipalities or autonomous regions have been covered, and the research is still going on.

It is therefore interesting to note that, at the beginning of July, a national conference was held in Peking on the epidemiology of malignant tumours (*Hsinhua News Agency*, July 26; *Tai-kung-pao*, September 4, special report). Some 300 workers attended, including delegates from regional cancer research institutes, rural barefoot doctors, and practitioners of both traditional Chinese medicine and Western medicine. Experience was exchanged and summed up, theses were put forward and debated on, and before the meeting concluded a work programme for 1977/80 was mapped out.

Carcinoma of the oesophagus remained one of the main concerns at the conference. Significant advances

review article

Recent developments in phase transitions and critical phenomena

David R. Nelson*

Universal dimensionless numbers characterise singularities at the critical point in magnets, binary mixtures and alloys, superfluids, and in a variety of other systems. A desire to understand these numbers has stimulated a significant breakthrough in the analysis of statistical mechanical partition sums. Critical phenomena can now be understood in terms of universality, Hamiltonian flows, and renormalisation group recursion relations.

PHASE changes of matter in thermodynamic equilibrium are often accompanied by sharp discontinuities in quantities such as the magnetisation in a ferromagnet, the density in a liquid-vapour system, or the concentration in a binary mixture. Usually, these phase transitions can be made continuous by adjusting some external parameter such as the temperature. The point at which the discontinuity vanishes is called a critical point. At temperatures above the critical point, the spontaneous magnetisation of a ferromagnet is zero, the liquid and vapour phases of a fluid become indistinguishable, and the chemical species present in a binary fluid mixture are completely miscible. One usually associates the discontinuities below the critical temperature with some sort of broken symmetry¹⁻³.

Although critical points were discovered in the nineteenth century, they have been the focus of particularly intense scientific investigation during the past few decades¹⁻³. A rather precise characterisation of critical point singularities by experiments⁴ and perturbation techniques such as high temperature series expansions⁵ was followed by a detailed theory due to Wilson⁶. In this review, I shall describe Wilson's renormalisation group approach to critical phenomena, and illustrate its utility with examples chosen from the recent literature.

The spontaneous magnetisation and susceptibility of a ferromagnet are plotted schematically in Fig. 1. Below the Curie temperature, a substance such as iron or nickel will support a spontaneous magnetisation $M_0(T)$, which accounts for the well known magnetic properties of these substances. As the temperature is raised, however, the magnetisation drops to zero at T_c as shown in the figure. The magnetic susceptibility in zero field, $\chi_0(T)$, which is a measure of the response of the magnetisation to a small magnetic field, actually diverges to infinity as one approaches the Curie temperature from above or below.

The extraordinary thing about the anomalies shown in Fig. 1 is that they seem to be universal. That is, a wide variety of ferromagnets displays spontaneous magnetisations which tend to zero and susceptibilities which apparently diverge in precisely the same way. Despite differing chemical compositions, lattice structures, and Curie temperatures, one finds that $M_0(T)$ behaves as

$$M_0(T) \sim |T - T_c|^\beta \quad (1)$$

near T_c , while $\chi_0(T)$ diverges according to

$$\chi_0(T) \sim |T - T_c|^{-\gamma} \quad (2)$$

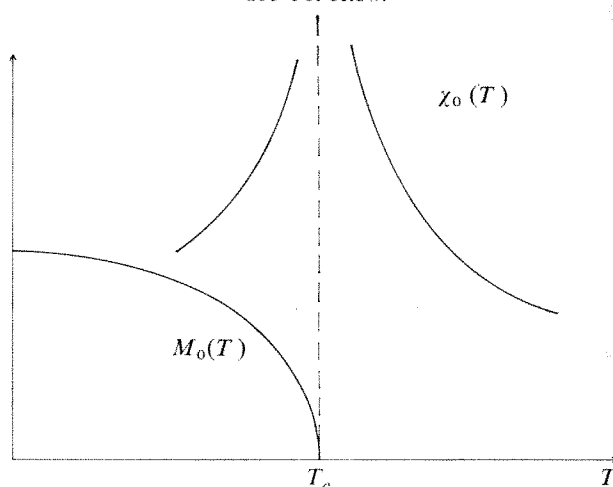
where the critical exponents β and γ are essentially the same for all magnets. A second curious fact is that the universal numbers β and γ are not simply one-half or unity, although most simple theories lead inevitably to these values¹⁻³. In fact, experiments⁴ and evidence from high-temperature series expansions⁵ suggest that β and γ lie in the ranges,

$$0.31 \lesssim \beta \lesssim 0.38 \quad 1.25 \lesssim \gamma \lesssim 1.38 \quad (3)$$

in most substances. Although equation (3) indicates some variation in the observed values of β and γ , it now seems that magnets with the same symmetry should have precisely the same exponents: that is, if we imagine that a magnetic crystal is described by a lattice of classical spin vectors (see Fig. 2a), there is a weak dependence of critical exponents on the number of spin components n which actively participates in the phase transition. The number of such spin components is related to the symmetry group of the crystal.

The universality of critical phenomena is not limited to ferromagnetism. Analogous quantities to the magnetisation and

Fig. 1 The magnetisation $M_0(T)$ and zero field susceptibility $\chi_0(T)$ of a ferromagnet. The magnetisation goes to zero at the Curie temperature T_c , while $\chi_0(T)$ diverges as this temperature is approached from above or below.



*Lyman Laboratory of Physics, Harvard University, Cambridge, Massachusetts 02138

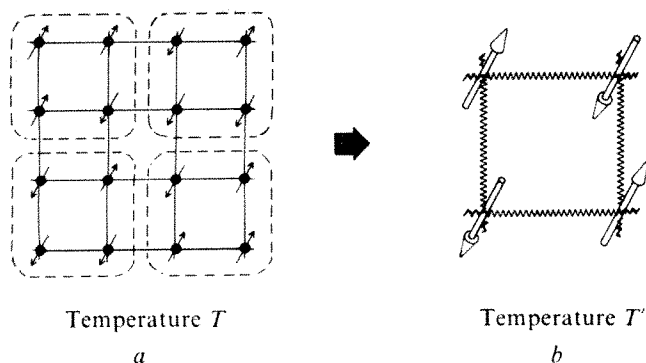


Fig. 2 Kadanoff block scheme. Dashed lines enclose groups of spins on lattice (a), which is at temperature T . Out of each block, we construct a collective spin variable by integrating over the internal degrees of freedom. The new spin degrees of freedom populate a lattice (b) with twice the old lattice spacing, and interact as if they were at temperature T' .

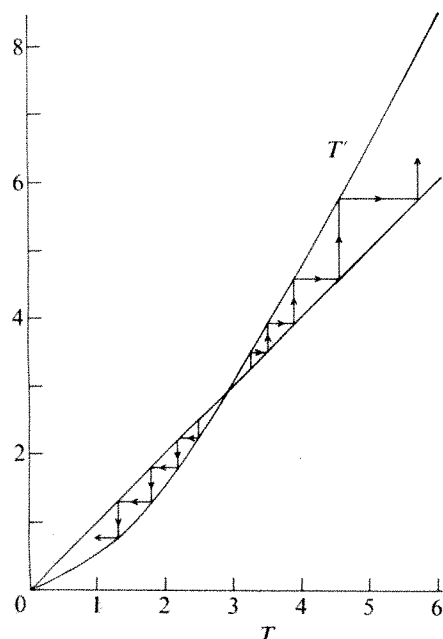
susceptibility exhibit singularities at the critical point of liquid-gas systems, binary mixtures, and alloys, at the λ -transition in superfluid helium, and at the Néel temperature in antiferromagnets¹⁻³. Exponents such as β and γ which characterise the anomalous behaviour are very close to the numbers found for ferromagnets. A variety of other exponents can be defined for systems near a critical point, and they, too, seem to be universal.

The behaviour of a liquid-gas system near its critical point is particularly striking. The fluid suddenly becomes milky and opaque, and scatters laser light intensely in the forward direction. It seems clear that some sort of cooperative phenomenon is taking place, and that the particles of fluid are strongly correlated over distances comparable with the wavelength of the laser light. The correlation length $\xi(T)$ of the fluid, which is a measure of the cooperativity, is expected to diverge strongly at T_c .

$$\xi(T) \sim |T - T_c|^{-\nu} \quad (4)$$

The exponent ν is in the range

Fig. 3 Approximate recursion relation for $n = 1$ or Ising spins, with a fixed point at $T^* = T_c = 2.98$. The 'ladder' construction shows successive temperatures produced by repeated iterations of the transformation. The slope of the function $T'(T)$ through its fixed point is related to the critical exponent ν .



$$0.64 \leq \nu \leq 0.70 \quad (5)$$

in most substances.

Where do the universal exponents β , γ , and ν come from? What is their precise numerical value? Can we account for slight variations, if any, in β , γ , and ν from system to system? A desire to answer these puzzling questions culminated in a major breakthrough in 1971. Wilson's renormalisation group theory of critical phenomena⁶. Building on work by Kadanoff⁷, Wilson showed why the exponents were universal and how to calculate them. In doing so, he placed at the disposal of condensed matter physicists a powerful mathematical tool, the renormalisation group.

Kadanoff blocks and lattice renormalisations

If critical exponents are indeed universal, one might hope to understand them using only a very simple model of magnetism. Consider for concreteness a d -dimensional hypercubic lattice populated with fixed length, classical spin vectors S_i (see Fig. 2). To each configuration $\{S_i\}$ of spins we assign an energy,

$$H[\{S_i\}] = -J \sum_{\langle i,j \rangle} S_i \cdot S_j \quad (6)$$

where i and j label the sites of the lattice, and the sum is restricted to nearest-neighbour pairs of spins. Each spin has n components, where n can be different from d , the dimensionality of space. According to the rules of statistical mechanics, the macroscopic properties of such a system follow from a calculation of the partition function,

$$Z = \text{Tr}_{\{S_i\}} \exp(-\beta H[\{S_i\}]) \quad (7a)$$

$$\equiv \text{Tr}_{\{S_i\}} \exp\left(-\frac{1}{T} \sum_{\langle i,j \rangle} S_i \cdot S_j\right) \quad (7b)$$

In passing from (7a) to (7b), we have combined β (not to be confused with a critical exponent!) and the coupling constant J into a dimensionless 'temperature', $T \equiv 1/\beta J$. The trace operation (Tr) means an integration over all spin configurations $\{S_i\}$ consistent with the fixed length requirement, namely

$$|S_i| = 1, \text{ all } i \quad (8)$$

The partition sum (7) can be computed exactly in one dimension for all values of n (ref. 8) but does not exhibit a finite temperature phase transition. Onsager obtained the partition function in two dimensions⁹ for the special case of the Ising model, or $n = 1$. Onsager's work, which remains today a true mathematical *tour de force*, eventually led to the exponent predictions¹⁻³

$$\beta = \frac{1}{8}, \quad \gamma = \frac{7}{4}, \quad \nu = 1 \quad (9)$$

An analytical evaluation of (7) for other values of n and d seems to be a hopeless task.

Rather than attempting to evaluate (7) exactly, we can, in fact, make progress by merely thinning out the spin degrees of freedom slightly. To do this, we follow the original suggestion of Kadanoff⁷ and group the spins on the original lattice (which is at temperature T) into blocks as shown in Fig. 2. With some ingenuity, it turns out to be possible to integrate approximately over the internal degrees of freedom within each block. We are then left with a new statistical mechanical problem with twice the lattice spacing. Provided the interactions between spins in the new lattice are of the same form as in the old, couplings in the new lattice can be characterised simply by a new temperature T' .

This sort of program was first carried successfully for Ising ($n = 1$) spins on a triangular lattice by Niemeyer and van Leeuwen¹⁰. A very primitive version of their transformation gives a relationship between the new and old temperatures, namely¹⁰

$$T' = \frac{1}{2} T (e^{3/T} + 3e^{-1/T})^2 / (e^{3/T} + e^{-1/T})^2 \quad (10)$$

Equation (10) is depicted graphically in Fig. 3, together with a

'ladder' construction which shows how the temperature changes with repeated iterations of the transformation. The recursion formula (10) has a fixed point at $T^* \approx 2.98$, and it seems natural to identify this isolated point with the Curie temperature T_c of the spin system.

We can see the utility of this procedure of 'thinning out' spins as follows: even an approximate calculation of partition function (7) is difficult near T_c because of the large correlation length $\xi(T)$ in this region. Note from Fig. 3, however, that the renormalisation transformation increase temperatures which, initially, are slightly above T_c . By repeatedly applying the transformation (10), we can relate a difficult calculation near T_c to a more tractable high temperature problem. A very accurate calculation of the partition sum (7) is possible at high temperatures by expanding in powers of $1/T$. No information about Z is lost during the thinning out procedure, because there is a very precise relationship between the partition function calculated at temperature T , and the corresponding quantity calculated at temperature T' (refs 6 and 10–12). In a similar fashion, we see that the transformation (10) decreases temperatures initially slightly below T_c , and eventually produces a more manageable (low temperature) problem.

As emphasised by Wilson¹⁴, the physics behind a renormalisation transformation is in the reduction of the correlation length. If the lattice spacing is changed by a factor b by the thinning out process, it follows the correlation length transforms according to¹⁴

$$\xi(T') = \xi(T)/b \quad (11)$$

For the square lattice spin system shown in Fig. 2, $b = 2$, while for the triangular lattice of Niemeyer and van Leeuwen (it turns out that)¹⁰ $b = \sqrt{3}$. Equation (11) can be used in conjunction with (10) to produce a critical exponent. Near the fixed point $T^* = T_c = 2.98$, equation (10) can be written in the approximate form,

$$T' - T_c = b^{0.89} (T - T_c) \quad (12)$$

which when combined with (11) gives a functional equation for $\xi(T - T_c)$,

$$\xi(T - T_c) = b \xi[b^{0.89} (T - T_c)] \quad (13)$$

It is easy to check that the solution of this functional equation is $\xi(T) \sim |T - T_c|^{-\nu}$ with $\nu \approx 1.12$, which is not too different from the exact result $\nu = 1.0$.

In more sophisticated calculations^{10,15–18}, it is not possible to describe a renormalisation transformation in terms of a single coupling constant. More complicated couplings such as next-nearest-neighbour and multispin interactions are generated after one iteration. When these extra couplings are taken into account^{10,15–18}, they not only produce more accurate estimates of critical exponents, but also provide a qualitative explanation of universality: if several couplings are included in the calculation, the renormalisation transformation can be viewed as a mapping of one Hamiltonian to another in a multidimensional coupling-constant space. As T is adjusted towards T_c , it turns out that any initial set of couplings 'flows' toward a unique fixed point under repeated applications of the transformation. Just as in the one-interaction-constant example, critical exponents are related to eigenvalues of the transformation about this fixed point. We are led to the conclusion that the exponents are independent of the precise values of the initial couplings, and depend instead on the 'universal' properties of the fixed point.

Calculations by the Kadanoff block method for Ising spins have now become quite refined. Niemeyer and Van Leeuwen¹⁰ presented a series of cluster approximations to an exact transformation which seems to converge to the exact results of Onsager. Wilson¹⁵ carried out renormalisations on a square lattice keeping track of 217 different interaction constants, and obtained critical exponents accurate to better than 1%. Kadanoff has proposed a variational principle^{17,18} which, when coupled with a simple block

spin transformation, gives exponents accurate to 0.1% in two dimensions, and results competitive with high temperature series expansions in three dimensions!

Despite some initial success, this field is still in its infancy. The principal achievement of the block spin analysis so far (beyond providing an intuitive understanding of universality) has been to reproduce the results of Onsager and high temperature series expansions. Nevertheless, lattice renormalisation groups may eventually become a powerful computational tool, similar to various methods used to evaluate integrals numerically in ordinary calculus. The partition sum (7) can be viewed as a complicated functional integral which until now could only be computed in a few fortuitous cases. Renormalisation theory suggests that such sums can be evaluated routinely and very accurately at any temperature, without relying on a mathematical *tour de force* such as Onsager's solution⁹ of the two-dimensional Ising model.

Continuation in dimensionality

Wilson has characterised developments in the theory of critical phenomena as a "search for analyticity". The idea is to find a mathematical framework in which critical point singularities are described by smooth, analytic functions. Landau¹⁹ proposed that critical phenomena could be understood in terms of an analytic expansion of the free energy in powers of a local magnetisation variable. Although Landau's theory has led to a qualitative understanding of a wide variety of critical points, the exponent predictions disagree with experiments^{1–3}.

The proposal that critical points can be understood in terms of analytical recursion relations⁶ seems to be the correct modification of Landau's theory. The method of construction ('thinning out' degrees in freedom) virtually guarantees that the recursion relations will be analytic at finite temperatures. Wegner¹⁹ has shown that a wide variety of critical point singularities follow from the analyticity of recursion relations about a fixed point.

Perhaps the most useful analyticity property of recursion relations is that they are remarkably easy to continue in dimensionality. The original continuation of Wilson and Fisher into $4-\epsilon$ dimensions (Landau's theory turns out to be correct above $d = 4$) led to quantitatively accurate estimates of exponents in three dimensions, and to a detailed understanding of a wide variety of complicated critical point phenomena. The formulas for β and γ as a function of n and d ($d < 4$) are^{21,22}

$$\beta = \frac{1}{2} - \frac{3}{2(n+8)}(4-d) + \frac{(n+2)(2n+1)}{2(n+8)^3}(4-d)^2 + \dots \quad (14a)$$

$$\gamma = 1 + \frac{n+2}{2(n+8)}(4-d) + \frac{(n+2)(n^2+22n+52)}{4(n+8)^3}(4-d)^2 + \dots \quad (14b)$$

Although expansions like these in $\epsilon = 4-d$ (and, to a lesser degree, expansions in $1/n$)^{23–25} probably represent the most powerful and versatile application of Wilson's theory, this work has been extensively reviewed elsewhere^{14,26–28}. We shall turn instead to more recent investigations of critical points in $2+\epsilon$ dimensions.

Critical phenomena in $(2+\epsilon)$ dimensions

To a first approximation, the action of a renormalisation group on a Hamiltonian can be compared with the motion of a ball rolling down a hill⁶. One assumes that the differential change in temperature $dT \equiv T' - T$ associated with a block spin transformation that changes the lattice spacing by a factor $d/l \equiv db/b$ is described by a potential function $V(T)$ (ref. 6)

$$\frac{dT(l)}{dl} = -\frac{dV(T(l))}{dT(l)} \quad (15)$$

Here, d/l is an infinitesimal change in the logarithm of the Kadanoff block size, and $T = T(l)$ is the effective temperature produced by

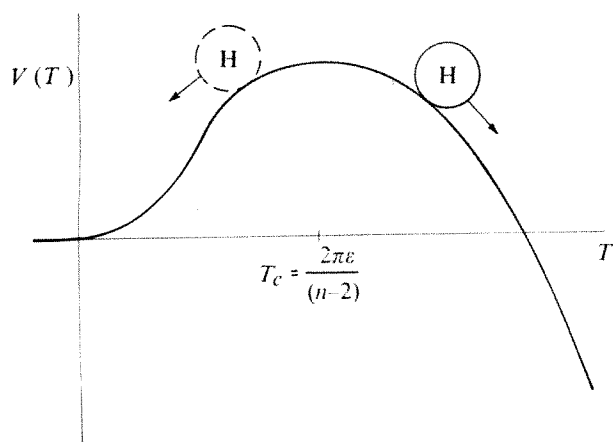


Fig. 4 Potential function $V(T)$ for fixed length spins in $2+\epsilon$ dimensions, with ϵ small and $n \geq 3$. Hamiltonians initially to the right of T_c 'roll' toward higher temperatures, while systems with temperatures less than T_c roll toward a zero temperature fixed point.

blocks of size $b = e^l$. Although a differential change in the block size is hard to visualise, it turns out to be fairly easy to effect this transformation mathematically.

Recent studies of the fixed length spin Hamiltonian (7) near two dimensions²⁹⁻³⁴ provide a particularly simple realisation of these ideas. The potential $V(T)$ has now been calculated by using a low-temperature spin-wave theory, with the result^{29,31}

$$V(T) = \frac{1}{2}(d-2)T^2 - \frac{n-2}{6\pi}T^3 + \dots \quad (16)$$

As shown in Fig. 4, Hamiltonians at temperatures initially to the right of the crest in this potential roll toward high temperatures, while Hamiltonians at lower temperatures roll toward the minimum at $T = 0$. There is no oscillatory motion about this minimum because the 'equation of motion' (15) contains only one derivative in the time-like parameter, l .

This is precisely the behaviour we found using the discrete recursion formula (10), and it is natural to associate the crest of the potential hill with the critical temperature,

$$T_c = 2\pi(d-2)/(n-2) \quad (17)$$

In contrast to the $n = 1$ or Ising model, T_c tends to zero as d approaches two from above for $n \geq 3$. Linearisation of the equation (15) about the maximum in the potential leads to the correlation length exponent prediction²⁹⁻³¹

$$\nu = \frac{1}{(d-2)}[1 + O(d-2)] \quad (18)$$

Higher order corrections to this result are determined in ref. 31.

Note that for the interesting case of an XY model ($n = 2$) in precisely two dimensions, the potential (16) is perfectly flat. This result (which holds to all orders in T) suggests that such a system would exhibit a line of critical points with continuously variable critical exponents³⁵⁻³⁶. Work by Berezinskii³⁷ and by Kosterlitz and Thouless^{38,39} gives convincing evidence that this line actually ends at some finite temperature. Renormalisation group flow patterns for the XY model obtained using a more sophisticated theory by Kosterlitz³⁹ and later by José *et al.*⁴⁰ are plotted in Fig. 5, which shows the motion of the temperature coupling $T(l)$ together with the motion of a new parameter $y(l)$, which gives the probability of a vortex pair excitation in the system. These flows suggest that vortices become unimportant at low temperatures, so that spin-wave theory (which neglects vortices) should be qualitatively correct in region I. The instability in the flows in regions II and III, however, suggests a finite temperature transition into a phase not describable by spin-wave theory. It is possible that this bizarre phase transition may actually be observable in superfluid ⁴He films.

Concluding remarks

We have seen that the renormalisation group is a rather powerful and sophisticated tool for understanding the behaviour of matter near a critical point. Much of the progress in this area resulted from a rather fertile interaction of statistical mechanics with quantum field theory, where the renormalisation group was first developed⁴¹. Renormalisation groups constructed for problems in statistical mechanics may eventually be of some use in understanding certain lattice quantum field theories⁴².

Modern renormalisation theory may also be useful in other areas of condensed matter physics. Anderson⁴³ and Wilson¹⁵ have applied renormalisation group ideas to the Kondo problem, while Licciardello and Thouless⁴⁴ and Wegner⁴⁵ have used these techniques to study localisation in metals. Equilibrium time-dependent critical phenomena have been successfully treated⁴⁶ using Wilson's ideas, and there is now some effort to extend these techniques to non-equilibrium problems. One might hope that they would be of some utility in understanding, for example, properties of turbulent fluids at high Reynold's number.

Much of my understanding of static critical phenomena is the result of stimulating interactions with Michael Fisher, Kenneth Wilson, and Leo Kadanoff.

This research was supported in part by the National Science Foundation.

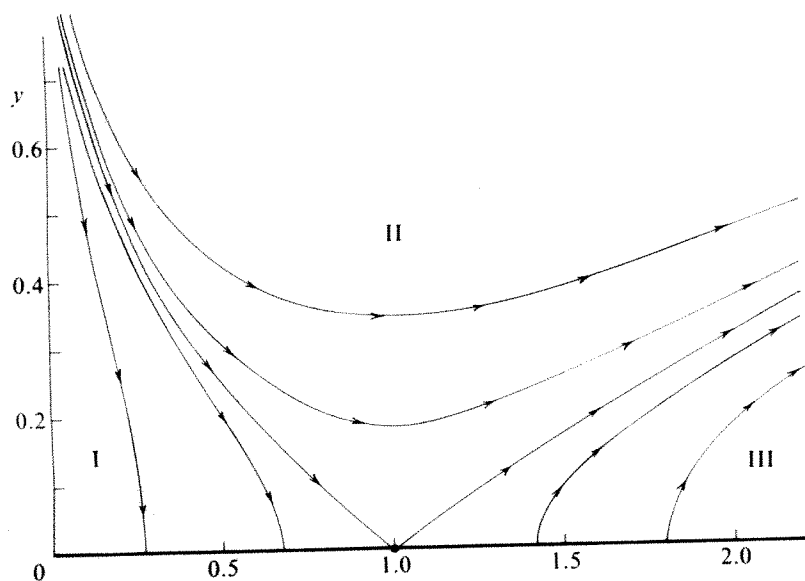


Fig. 5 Hamiltonian flows for the two-dimensional XY model. The flowlines show the motion of the coupling constants T and y under repeated iterations of a renormalisation group transformation. The parameter y measures the probability of exciting a vortex pair in the system. The flowlines are both attracted and repelled by a line of fixed points at $y = 0$. Two separating trajectories divide the plane of possible Hamiltonians into three distinct regions. Vortices are unimportant to the critical properties in region I, but become increasingly important in regions II and III.

- ¹ Fisher, M. E. *Rept. Progr. Phys.* **30**, 615-730 (1967).
- ² Kadanoff, L. P. *et al. Rev. mod. Phys.* **39**, 395-431 (1967).
- ³ Stanley, H. E. *Introduction to Phase Transitions and Critical Phenomena* (Oxford, New York and Oxford, 1971).
- ⁴ Heller, P. *Rept. Progr. Phys.* **30**, 731-826 (1967).
- ⁵ Domb, C. & Green, M. S. (eds) *Phase Transitions and Critical Phenomena* **3**, (Academic, New York, 1975).
- ⁶ Wilson, K. G. *Phys. Rev.* **B4**, 3174-3183 and 3184-3205 (1971).
- ⁷ Kadanoff, L. P. *Physics* **2**, 263-272 (1966).
- ⁸ Stanley, H. E. *Phys. Rev.* **179**, 570-577 (1969).
- ⁹ Onsager, L. *Phys. Rev.* **65**, 117-149 (1944).
- ¹⁰ Niemeyer, Th. & van Leeuwen, J. M. J. *Phys. Rev. Lett.* **31**, 1411-1414 (1973); *Physica* **71**, 17-40 (1974).
- ¹¹ Rudnick, J. *Phys. Rev.* **B11**, 363-376 (1975).
- ¹² Nauenberg, M. & Nienhuis, B. *Phys. Rev. Lett.* **33**, 1598-1601 (1974).
- ¹³ Nelson, D. R. *Phys. Rev.* **B11**, 3504-3519 (1975).
- ¹⁴ Wilson, K. G. & Kogut, J. *Phys. Rep.* **12C**, 77-199 (1974).
- ¹⁵ Wilson, K. G. *Rev. mod. Phys.* **47**, 773-840 (1975).
- ¹⁶ Kadanoff, L. P. & Houghton, A. *Phys. Rev.* **B11**, 377-386 (1975).
- ¹⁷ Kadanoff, L. P. *Phys. Rev. Lett.* **34**, 1005-1008 (1975).
- ¹⁸ Landau, L. D. *Phys. Zurn. Sovetskijunon* **11**, 26-35, 545-567 (1937) [English translation: ter Haar, D. (ed.) *Mon. of Physics*, L. D. Landau, vol. II (Pergamon, Oxford, 1969)].
- ¹⁹ Wegner, F. J. *Phys. Rev.* **B5**, 4529-4536 (1972).
- ²⁰ Wilson, K. G. & Fisher, M. E. *Phys. Rev. Lett.* **28**, 240-243 (1972).
- ²¹ Wilson, K. G. *Phys. Rev. Lett.* **28**, 548-551 (1972).
- ²² Ma, S.-K. *Phys. Rev. Lett.* **29**, 1311-1314 (1972).
- ²³ Abe, R. *Prog. theor. Phys.* **48**, 1414-1415 (1972); *ibid.* **49**, 113-128 (1973).
- ²⁴ Abe, R. & Hikami, S. *Prog. theor. Phys.* **49**, 442-452 (1973).
- ²⁵ Ma, S.-K. *Rev. mod. Phys.* **45**, 589-614 (1973).
- ²⁶ Fisher, M. E. *Rev. mod. Phys.* **46**, 597-616 (1974).
- ²⁷ Barber, M. *Phys. Rep.* **29C**, 3-84 (1977).
- ²⁸ Polyakov, A. M. *Phys. Lett.* **59B**, 79-81 (1975).
- ²⁹ Migdal, A. A. *Zh. E. T. F.* **69**, 1457-1463 (1975).
- ³⁰ Brezin, E. & Zinn-Justin, J. *Phys. Rev. Lett.* **36**, 691-694 (1976); *Phys. Rev.* **B14**, 3110-3120 (1976).
- ³¹ Pelcovits, R. A. & Nelson, D. R. *Phys. Lett.* **57A**, 23-25 (1976); *Phys. Rev. B* (in the press).
- ³² Brezin, E., Zinn-Justin, J. & Le Guillou, J. C. *Phys. Rev.* **B14**, 4976-4978 (1976).
- ³³ Kadanoff, L. P. *A. Phys.* **100**, 359-394 (1976).
- ³⁴ Wegner, F. J. *Z. Phys.* **206**, 465-470 (1967).
- ³⁵ Berezinskii, V. L. *Zh. Eksp. Tero. Fiz.* **59**, 907-920 (1970) [*Sov. Phys. JETP* **32**, 493-500 (1971)].
- ³⁶ Berezinskii, V. L. *Zh. Eksp. Tero. Fiz.* **61**, 1144-1156 (1971) [*Sov. Phys. JETP* **34**, 610-616 (1971)].
- ³⁷ Kosterlitz, J. M. & Thouless, D. J. *J. Phys.* **C6**, 1181-1203 (1973).
- ³⁸ Kosterlitz, J. M. *J. Phys.* **C7**, 1046-1060 (1974).
- ³⁹ Jose, J., Kadanoff, L. P., Kirkpatrick, S. & Nelson, D. R. *Phys. Rev. B* (in the press).
- ⁴⁰ Gell-Mann, M. & Low, F. E. *Phys. Rev.* **95**, 1300-1312 (1954).
- ⁴¹ Wilson, K. G. *Phys. Rev.* **D10**, 2445-2459 (1974).
- ⁴² Anderson, P. W. *J. Phys.* **C3**, 2436-2441 (1970).
- ⁴³ Licciardello, D. C. & Thouless, D. J. *Phys. Rev. Lett.* **35**, 1475-1478 (1975).
- ⁴⁴ Wegner, F. J. *Z. Phys.* **B25**, 327-337 (1976).
- ⁴⁵ Hohenberg, P. C. & Halperin, B. I. *Rev. mod. Phys.* **49**, 435-479 (1977).

articles

The Messinian salinity crisis and evidence of late Miocene eustatic changes in the world ocean

C. G. Adams

Department of Palaeontology, British Museum (Natural History), Cromwell Road, London SW7, UK

R. H. Benson

Department of Paleobiology, Smithsonian Institution, United States National Museum, Washington DC 20560

R. B. Kidd

Institute of Oceanographic Sciences, Wormley, Godalming, Surrey, UK

W. B. F. Ryan

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York, 10964

R. C. Wright

Department of Geology, Florida State University, Tallahassee, Florida 32306

The Mediterranean Sea became isolated from the world ocean in the late Miocene and underwent a 'crisis of salinity' during which vast deposits of evaporites were laid down in pre-existing depressions. The final connection with the Atlantic is believed now to have been the Betic Strait entering into the Mediterranean from Andalusia, Spain. Although the closing of this connection, known as the Iberian Portal, is related to large-scale plate movements which brought Africa into direct contact with southern Europe, its final severing may have resulted from glacial-eustatic lowering of the global ocean. Further stratigraphic resolution of late Miocene sea-level and ice-volume changes is sought to verify the eustatic-fall hypothesis.

RECENT research, particularly that of specialists contributing reports on the DSDP legs 13 (refs 1, 2) and 42A (ref. 3) has revealed the existence of 1.5-2 km of evaporites throughout the deep Mediterranean basins and has strongly supported Ruggieri's⁴ suggestion that a 'crisis of salinity' overwhelmed the faunas of the ancient Tethys Sea in latest Miocene (Messinian) time some 6.5-5 Myr ago (Fig. 1).

The salinity crisis occurred after the western Tethys lost its last connection with the Atlantic about 6.2 Myr BP (ref. 5), its connection with the Indo-Pacific having been severed at about the end of the Early Miocene⁶ some 10 Myr earlier. The Iberian Portal, as the hypothetical last connection with the Atlantic is often known, must have existed somewhere between the Iberian Meseta and the Moroccan Meseta and could have been any one of the former Betic, Gibraltar, or Rif straits, although Benson⁷ now believes that its connection to the Atlantic lay across Andalusia and was therefore the northernmost of the three passages. Isolation of the ancient Mediterranean from the world ocean resulted in the precipitation of more than 10⁶ km³ of gypsum, halite and other salts from a volume of seawater estimated to be equivalent to thirty times that contained in the present Mediterranean basins⁸.

The connection between the Atlantic and the Mediterranean may have been broken by plate movements bringing Africa into direct contact with southern Europe⁹ or by uplift of the sea floor associated with the Alpine Orogeny (doubtless two aspects of the same basic process). It is possible, however, that the final separation of the Mediterranean from the Atlantic could have been brought about by a fall in the level of the world ocean¹⁰ or by a combination of local uplift of the Iberian Portal and global eustatic

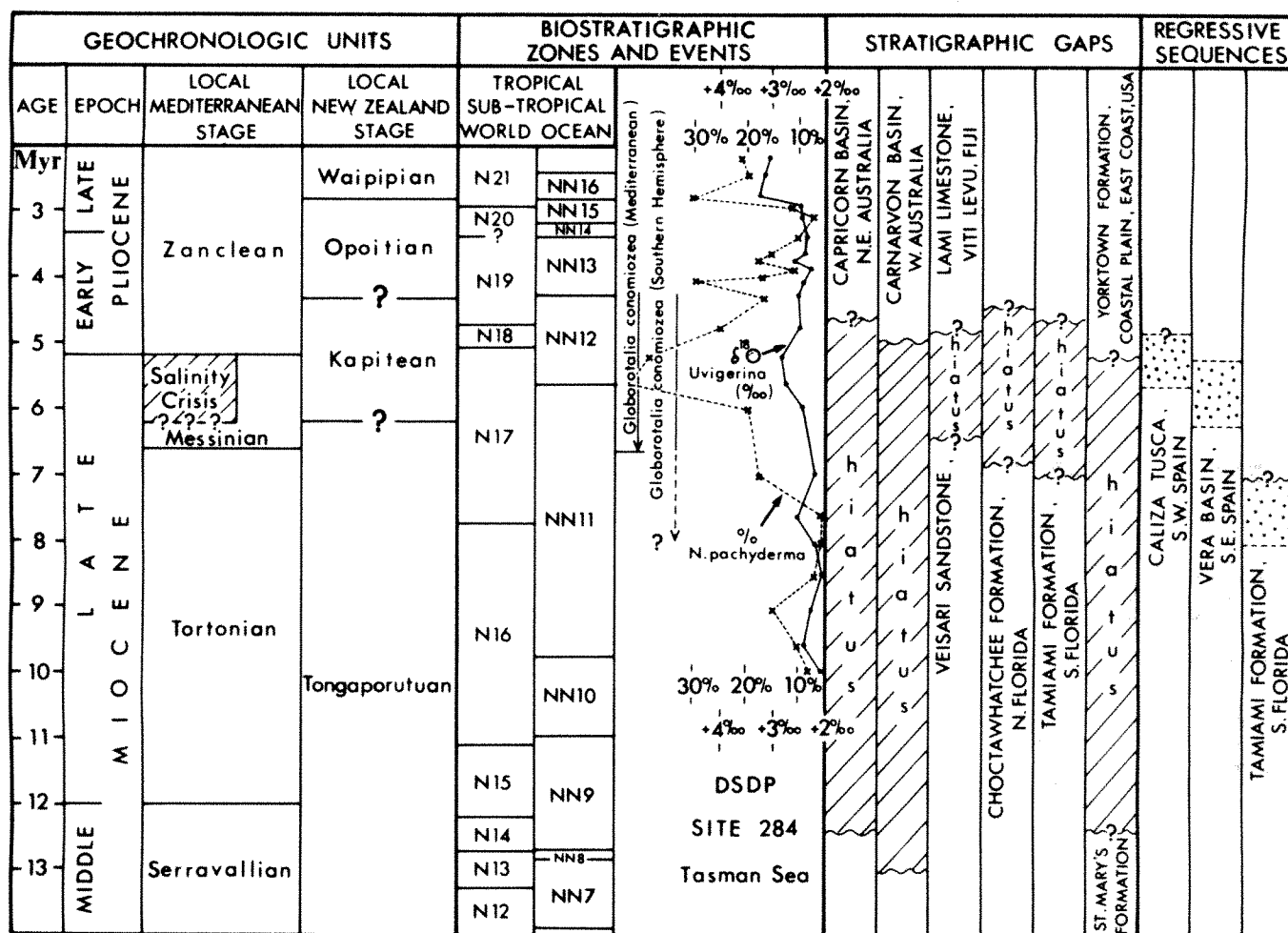


Fig. 1 The Miocene/Pliocene stratigraphic interval with position of stage boundaries and the Mediterranean salinity crisis relative to world ocean biostratigraphic zones, various climatic events, erosional gaps in shallow-water formations and regressive sequences discussed in the text.

oscillations. We examine here the evidence for eustatic change without implying that it was the only major physical process associated with the Messinian Salinity Crisis or that it was necessarily the most important.

The Gibraltar Sill has a depth of 320 m at present. This is sufficiently shallow to keep cold, deep Atlantic waters out of the Mediterranean, but not so shallow as to prevent the reflux of a higher salinity (38‰) intermediate water mass as a consequence of excess evaporation over river run off and precipitation in the Mediterranean region¹¹. Despite its great depth (> 3,000 m in places) the modern Mediterranean is thermospheric, that is, its bottom waters are everywhere warmer than 10 °C. Oceanic bottom waters on the other hand are psychrospheric, that is, 10 °C or less at depth. Some sediments of Eocene to Middle Miocene (Serravallian) age in the Mediterranean area contain psychrospheric ostracodes which, according to Benson¹², have their upper depth limits closer to the 8 °C than to 10 °C isotherm while those of Late Miocene (Tortonian and Messinian) age do not¹³. The marine sediments of latest Miocene age can therefore be regarded as deposits of a thermospheric sea.

The salinity crisis could not have been initiated until the Iberian Portal became shallow enough to cause the Mediterranean progressively to concentrate salt as a consequence of ending the present-day type of brine reflux. Once this stage was reached communication through the Iberian Portal would essentially be one way, and the balance between rate of supply and the rate of evaporation would dictate when and if drawdown would start in the now isolated Mediterranean¹⁴. Normal or near normal marine

planktonic faunas would perhaps persist for some time until increasing salinity led to their ultimate disappearance as when the Red Sea became isolated from the Gulf of Aden at 22,000 BP during the last ice age^{15,16}.

Before the total isolation of the Mediterranean, the Iberian Portal might have been a wide, shallow channel through which a permanent current flowed from west to east. One of us (W.B.F.R.) suggests that in response to eventual drawdown of the Mediterranean, the portal may have evolved into a series of interconnected marine waterways lying at different base levels relative to the Atlantic and separated by cataracts. Evaporation along the route through the portal may have been minimal accounting for the observation of H. Dronkert in the Vera Basin of southern Spain of shallow-water corals and bioherms sandwiched between Messinian gypsum with bird tracks on bedding planes¹⁷ and deep-water pelagic marls and turbidites containing *Globorotalia conomiozea*¹⁸. Parts of the cascading passageway would eventually have been subjected to tectonic modification and subaerial erosion before its remnants were filled with Pliocene or later marine sediments.

There are several supposedly continuous Miocene/Pliocene marine successions in the Mediterranean region (Chelif Basin, Algeria; the circum-Troodos area of Cyprus; and the central and western parts of Crete) which on present evidence do not seem to support the deep basin desiccation hypothesis as formulated after the 1970 deep-sea drilling cruise² and later sustained by further drilling in 1975 (ref. 19). Of the land areas mentioned above, the first two are inadequately known and the third is not noted for the quality of its exposures and faunas at the critical

level. These anomalies require further careful investigation as does the succession recently described by Montenat *et al.*²⁰ in the Vera Basin, Spain.

The flow of normal marine water through the shallow Iberian Portal envisaged above, would have been affected by changes in sea level. A substantial global fall might have led to a total restriction of the Mediterranean and to consequent desiccation^{21,22}. Thus, it is pertinent to examine the available sedimentary and faunal evidence for Late Miocene alterations in global sea level.

The deposition of shallow-water marine carbonates (inter-tidal, subtidal, platform and shelf facies, etc.) was a normal and continuing process within the circumtropical zone throughout most of Cenozoic time, fossiliferous limestones of Palaeocene to Middle Miocene age being common in the central Americas, Mediterranean and Indo-Pacific regions. Upper Miocene limestones are, however, rare everywhere^{23,24}, and there seem to be no described shallow-water carbonates spanning the Miocene/Pliocene boundary. Even in the Pacific (for example, Bikini and Eniwetok atolls) where continuous deposition across this boundary was once thought to have occurred^{25,26}, reappraisal of the foraminiferal faunas has shown that assemblages characteristic of the latest Miocene are absent²⁷. In the Capricorn Basin of NE Australia, a faunal and physical break has been demonstrated between shallow-water carbonates of N14 and N19 age in the Wreck Island Bore²⁷, while in the off-shore part of the Carnarvon Basin, western Australia, a major disconformity separates the shallow-water N13 carbonates penetrated in the Tryal Rocks No. 1 bore from the overlying limestones of N18 age²⁸. The most comprehensive review of the stratigraphical succession in Indonesia²⁹ includes no examples of limestones spanning the Miocene/Pliocene boundary. Baumann³⁰ has, however, recently published a diagram (ref. 30, Fig. 2) showing this boundary within the Karren Limestone of NE Java but without adducing any supporting data. There is, in fact, no faunal evidence that any part of the limestone is Miocene, and, if it were, it would still be necessary to prove continuous deposition across the Miocene/Pliocene boundary in order to affect the present argument. A recently completed study of the Miocene/Pliocene succession in Viti Levu, Fiji (Adams and Rodda; in preparation) proves that an angular unconformity separates N17 from N18/19 sediments. A clearly recognisable stratigraphic hiatus exists on the Sea of Japan side of Honshue (Lloyd H. Burckle, personal communications). The Caliza Tosca, a shallow-water limestone some 60 m thick in Andalusia, Spain, is generally regarded³¹ as Messinian in age and therefore coeval with the Mediterranean evaporites. This age determination, however, is based on stratigraphic position and the absence from a critical sample (C12) of *Discoaster quinqueramus*, rather than on positive evidence, and is therefore questionable. There seems to be no palaeontological proof that it is not early Messinian, that is, pre-evaporitic. The thick succession of carbonates and evaporites which make up the Bahamas Platform in the West Indies extends from the Recent down at least to the Lower Cretaceous, but the Andros deep well (14,585 feet) yielded no evidence for the position of the Miocene/Pliocene boundary³² and none for or against continuous carbonate deposition throughout the Late Miocene.

Examination of the Upper Miocene—Lower Pliocene carbonate-clastic sequence in northern Florida, USA³³ shows a pronounced erosional unconformity between the Choctawhatchee Formation (N17) and the overlying N19 sediments. In southern Florida, the Tamiami Formation, a shallow water carbonate unit, is broken by an unconformity separating N17 from N19 sediments³⁴. A similar unconformity occurs in the coastal plain of Maryland, Delaware and New Jersey separating yellow-white glauconitic sands and silts of the Yorktown Formation of latest

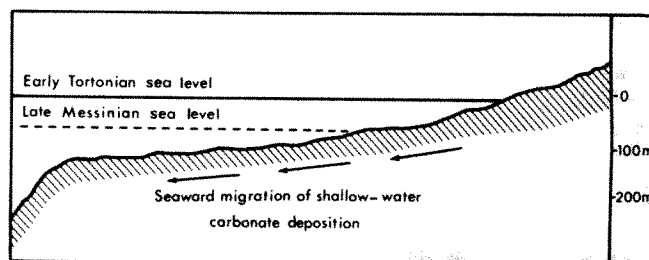


Fig. 2 Hypothetical migration of the locus of shallow-water carbonate deposition towards the shelf-edge during the suggested Messinian age eustatic sea-level fall of 40–70 m in the world ocean.

Miocene to Pliocene (?) age from bluish-grey clay belonging to the St Mary's Formation of Middle Miocene age^{35,36}.

Since shallow-water deposits of the type we have been referring to are deposited within the photic zone, a fall in the level of the world ocean would ensure that their area of deposition migrated towards the edge of the continental shelves. The greater the eustatic drop, the farther the zone of deposition would move. Nearshore limestones of pre-Messinian age would either be eroded or buried by clastic sediments deposited in brackish or freshwater milieux. New limestones would form farther away from the old coast lines (Fig. 2) and, where sedimentation was continuous, would rest on somewhat deeper-water sediments of Tortonian age. Changes in sedimentation would occur across the continental shelves of the entire world, and the lowering of sea level would be reflected in their faunas. Only where considerable uplift has occurred could such limestone be found on the land surface today.

The possibility of a Late Miocene eustatic change is also suggested by the facies changes in ostracode faunas and sediments of the Caribbean and on the Atlantic Coast of the United States. The ostracodes of these regions are well known (refs 37, 38). Of special interest are those from Blow's zones N13 to N21 from Cuba, Hispanola, Puerto Rico and Trinidad, although faunas of the same age and latitude are known from Central America. In almost every case the palaeoenvironmental succession indicates a rapid shallowing upward from N16, often followed by a hiatus, then by a deepening. The exceptions occur in Panama, where the deposition of the shallow Gatun Formation was followed by continental deposition, and in Costa Rica, where shallowing was slow and continuous. Often the stratigraphic control of these shallow beds is through inference or benthic microfaunal successions as planktonics are rare. In Trinidad, the Springvale Formation is a clay-glaucconitic sand mixture; carbonates of N17 age are rare, stratigraphically doubtful or absent. The Jamaican section is carbonate, but deep; it nevertheless shows noticeable shallowing during the Late Miocene.

There is good evidence from widely separated areas that marked faunal and floral changes occurred during the late Miocene. In California^{39,40,41}, Alaska⁴², the Atlantic⁴³, the Pacific⁴⁴, the Antarctic and southern oceans where foraminiferal assemblages and the isotopic composition have suggested^{45,46} both cooling and a drop in sea level. There is also evidence from the land floras from a cooling in the Late Miocene of NW America⁴⁷.

The benthic foraminiferal fauna of the south Florida Tamiami Formation, underlying the N17–N19 unconformity indicates a shoaling environment which became increasingly restrictive with time³⁴. This fauna is replaced by a lacustrine diatom assemblage at the Miocene/Pliocene boundary. Rocks of the same age in northern Florida also indicate a prograding succession of events that may also be due to a Late Miocene drop in sea level³³.

Changes in the frequency^{42,48}, coiling direction^{49,51} and $\delta^{18}\text{O}$ composition of planktonic foraminifera indicate an influx of cold water into the New Zealand area during a

stratigraphic interval known locally as the Kapitean stage. Reinterpretation of the palaeomagnetic stratigraphy on the Mangapoi and Blind River sections⁵² suggests that regressive sediments and discontinuities in the Kapitean are time correlative⁵ with the Messinian stratotype of Sicily. Coiling ratios from strata off NW Australia referable to zone N18 also indicate cooling of the higher latitude seas at the close of the Late Miocene.

This cooling and accompanying regression have been attributed to an expansion of Antarctic glaciation leading to a fall in sea level of perhaps 50–70 m, recognisable during the later part of the Andalusian³¹ and consistent with the magnitude of ice volume change recorded in the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ isotopic data on *Uvigerina* from DSDP Site 284, south Pacific⁴⁵.

A substantial fall in the level of the world ocean, that is more than about 50 m, would be sufficient to convert some shallow shelf areas into inland lakes. The Sea of Japan would be isolated by a fall of 70 m, and it would be interesting to learn whether evidence for freshwater sediments of late Miocene age has been obtained from drill holes in this area.

The lowering of global sea level could have been the event which closed off the Iberian Portal, leading to the Mediterranean isolation and eventual desiccation^{10,33}. Conversely, the extraction of 6% of the dissolved salts in the world ocean brought about by the Mediterranean desiccation⁸ could have induced the late Miocene glaciation by lowering high latitude salinity sufficient to raise the freezing point of seawater. Other possible effects of the massive salt extraction are the abrupt change in the percentage carbonate (relating to the dissolution of calcareous tests) occurring in drill holes in the Pacific, Atlantic and Caribbean areas at a stratigraphic level very close to the boundary between Upper Miocene and Lower Pliocene sediments⁵ and the apparent absence of even shallow-water limestones at that time²⁴. It may be more probable, however, that the onset of glaciation was independent of Mediterranean events, and high latitude cooling was the principal mechanism to cause areas of carbonate deposition to migrate away from the land.

Precise dating of these numerous events of the Late Miocene is fraught with difficulties because of the short period of time involved and the consequent imprecision of stratigraphical correlation using palaeobiological methods. A general lowering of sea level, however, of the order of 40–70 m would certainly have had a dramatic effect on the sediments and faunas of the continental shelves. Evidence of this should be preserved in bore-hole records, the investigation of which could reveal the duration and order of magnitude of any change. The Tortonian/Messinian eustatic-fall hypothesis is therefore eminently testable and can be verified or refuted by stratigraphers with access to drilling records in such areas as the Gulf of Mexico, China Sea and Persian Gulf.

We acknowledge W. C. Pitman III, B. C. Schreiber, L. H. Burckle and H. W. Ball for reviews and suggestions, and the US NSF and the Natural Environment Research Council of Great Britain for financial support. This article is contribution No 8 to IGCP Project 96.

Received 1 July; accepted 29 July 1977.

- ¹ Hsü, K. J., Cita, M. B. & Ryan, W. B. F. *Initial Reports of the Deep Sea Drilling Project*, U.S. Government Printing Office, Washington, D.C., 13, 1203–1231 (1973).
- ² Hsü, K. J., Ryan, W. B. F. & Cita, M. B. *Nature* **242**, 240–244 (1973).
- ³ Hsü, K. J. *et al.* *Initial Reports of the Deep Sea Drilling Project*, U.S. Government Printing Office (in the press).
- ⁴ Ruggieri, G. *Systematics Assoc. Publ. No. 7* (eds Adams, C. G. & Ager, D. V.) 283–290 (London, 1967).
- ⁵ Ryan, W. B. F. *et al.* *Riv. Ital. Paleont.* **80**, 631–687 (1974).
- ⁶ Adams, C. G. *Systematics Assoc. Publ. No. 7* (eds Adams, C. G. & Ager, D. V.) 195–217 (London, 1967).
- ⁷ Benson, R. H. *Marine Micropal.* (in the press).
- ⁸ Ryan, W. B. F. *Messinian Events in the Mediterranean* (ed. Drooger, C. W.) 26–29 (Konink. Ned. Akad. Wetensch. Amsterdam, 1973).
- ⁹ Cita, M. B., Wright, R. H., Ryan, W. B. F. & Longinelli, A. *Initial Reports of the Deep Sea Drilling Project*, 424, U.S. Government Printing Office, Washington, D.C. (in the press).
- ¹⁰ Nesteroff, W. D. & Glacon, R. *Rapp. Comm. Int. Mer. Médit.* **23**, 153–154 (1975).
- ¹¹ Lacombe, H. & Tchernia, P. *The Mediterranean Sea* (ed. Stanley, D. J.) 25–36 (Dowden Hutchinson and Ross, Stroudsburg, Pa. 1972).
- ¹² Benson, R. H. *The Mediterranean Sea* (ed. Stanley, D. J.) 63–73 (Dowden, Hutchinson and Ross, Stroudsburg, Pa., 1972).
- ¹³ Benson, R. H. *Micropaleontology*, Special Pub. No. 1, 41–48 (1975).
- ¹⁴ Maiklem, W. R. *Bull. Can. Petrol. Geol.* **19**, 487–503 (1971).
- ¹⁵ Deuser, W. G., Ross, E. H. & Waterman, L. S. *Science* **191**, 1168–1170 (1976).
- ¹⁶ Berggren, W. A. & Boersma, A. *Hot Brines and Recent Heavy Metal Deposits in the Red Sea* (eds Degens, E. T. & Ross, D. A.), 282–298 (Springer-Verlag, New York, 1969).
- ¹⁷ Smith, L. A. *Geotimes* **22**, No. 3, 20–23 (1977).
- ¹⁸ Bizon, G., Bizon, J. J. & Montecat, Ch. *Revue de l'Inst. Français du Pétrole*, **27**, 831–863 (1972).
- ¹⁹ Hsü, K. J. *et al.* *Nature* **267**, 399–403 (1977).
- ²⁰ Montecat, C., Bizon, G. & J. J. *Revue de l'Inst. Français du Pétrole* **31**, 613–663 (1976).
- ²¹ Hsü, K. J. *Earth-Sci. Rev.* **8**, 371 (1972).
- ²² Hsü, K. J. *Sci. Am.* **277**, 27–36 (1972).
- ²³ Adams, C. G. *Bull. Brit. Mus. Nat. Hist. (geol.)* **19**, 85–137 (1970).
- ²⁴ Adams, C. G. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **20**, 47–66 (1976).
- ²⁵ Cole, W. S. *Prof. Pap. U.S. Geol. Surv.* 260-O, 569–605 (1954).
- ²⁶ Cole, W. S. *Prof. Pap. U.S. Geol. Surv.* 260-V, 743–784 (1958).
- ²⁷ Palmieri, V. *Rep. Geol. Surv. Qld.* **86**, 1–14 (1974).
- ²⁸ Quilty, P. G. *J. Geol. Soc. Aust.* **21**, 301–318 (1974).
- ²⁹ Bemmelen, R. W. van *The Geology of Indonesia* Government Printing Office, The Hague, 1A, xxiii + 732pp. (2nd edition, 1970).
- ³⁰ Baumann, P. *Bull. Nat. Inst. Geology and Mining* **5**, 13–28 (Bandung, 1975).
- ³¹ Berggren, W. A. & Haq, B. U. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* **20**, 67–129 (1976).
- ³² Spencer, M. *Bull. Am. Soc. petrol. Geol.* **51**, 263–268 (1967).
- ³³ Huddleston, P. F. & Wright, R. C. *Gulf Coast Assoc. geol. Soc. Trans.* **27** (in the press).
- ³⁴ Peck, D. M., Missimer, T. M., Wise, S. W. & Wright, R. C. *Fla. Scient.* **40**, 22–23 (1977).
- ³⁵ Kraft, J. C., Sheridan, R. E. & Maisano, M. *Am. Assoc. petrol. Geol.* **55**, 658–679 (1971).
- ³⁶ Colquhoun, D. J. & Johnson, H. S. *Palaeogeogr., Palaeoclim., Palaeoecol.* **5**, 105–126 (1968).
- ³⁷ Bold, W. A. van den *Mem. VI Conferencia Geologica del Carib-Margarita, Venezuela*, 485–490 (1972).
- ³⁸ Swain, F. M. *Prof. Pap. U.S. Geol. Surv.* 573-D (1968).
- ³⁹ Ingle, Jr. J. C. *Bull. Am. Paleontology* **52**, 217–394 (1967).
- ⁴⁰ Bandy, O. L. & Ingle, J. C., Jr. *Geol. Soc. Am. Spec. Paper* **124**, 131–172 (1970).
- ⁴¹ Barrow, J. A. *Palaeogeogr., Palaeoclim., Palaeoecol.* **14**, 277–291 (1973).
- ⁴² Bandy, O. L., Butler, E. A. & Wright, R. C. *Science* **166**, 607–609 (1969).
- ⁴³ Lietz, J. & Schminke, H. U. *Palaeogeogr., Palaeoclim., Palaeoecol.* **18**, 213–239 (1975).
- ⁴⁴ Douglas, R. C. & Savin, S. M. *Initial Reports of the Deep Sea Drilling Project* **32**, 509–520 (U.S. Government Printing Office, Washington, D.C., 1975).
- ⁴⁵ Shackleton, N. J. & Kennett, J. P. *Initial Reports of the Deep Sea Drilling Project* **29**, 801–807 (U.S. Government Printing Office, Washington, D.C., 1974).
- ⁴⁶ Hayes, D. E. & Frakes, L. A. *Initial Reports of the Deep Sea Drilling Project* **28**, 919–942 (U.S. Government Printing Office, Washington, D.C., 1975).
- ⁴⁷ Wolfe, J. A. & Hopkins, D. M. *Proc. 11th Pacific Sci. Congr. Tokyo*, 1966, 67. Sasaki, Sendai (1967).
- ⁴⁸ Kennett, J. P. & Vella, P. *Initial Reports of the Deep Sea Drilling Project* **29**, 769–799 (U.S. Government Printing Office, Washington, D.C., 1974).
- ⁴⁹ Kennett, J. P. *N. Z. J. Geol., Geophys.* **10**, 1051–1063 (1967).
- ⁵⁰ Kennett, J. P. *Micropaleontology* **14**, 305–318 (1968).
- ⁵¹ Bandy, O. *Giorn. Geol.* **35**, 277–290 (1968).
- ⁵² Kennett, J. P. & Watkins, N. D. *Bull. Geol. Soc. Am.* **85**, 1385–1398 (1974).
- ⁵³ Van Couvering *et al.* *Marine Micropaleontology* **1**, 263–286 (1976).

Evidence for a sensitising pigment in fly photoreceptors

K. Kirschfeld & N. Franceschini

Max-Planck-Institut f. biol. Kybernetik, Spemannstrasse 38, 7400 Tübingen, West Germany

B. Minke

Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem Israel

Many photoreceptor cells in invertebrates have a dual-peak spectral sensitivity. Evidence is presented that in fly photoreceptors the ultraviolet peak is due to a photostable pigment that absorbs light quanta and transfers the energy to the blue-absorbing visual pigment.

The primary process induced by absorption of a light quantum in a visual-pigment molecule is the isomerisation of the chromophore retinaldehyde from the 11-*cis* to the all-*trans* form¹. Subsequent dark reactions finally lead to an excitation of the receptor cell. We present here evidence that in addition to this direct interaction between light and visual pigment,

another process can take place—the light quantum can be absorbed by an accessory pigment which then transfers the energy to the visual pigment. The photostable accessory pigment is therefore acting as a 'sensitising pigment'. Sensitisation is known in photography where it is used to extend the spectral sensitivity of silver halides². In plant cells also, photostable accessory pigments (for example carotene) absorb light and transfer the energy to the effector pigment, chlorophyll *a*, for use in photosynthesis³.

Fly photoreceptors

In the compound eye of flies six of the eight receptor cells in each ommatidium have a receptor-potential action-spectrum with two maxima of similar height, one close to 500 nm, the other in the near ultraviolet, close to 360 nm (refs 4, 5). Dual-peak sensitivity of this type cannot be explained on the basis of extinction spectra of known rhodopsins: these pigments have only a small peak at shorter wavelengths, of less than 25% of the maximum (β -peak)⁶.

On the basis of electrophysiological experiments using selective chromatic adaptation, Burkhardt⁴ concluded that there was only one visual pigment present in each photoreceptor. Later data were thought to suggest the existence of two different rhodopsins and metarhodopsins in one and the same receptor cell^{5,7}, but the most recent results⁸ again support the view that there is only one visual pigment in these receptors.

Receptor-potential recordings are not a direct method of characterising visual pigments, for two reasons, first, it is not possible to exclude electrical interactions between different receptor cells with any certainty; and second, it is not possible to discriminate between effects of visual pigment *per se* and effects of accessory pigments which could modify the properties of photoreceptors in several ways⁹.

Microspectrophotometry provides more direct data on visual pigments. Difference spectra in receptors 1 to 6 have been measured in the visible range ($\lambda = 400\text{--}700\text{ nm}$)^{10–13}. These data (from *Calliphora*) show that there is only one isosbestic point at 510 nm, irrespective of which combination of two wavelengths of the spectrum is used for the conversion of rhodopsin into (dark-stable) metarhodopsin and *vice versa*. The difference spectrum is consistent with the existence of a rhodopsin with peak absorption at 490 nm, which nicely fits the spectral sensitivity in the visible, and a metarhodopsin with peak absorption at 580 nm. The fixed isosbestic point also indicates that there should be only one visual pigment in these receptors. But, it does not exclude with certainty the existence of a second visual pigment, since this could be non-absorbing in the limited spectral range investigated so far, or its spectrum could strongly overlap that of its metarhodopsin and hence exhibit a null difference spectrum. Results from microspectrophotometry so far give no explanation for the high ultraviolet sensitivity measured electrophysiologically.

Since there must be absorption of light in the ultraviolet if the receptor cells show high ultraviolet sensitivity, we decided to record not only the difference spectrum but also an absolute spectrum and to extend the measurements into the ultraviolet spectral range.

Microspectrophotometry

For microspectrophotometry, dipteran photoreceptors have the advantage that—in contrast to the situation in for example, locust or bee—the rhabdomeres in each ommatidium are not fused together but are separated from each other over their whole length, so that they act as individual light guides. It is therefore possible to measure the extinction of individual rhabdomeres in an eye cup preparation as was first done by Langer¹⁴. These structures are only 1–2 μm in diameter, so waveguide effects slightly modify the measured extinction spectra; however, this minor effect¹⁵ is within the limits of error of our data and no correction was applied.

Figure 1a shows two extinction spectra of type R_{1-6} receptors

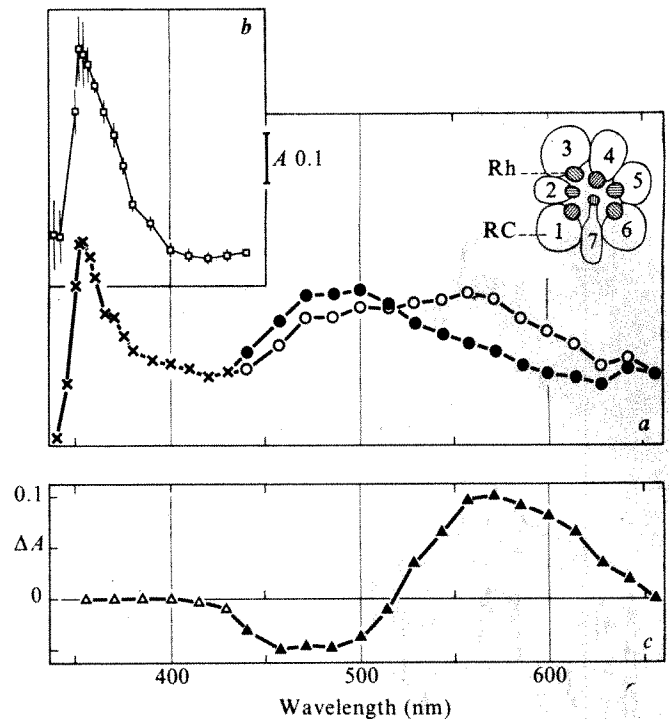


Fig. 1 *a* and *b*, Extinction spectrum of rhabdomeres type R_{1-6} (*Musca* ♀, eye-cup preparation of a white-eyed mutant) as measured with a technique similar to that of Langer¹⁴, but using a single beam instrument (Leitz MPV2, with Zeiss ultrafluor-optics, selected for minimal chromatic aberration). During measurements in the spectral range from 440–660 nm the preparation was illuminated with strong adapting lights of 365 nm (○) or 583 nm (●) at regular intervals, in order to maintain a stable equilibrium of rhodopsin/metarhodopsin. During adaptation the photomultiplier was protected by a mechanical shutter. Since other experiments showed that there is only a slight change or no change in extinction for wavelengths shorter than 430 nm after ultraviolet or orange adaptation, the spectrum in this range was measured without adaptation (×). ○, ●, ×, Data from rhabdomeres no. 2, 3 and 4 (measured together) of one ommatidium. In order to get a more accurate spectrum in the ultraviolet, rhabdomeres (no. 4 and 5) from 5 ommatidia of another eye were measured and the mean spectrum of all 6 ommatidia calculated (*b*); the given standard error was obtained after shifting the spectra along the ordinate to coincide at $\lambda = 440\text{ nm}$. Zero extinction is not defined in this kind of measurement, since the measuring beam passes the convergent cornea-lens of the ommatidium, whereas the reference beam does not. The cornea was shown to transmit uniformly in the spectral range analysed and fluorescence did not affect the measured spectra. Temperature of the preparations was 6–8 °C. Inset: cross section through ommatidium indicating receptor cells RC and rhabdomeres Rh. *c*, Difference of spectra from *a* (▲). Additional measurements at the short wavelengths (Δ) were carried out in the same rhabdomeres. Other rhabdomeres R_{1-6} sometimes showed reproducible small negative differences in the ultraviolet.

from a white-eyed *Musca* ommatidium, adapted with orange ($\lambda = 583\text{ nm}$) and ultraviolet ($\lambda = 365\text{ nm}$) light, respectively. It is obvious that there is a high extinction in the ultraviolet, which is confirmed by data from five more ommatidia (Fig. 1*b*). A second maximum occurs either near 500 nm or near 560 nm, respectively, depending on the pre-adapting light. The difference (Fig. 1*c*) between the two curves of Fig. 1*a* represents the difference spectrum between metarhodopsin (M 580) and rhodopsin (R 490), which corresponds to that obtained in intact animals¹¹ and in wild-type flies (*Calliphora*)¹³. We have confirmed that the difference spectrum in *Musca* changes only in amplitude, not in shape, when the wavelengths used for shifting the pigment from one stable state (R 490) to the other (M 580) are changed, and we have extended the measurements into the ultraviolet.

In spite of the fact that there is a high absorption in the ultraviolet (Fig. 1*a, b*) and that ultraviolet light induces a shift from rhodopsin to metarhodopsin, there is no conspicuous

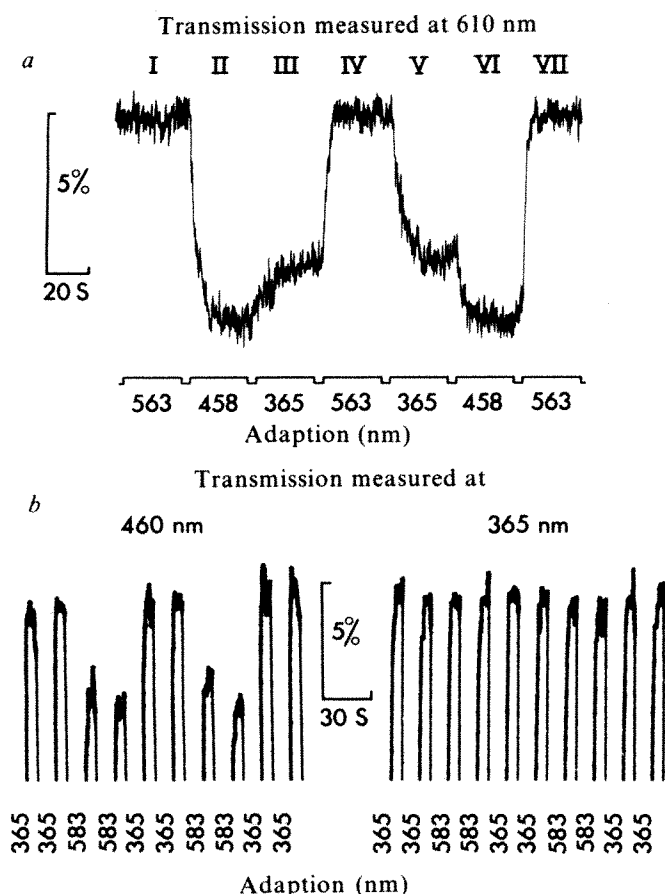


Fig. 2 *a*, Transmission of rhabdomeres no. 2+3+4 (*Musca* ♀, white-eyed) measured at 610 nm. Adapting lights of different colours were used as indicated. Appropriate edge filters prevented transmission of adapting light to the photomultiplier. *b*, Transmission measured in the blue (460 nm) and ultraviolet (365 nm) following various adapting lights as indicated. In contrast to Fig. 2*a* the adapting light was switched off during the measurement.

decrease in the ultraviolet extinction. As this result was unexpected we checked directly whether the blue and ultraviolet lights shift the pigment to the same or to two different metarhodopsins, both absorbing in the red. The temporal change in metarhodopsin concentration was monitored by measuring transmission at 610 nm: the higher the transmission, the lower the metarhodopsin concentration (Fig. 2*a*).

After an equilibrating pre-adaptation with 563-nm light which shifts the pigment to the rhodopsin state, both blue (458 nm, Fig. 2*a* I→II) and ultraviolet (365 nm, Fig. 2*a* IV→V) lights shift the pigment back to metarhodopsin, as indicated by the reduction in transmission at 610 nm. The equilibrating blue light shifts more than the equilibrating ultraviolet light.

In fact, ultraviolet light following blue light (Fig. 2*a* II→III) leads to a conspicuous increase in transmission at 610 nm. If there had been two different red light-absorbing metarhodopsins in the one photoreceptor, this ultraviolet stimulus should have led to a further decrease in transmitted red light, due to the formation of the second metarhodopsin. The observed increase indicates that, during ultraviolet illumination, a new equilibrium between rhodopsin and metarhodopsin is created which yields less metarhodopsin than does blue illumination. These results are evidence that the metarhodopsin formed by ultraviolet light is the same as that formed by blue light.

Figure 2*b* illustrates directly the fact that ultraviolet light following orange (583 nm) light reduces the rhodopsin concentration, as shown by an increase in transmission in the blue (460 nm), despite the fact that there is no change or only a slight change, in ultraviolet absorption in equivalent conditions (Fig. 2*b*).

The results obtained with microspectrophotometry can be interpreted in at least three ways. (1) There is a second visual pigment that absorbs in the ultraviolet, and has a metarhodopsin also absorbing in the ultraviolet. Their strongly overlapping spectra would explain the odd absence of extinction change in the ultraviolet (Fig. 1). (The shift of pigment to M 580 by ultraviolet illumination is then due to the β peak of R 490). (2) The rhodopsin and metarhodopsin are unusual insofar as they both absorb strongly in the ultraviolet. (3) There is a photostable pigment absorbing in the ultraviolet and transferring the energy to the rhodopsin. It then acts as a sensitising pigment.

One possible mechanism of energy transfer is by means of dipole-dipole interactions (case 3*a*), according to Förster's theory¹⁶. In this case the photostable pigment is called an 'antenna' pigment, as in photosynthesis. Other kinds of energy transfer are also possible, for example, a photostable chromophore could be attached to the rhodopsin molecule, giving rise to a high ultraviolet absorption in rhodopsin (as well as in metarhodopsin) (case 3*b*). Such a mechanism, in a way, is rather similar to the situation in photographic sensitisation, in which a dye must be adsorbed on to the silver halides in order to act as a sensitiser². The trivial case in which light quanta, emitted due to fluorescence of a photostable substance, are reabsorbed by the rhodopsin seems rather unlikely because of the low efficiency of this process within a rhabdome.

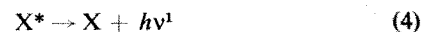
According to electrophysiological data, hypothesis (1) is improbable, since intracellular recordings made in cells 1-6 under selective chromatic adaptation have revealed that blue-light adaptation depresses sensitivity in the blue and the ultraviolet to the same extent and vice versa^{4,8}. We present here experiments that exclude hypothesis (2) as well as (1) and give evidence that hypothesis (3) is realised.

Evidence for a sensitising pigment

The sensitising-pigment hypothesis can be formulated as follows:



Due to the absorption of a light quantum, the unspecified molecule X is converted into the excited state X^* (equation 1). In a secondary process it then may interact with a rhodopsin molecule R , converting it into an excited state R^* (equation 2), which finally leads to metarhodopsin M (equation 3). Alternatively, the molecule X^* may lose the extra energy by fluorescing



or by suffering deactivating collisions with other molecules. In the latter case the energy is dissipated as heat. If the energy transfer occurs by means of a dipole-dipole interaction (case 3*a* as discussed above), the concentration of the molecules present determines whether the secondary process occurs according to equations (2) and (3) or to equation (4), assuming that the lifetime of the excited state is sufficiently short. This is because these concentrations affect the probability that a molecule X^* meets a molecule R ¹⁷. The relationship between the percentage of rhodopsin still present and the number of bleaching quanta should be a decreasing exponential for blue as well as for ultraviolet quanta, as long as the concentration of R and X is comparatively high, since the kinetics in both cases corresponds to classical first-order photochemical bleaching.

But, if we reduce sufficiently the concentration of X and/or R we expect to observe the following two phenomena: (1) The ratio of the number of ultraviolet quanta to blue quanta necessary to shift a given percentage of rhodopsin into metarhodopsin must be increased with a sufficiently decreased concentration of R and/or X . (2) An exponential decrease of rhodopsin concentration due to bleaching is still expected for blue light,

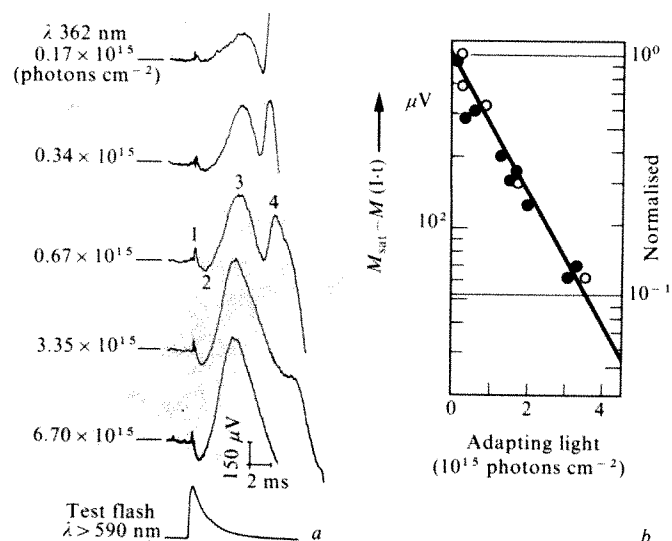


Fig. 3 *a*, Initial part of electroretinograms on a rapid time scale obtained with high-energy orange test flashes of constant intensity from a white-eyed *Drosophila* raised on Sang's synthetic diet medium²⁴ with 2.4 mg per 100 ml β -carotene added (8 mg per 100 Δ 100%). The flash light-source (photographic flash, Braun F 900) was used in conjunction with a Schott OG 590 edge filter and a Schott KG1 heat filter. The energy of the flash when using a monochromatic 584-nm interference filter (Schott, Depal) together with KG1 heat filter, was 5.4×10^{16} photons cm^{-2} at the level of the preparation. Before each measurement the eye was given two orange flashes that shift the pigment to the rhodopsin state. After 1 min in the dark the amount of monochromatic 362 nm ultraviolet light (Schott, uv-pil) indicated on the left of each trace was applied. After 1 min in the dark the orange test flash (590 nm) of constant intensity was given, inducing the five responses shown. Each orange flash elicits a mass response composed of several components indicated by numbers on the third trace: 1, stimulus artefact, indicating the onset of the flash; 2 and 3, biphasic fast photovoltage proportional to the concentration of pigment in the metarhodopsin state. This response called the M-potential, can be subdivided into a negative phase (2) and a positive phase (3)¹⁸. 4, 'on' transient of the ERG which arises from the response of the second-order neurones. *b*, The ordinate gives the difference between the peak M-potential amplitude following saturating ultraviolet light (M_{sat} , bottom M-potential trace in *a*) and the M-potential amplitude $M(I-t)$ following various amounts ($I-t$) of adapting 362-nm ultraviolet (●) and 473-nm blue (○) light. The curve actually represents the decay of rhodopsin concentration as a function of the amount of adapting light.

but no longer for ultraviolet light. This is because the concentration of rhodopsin, further reduced by an increasing number of absorbed ultraviolet quanta, reduces the efficiency or energy transfer between X^* and R. The kinetics can then no longer be first order.

To test these predictions we measured the concentrations of fly visual pigments by an electrophysiological technique. This independent and sensitive method has the advantage of using intact flies whereas the microspectrophotometric measurements were carried out with eyecup preparations. The pigment conversion in receptors R_{1-6} was measured by a fast photovoltage, the M-potential¹⁸, which is a response proportional to the metarhodopsin concentration [M] in R_{1-6} , as shown by the following evidence. (1) The action spectrum of the M-potential corresponds to the metarhodopsin extinction spectrum of receptors R_{1-6} (ref. 18). (2) No M-potential has been observed in mutants lacking receptors R_{142} (S. R. Grabowski and W. L. Pak, personal communication). (3) In a dark-adapted eye, the M-potential amplitude increases exponentially with the amount of incident blue quanta and reaches saturation at the same level at which metarhodopsin formation saturates (Minke *et al.*, unpublished).

M-potentials were recorded from a white-eyed *Drosophila* with an approximately normal rhodopsin concentration, in

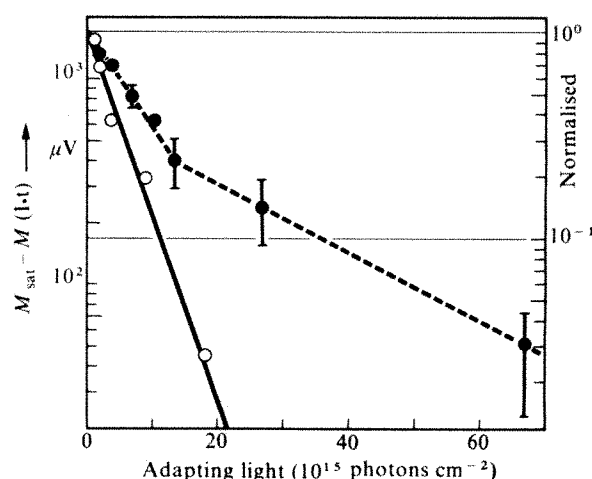
response to a strong orange test flash, following various amounts of 362-nm ultraviolet pre-adapting light (Fig. 3*a*). Figure 3*b* shows a plot of the difference between the M-potential from rhodopsin to metarhodopsin. This experiment is a control amplitude measured after an equilibrating ultraviolet illumination and the M-potential amplitude measured after different amounts of pre-adapting ultraviolet light. This method of presentation actually illustrates the decay of rhodopsin concentration [R] with increasing ultraviolet illumination, since $[R] = 1 - [M]$.

Equivalent data for 473-nm blue adaptation are also included in Fig. 3*b*. The data show that the rhodopsin concentration decreases exponentially with the amount of adapting blue or ultraviolet light. In this case the 362-nm ultraviolet light is as efficient as the 473-nm blue light in shifting the visual pigment from rhodopsin to metarhodopsin. This experiment is a control experiment for normal flies. In the critical experiment we need flies with rhabdomeres in which the concentrations of rhodopsin and/or the presumed sensitising pigment are reduced.

The concentrations of rhodopsin within the rhabdomeres can be reduced by raising flies in a vitamin A-deficient medium^{12,19,21,22}. Figure 4 shows the decay of the rhodopsin concentration, again measured by the M-potential, as a function of the amount of adapting 473-nm blue and 362-nm ultraviolet light, measured in a vitamin A-deprived fly. First, it seems that about twice as many ultraviolet quanta as blue quanta are now necessary to produce a criterion M-potential of 50% of the maximum (in three other experiments two to nine times as many ultraviolet quanta were necessary). Second, the dependence of the rhodopsin concentration on the amount of adapting ultraviolet light is no longer exponential. This is in contrast to the dependence on blue-adapting light (measured in the same fly), which remains exponential.

Thus in vitamin A-deprived flies, the effectiveness for converting rhodopsin into metarhodopsin is selectively reduced in the ultraviolet. This is in agreement with the result that the sensitivity of the receptors as determined with the ERG in vitamin A-deprived flies is selectively reduced in the ultraviolet^{19,23}. This finding, as well as the fact that the kinetics of the decrease of rhodopsin concentration under ultraviolet illumination no longer corresponds to a first-order reaction, fits the predictions of the 'antenna' pigment model.

Fig. 4 Concentration of pigment in the rhodopsin state, as measured by the M potential, in response to the orange test flashes of constant intensity, as a function of the amount of adapting 362-nm ultraviolet (●) and 473-nm blue (○) light. All the measurements were from a single white-eyed *Drosophila* raised on vitamin-A-deficient medium (Sang's synthetic diet medium with 0.8 mg per 100 ml β -carotene). The vertical bars are standard deviations of the mean calculated from three or four measurements. Most of the points without bars are the average of two measurements.



Our results are not compatible with the hypothesis of a second rhodopsin-metarhodopsin, absorbing in the ultraviolet (model 1). This model would predict neither the high efficiency of ultraviolet quanta for the production of M 580, as shown in Fig. 3b, nor the modifications seen in flies raised on vitamin A-deprived media (Fig. 4). An unusual rhodopsin (model 2) would also be unable to generate the modifications observed in vitamin A-deprived flies. Although the antenna-pigment model is sufficient to explain the experimental results, this kind of sensitisation is not necessarily the only possible one. With several special assumptions, a mechanism of energy transfer according to case 3b, as mentioned above, could also be realised. There is some evidence that the absorption in the ultraviolet is due to a derivative of vitamin A (J. Schwemer, personal communication). If this substance is attached to all rhodopsin molecules as a second chromophore only at sufficiently high vitamin A concentrations, then at lower vitamin A concentrations, two populations of rhodopsin will be present: one with a high ultraviolet absorption due to the second chromophore, and another which lacks the second chromophore and therefore has only low ultraviolet absorption (due to the low β peak of the rhodopsin). The kinetics of the decay of both rhodopsins will be the same if blue light is used for bleaching. There will be two different decay rates, however, if ultraviolet light is used, since the absorption coefficients of the two substances differ in the ultraviolet. This is also in accordance with Fig. 4 (ultraviolet illumination), since the deviation from the pure exponential can be interpreted as being due to the sum of two exponentials with different decay constants.

We conclude that the photoreceptors 1-6 in the fly achieve their high ultraviolet sensitivity thanks to an accessory photostable pigment that acts as a sensitiser for rhodopsin. The mechanism of the energy transfer has still to be worked out in detail. The functional consequence of the sensitising pigment for

the photoreceptors is clear—it broadens the spectral range of the visual pigment and thus increases absolute sensitivity.

We thank Drs E. Buchner, K. G. Götz, K. Hamdorf, P. Hillman, S. Hochstein, B. Pick and C. Wehrhahn for discussions, Mr R. Feiler for technical assistance, Mr M. Heusel for preparing the figures and Dr P. McIntyre for reading the manuscript. This research was supported in part by a grant from the United States-Israel Binational Science Foundation, Jerusalem, Israel (to B.M.).

Received 14 March; accepted 21 July 1977.

- ¹ Wald, G. *Nature* **219**, 800-807, (1968).
- ² West, W. & Carroll, B. H. in *The Theory of the Photographic Process* 3rd edn (ed. James, T. H.), 233-277 (Macmillan, New York, 1966).
- ³ Govindjee in *Bioenergetics of Photosynthesis* (ed. Govindjee), (Academic, New York, 1975).
- ⁴ Burkhardt, D. *Symp. Soc. exp. Biol.* **16**, 86-109 (1962).
- ⁵ Horridge, G. A. & Mimura, K. *Proc. R. Soc. B* **190**, 211-224 (1975).
- ⁶ Rodieck, R. W. *The Vertebrate Retina, Principles of Structure and Function*, (Freeman, San Francisco, 1973).
- ⁷ Rosner, G. *J. comp. Physiol.* **102**, 269-295 (1975).
- ⁸ Tsukahara, Y. & Horridge, G. A. *J. comp. Physiol.* **114**, 233-251 (1977).
- ⁹ Kirschfeld, K. & Franceschini, N. *Biophysics of Structure and Mechanism* **3**, 191-194 (1977).
- ¹⁰ Hamdorf, K., Paulsen, R. & Schwemer, J. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 155-166 (Springer-Berlin, Heidelberg & New York, 1973).
- ¹¹ Stavenga, D. G., Zantema, A. & Kuiper, J. W. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 175-180 (Springer Berlin, Heidelberg & New York, 1973).
- ¹² Razmjoo, S. & Hamdorf, K. *J. comp. Physiol.* **105**, 279-286 (1976).
- ¹³ Stavenga, D. G. *J. comp. Physiol.* **111**, 137-152 (1976).
- ¹⁴ Langer, H. *Verhandlungen der Deutschen Zoologischen Gesellschaft in Jena*, 329 (1965).
- ¹⁵ Kirschfeld, K. & Snyder, A. *Vision Res.* **16**, 775-778 (1976).
- ¹⁶ Förster, T. *Fluoreszenz Organischer Verbindungen* (Vandenhoeck und Ruprecht Göttingen, 1951).
- ¹⁷ Dartnall, H. J. A. in *The Visual Pigments* (ed. Dartnall, H. J. A.) 63 (Wiley, New York, 1957).
- ¹⁸ Pak, W. L. & Lidington, K. J. *J. gen. Physiol.* **63**, 740-756 (1974).
- ¹⁹ Goldsmith, T. H., Barker, R. J. & Cohen, C. F. *Science* **146**, 65-67 (1964).
- ²⁰ Stark, W. S. & Zitzmann, W. G. *J. comp. Physiol.* **105**, 15-27 (1976).
- ²¹ Boschek, C. B. & Hamdorf, K. *T Naturforsch.* **31c**, 763 (1976).
- ²² Harris, W. A., Ready, D. F., Lipson, E. D., Hudspeeth, A. J. & Stark, W. S. *Nature* **266**, 648-650 (1977).
- ²³ Stark, W. S., Ivanyshyn, A. M. & Hu, K. G. *Naturwissenschaften* **63**, 513-518 (1976).
- ²⁴ Doane, W. W. in *Methods in Developmental Biology* (eds Wilt, F. H. & Wessels, N. K.) 54 (Crowell, New York, 1967).

Nucleotide sequences of 5'-terminal ribosome-protected initiation regions from two reovirus messages

Marilyn Kozak

Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Sequences for two reovirus ribosome-protected fragments are presented. Comparison with four other reovirus initiation sites reveals only two common features: the 5'-terminal sequence m⁷GpppG^mCUA, and (located 15-33 nucleotides from the cap) the sequence AUGG. Both the cap and the AUG codon are included in the 40S-ribosome protected region from all six reovirus messages.

THERE has been considerable speculation about what features in mRNA direct attachment of ribosomes to the sites where translation will be initiated. Although rather sophisticated hypotheses have been advanced for messenger-ribosome interactions in prokaryotic systems^{1,2}, deductions about eukaryotic mechanisms have been limited by the absence of detailed information concerning the initiation sites from a variety of eukaryotic messages. The sequence extending from the 5' capped terminus up to and beyond the AUG initiation triplet was reported for one of the genome segments from brome mosaic virus³ and, more recently, for rabbit β -globin mRNA^{4,5}. We have previously described the nucleotide sequences of ribosome-protected regions from four reovirus messenger RNAs^{6,7}. We present here sequences for two additional reovirus ribosome binding sites. All six ribosome-protected frag-

ments from reovirus mRNA include the 5'-terminal methylated cap, which has been shown to strongly promote *in vitro* translation of various eukaryotic messages⁸.

Isolation of two ribosome-protected fragments

Reovirus mRNAs, labelled with α -³²P-ribonucleoside triphosphate precursors, were synthesised *in vitro* by virion-associated enzymes, in conditions described previously⁶. The *in vitro* transcription reaction catalysed by reovirus cores generates 10 presumably monocistronic messenger species which can be fractionated by sucrose gradient velocity sedimentation into small, medium and large size classes⁹. In this study, the small size class (s-RNA), consisting of four messenger species, was incubated in a wheat germ extract in conditions permitting formation of both 40S and 80S initiation complexes^{6,7}. After addition of T₁ RNase to hydrolyse the exposed regions of the RNA, the samples were sedimented through glycerol gradients^{6,7}. The ³²P-labelled material recovered from 40S ribosomal complexes consisted of a mixture of three cap-containing fragments, which were readily resolved by polyacrylamide gel electrophoresis followed by two-dimensional homochromatography⁷. Nucleotide sequences were previously determined for two of these s-RNA fragments⁷ (s54 and s45 in Fig. 1). The following section describes the sequence of the third fragment, designated s46; that is, a 46-nucleotide fragment

PARTIAL II				PARTIAL I			
T1				T6 (T7, T3)	T7	T2	
M7GpppG ^m CUAUUCGUGGUCAGUUAUGGCU				G(CG, CU)C	GCGUCCUAUUAAG		
				CGC(GC, U)			
				T3 (T7, T6)			

a) ANALYSIS OF T₁ RNase PRODUCTS FROM THE 46-NUCLEOTIDE FRAGMENT

T ₁ RNase primary product	Products of secondary digestion with pancreatic RNase				Sequence deduced
	[α- ³² P]CTP	[α- ³² P]UTP	[α- ³² P]GTP	[α- ³² P]ATP	
s46/T1	G, U, cap	AA, cap	C, cap	U	m7GpppG ^m CUAUUCG[C]
s46/T2	C, 2U	AA, C, U	AAG	AA, C, U	UUCUAUUAAG[A]
s46/T3	G, U	C	C	--	CUCG[C]
s46/T4	U	AG	AG	C	UCAG[U]
s46/T5	--	AU, U	AU, G	U	UUAG[C]
s46/T6	G	C	U, G(2:1)	--	CUG[C, G]
s46/T7	G	G	C	--	CG[C, U]
s46/T8	G	G	--	--	G[C, U]

b) ANALYSIS OF PANCREATIC RNase PRODUCTS FROM THE 46-NUCLEOTIDE FRAGMENT

Pancreatic RNase primary product	Products of secondary digestion with T ₁ RNase				Sequence deduced
	[α- ³² P]CTP	[α- ³² P]UTP	[α- ³² P]GTP	[α- ³² P]ATP	
s46/P1	--	--	AAG	AA	AAG[A]
s46/P2	G	C	G	--	GGC[U]
s46/P3	--	AG, U	AG	--	AGU[U]
s46/P4	U	G	G	--	GGU[C]
s46/P5	G	C	C	--	GC[G, U]
s46/P6	cap	cap	cap	--	m7GpppG ^m C[U]
s46/P7	--	AA	AA	--	AU[G, U]
s46/P8	--	G, U	--	--	GU[U]
s46/P9	C	C	C	C	C[C, U, G, A]
s46/P10	U	U	U	U	U[C, U, G, A]

c) SUPPLEMENTARY ANALYSES ON LARGE T₁ OLIGONUCLEOTIDES FROM FRAGMENTS s46 AND m30

T ₁ RNase primary product	Labelled precursor NTP	Products of U ₂ RNase digestion			Sequence of oligonucleotide
		Composition	Panc/CMCT products	Sequences of U ₂ products	
s46/T2	C	a) C, U b) U	a) UUC ^{AA} b) UUC	a) UUCUA[U] b) UUCA[G] c) G[A]	UUCUAUUAAG[A]
	U	a) C, A, U b) U	a) UUC, UA, C ^{AA} b) UUC		
	A	a) U b) C, A c) G	n.d.		
m30/T2	C	a) A b) G, U c) C, U	n.d.	a) CUUA[C] b) UCG[C] c) CA[U]	CUUACAUCG[C]
	U	a) C, U c) A	n.d.		

d) ANALYSIS OF PARTIALS DERIVED BY LIMITED T₁ RNase DIGESTION OF FRAGMENT s46

Partial product	Labelled precursor NTP	Products of complete digestion with T ₁ RNase	Products of complete digestion with pancreatic RNase
I	G	T2(AAG); T3(C); T5(AU, G); T6(U); T7(C)	AAG, GGC, GC, AU, U
	U	T2(AU, C, U); T3(C); T5(AU, U); T6(C); T7(G)	GGC, GC, AU, GU
II	G	T1(C, cap); T4(AG); T6(G, U)	cap, GGU, AG, C, U
	U	T1(AU, cap); T4(AG); T6(C); T8(G)	cap, GGU, AG, GC, AU

Nucleotide sequence of fragment m30

The T₁ RNase fingerprint of fragment m30 is shown in Fig. 2c and d. The sequence of each primary T₁ RNase product was deduced from the results of secondary digestion with pancreatic RNase, summarised in Fig. 4a. For the large oligonucleotide m30/T2, the sequence was confirmed by analysis of the U₂ RNase digestion products (Fig. 3c). Figure 4b summarises data on the primary pancreatic RNase products. Presence of panc oligonucleotide P5 (AG), which terminates with a G residue rather than with a pyrimidine, identifies T₁ oligonucleotide T4 (CAG) as the 3'

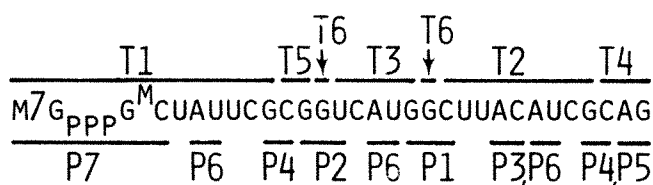
Fig. 3 Sequence analysis of fragment s46. The column headings α-³²P-CTP, α-³²P-UTP and so on indicate the labelled ribonucleoside triphosphate precursor. A double dash in any column means that the oligonucleotide in question was not labelled in the indicated conditions. The designation n.d. means a given analysis was not done. * Denotes the 3'-nucleoside monophosphate which retained the ³²P-radioactivity after alkaline hydrolysis. 'Cap' refers to the structure m7GpppG^mpCp, identified as in ref. 7. The nearest neighbour residue is enclosed in square brackets at the 3' end of each oligonucleotide. The sequence deduced in a for oligonucleotide s46/T2 takes into account the results described in c. In c, the secondary U₂ RNase products are identified as (a), (b) and (c), in order of increasing mobility on DEAE paper in 7% formic acid. The column headed 'Panc/CMCT products' lists the products obtained by pancreatic RNase digestion of each U₂ secondary product after modification of U and G residues with CMCT⁶. In d, the third column lists the T₁ oligonucleotides (identified as in a) obtained after complete T₁ RNase digestion: the products of secondary pancreatic RNase digestion are given in parentheses next to each T₁ oligonucleotide.

terminus of the ribosome protected fragment. Ordering of the remaining T₁ oligonucleotides is facilitated by beginning with T3, the only T₁ oligonucleotide that begins with a U residue. T3 must be preceded by P2 which is the only G-containing panc oligonucleotide that terminates with U. Since T3 ends with the sequence G[G], it is followed by P1 (GGC[U]), the only remaining panc oligonucleotide containing contiguous G residues. The resulting sequence, (5')GGUCAUGGCU(3'), must be followed by T2, which begins with CU. Furthermore, the 5' side of the sequence can be extended by adding one C residue, since oligonucleotide T5 (CG[G]) is the only remaining T₁ product that terminates in G[G]. Oligonucleotide T4 has already been placed at the 3' terminus of the fragment, leaving only the capped oligonucleotide T1, which obviously derives from the 5' end of the fragment. The complete 30-nucleotide sequence is shown at the top of Fig. 4.

Features of reovirus ribosome-binding sites

The two sequences described above, along with ribosome-protected portions of four other reovirus messages, are shown in Fig. 1. Each ribosome-protected fragment contains a single AUG triplet, with no termination codons following in phase. This AUG has not yet been proven to be the initiation codon, since the presence of blocked N termini in reovirus proteins¹⁴ has hampered amino acid sequence determinations. It is likely, however, that the single AUG triplet in each binding site is used for initiation, since the AUG occurs in a remarkably constant position within each 80S-protected fragment, as indicated in Fig. 1. The AUG triplet occurs in approximately the same position in the 80S-protected portion of rabbit β-globin mRNA¹⁵.

Each of the reovirus 5'-terminal fragments has been shown to rebind to wheat germ ribosomes with high efficiency^{7,10}, indicating that the isolated fragments retain the recognition signals needed for ribosome attachment. Thus, it is of interest to look for common features among the six reovirus sequences, as well as similarities with other eukaryotic initiation sites. Two of the reovirus fragments begin with the 5' terminal sequence m7GpppG^mCUAAAGU (see s45 and m44 in Fig. 1). In both of these fragments, the sequence GUC(or G)GUC occurs to the left of the AUG, and the sequence GCUUC follows directly after the AUG. The only part of this homology that occurs in other reovirus fragments is the sequence GCUU on the 3' side of the AUG codon in fragment m30. In fact, although fragment s45 differs, fragments m30 and m44 share an extensive 11-base sequence (GGUCAUGGCUU) that spans the putative initiation codon. A portion of this sequence (GGC... AUGGCU) also occurs in fragment s46, which further resembles m30 in that both fragments have the identical 10-residue 5'-terminal sequence. Thus, the 20-nucleotide sequence at the 5' end of fragment m30 is repeated (with two interruptions) in the first 25 residues of fragment s46. Such impressive homologies may reflect evolutionary relationships among reovirus genome segments, but the homologous regions are probably not directly related to the role of these fragments in initiating translation, since the same sequences are not seen in the remaining ribosome binding sites. Indeed, the only features common to all six reovirus initiation sites are a paucity of A residues, occurrence of a G residue directly after the AUG codon, and presence of the 5'-terminal sequence m7GpppG^mCUA. The

a) ANALYSIS OF T₁ RNase PRODUCTS FROM THE 30-NUCLEOTIDE FRAGMENT

T ₁ RNase primary product	Products of secondary digestion with pancreatic RNase				Sequence deduced
	[α- ³² P]CTP	[α- ³² P]UTP	[α- ³² P]GTP	[α- ³² P]ATP	
m30/T1	G, U, cap	AU, cap	C, cap	U	m7Gppp ^m CUAUUCG[C]
m30/T2	AU, AC, G	AU, C, U	C	U, AC	CUUACACG[C]
m30/T3	U	AU	AU, G	C	UCAUG[G]
m30/T4	--	AC	AC	C	CAG[U]
m30/T5	--	--	C, C	--	CG[G]
m30/T6	G	C	--	--	G[C, U]

b) ANALYSIS OF PANCREATIC RNase PRODUCTS FROM THE 30-NUCLEOTIDE FRAGMENT

Pancreatic RNase primary product	Products of secondary digestion with T ₁ RNase				Sequence deduced
	[α- ³² P]CTP	[α- ³² P]UTP	[α- ³² P]GTP	[α- ³² P]ATP	
m30/P1	C	C	C	--	GGC[U]
m30/P2	U	C	G	--	GGU[C]
m30/P3	AC	--	--	AC	AC[A]
m30/P4	C	--	C	C	GC[A, G]
m30/P5	--	AC	AG	--	AG[U]
m30/P6	AU	AU	AU	--	AU[C, U, G]
m30/P7	cap	cap	cap	--	m7Gppp ^m C[U]
m30/P8	--	--	C	C	C[G, A]
m30/P9	U	U	--	U	U[C, U, A]

Fig. 4 Analysis of T₁ and pancreatic RNase products from fragment m30. See Fig. 3 legend for explanations.

sequence G^mCUA adjacent to the cap is not seen, however, in many other messages, including brome mosaic virus RNA 4 (m7GpppGUAU)³, vesicular stomatitis virus mRNA (m7GpppA^mAC)¹⁶, silk fibroin (m7GpppA^mU^mC)¹⁷ and rabbit globin (m7GpppA^mC^mAC)¹⁸. The sequence AUGG, common to all six reovirus messages as well as globin mRNA^{4,5}, has the striking potential to form four base pairs with the anticodon loop (CCAU) of mammalian initiator tRNA²². In brome mosaic virus RNA 4, however, the AUG initiation codon is not followed by a G residue³. Thus, homology among the eukaryotic regions so far sequenced is limited to presence of an AUG triplet and a 5'-terminal cap.

It is difficult to detect any other significant features common to eukaryotic initiation sites. For example, eukaryotic ribosome binding regions differ from one another in that the AUG triplet does not always occur in the same phase, determined by measuring off three nucleotides at a time, from the AUG leftwards to the cap. The distance between the cap and the AUG also varies, from 11 nucleotides³ to at least 55 nucleotides^{4,5}. Furthermore, nonsense codons, which commonly occur on the 5' side of the initiation triplet in prokaryotic ribosome binding sites¹¹, are not a universal feature of eukaryotic initiation sites, since reovirus fragments s54, s46 and m30 lack such codons. The suggestion has been made that a specific interaction, involving a region near the AUG codon which is complementary to the 3' end of 18S ribosomal RNA, might be characteristic of eukaryotic (as well as prokaryotic) initiation sites². Between the reovirus 5'-terminal fragments and the 3'-terminal eight residues of 18S rRNA¹⁹, however, there are only minimal opportunities for base pairing (involving only two base pairs in fragments s54, s46 and m30; three base pairs in s45 and m44; and four A-U base pairs adjacent to the cap in fragment m52). Although absence of significant complementarity cannot be proved until additional portions of the 18S rRNA sequence

become known, there is no evidence in eukaryotic systems for even a moderately-stable, specific interaction between messenger and 18S ribosomal RNA. This seems to be true not only for reovirus, but also for the 5' non-coding regions of rabbit globin^{4,5}, brome mosaic virus³ and SV40 VP1 messages²⁰. Although base pairing with ribosomal RNA has been postulated for those three messages, the proposed interactions (involving primarily A-U base pairs, interrupted by unpaired residues in the case of SV40 VP1) are predicted to be far less stable than those demonstrated with prokaryotic messages². Furthermore, while the region postulated to undergo base pairing with ribosomal RNA is located in a rather constant position relative to the AUG in prokaryotic messages, this is not true of the eukaryotic initiation sites⁵. Thus, the suggestion that base pairing with ribosomal RNA might help to position the ribosome on eukaryotic messages seems to derive more from analogy with prokaryotic systems than from predication based on the available eukaryotic ribosome binding sequences. Although eukaryotic cellular mechanisms often parallel those observed with prokaryotes, perhaps the process of translational initiation is exceptional, due to the structural differences between prokaryotic and eukaryotic messages. With polycistronic prokaryotic messages, which require discrimination between the few internal AUG and GUG codons that actually serve for initiation and the much larger set of internal AUG and GUG codons which normally do not function as initiation triplets, a relatively intricate mechanism may be required for selection of the correct initiation sites by ribosomes. With monocistronic eukaryotic messages, on the other hand, initiation requires recognition of a single AUG codon, and inspection of the available eukaryotic sequences suggests that initiation begins at the AUG nearest the 5' end of the message. (Although the SV40 VP1 sequence²⁰ seems to be an exception, the possible occurrence of very limited processing of the VP1 protein, involving removal of a few N-terminal amino acids, would hamper the correct identification of the initiation region, which was based not on ribosome binding but on a comparison of the nucleotide sequence of the DNA with the amino acid sequence of the mature virion protein.) Inclusion of the 5' terminus as well as the AUG triplet within all six 40S ribosome-protected reovirus fragments is consistent with the possibility that these two elements are necessary features (recognition signals) in eukaryotic initiation sites. The absence of other detectable homologies among the limited set of eukaryotic initiation regions sequenced to date may mean that the presence of an AUG triplet near a 5' terminus (usually, but not necessarily capped) is sufficient to direct binding of eukaryotic ribosomes. This 'minimal recognition' hypothesis will undoubtedly require modification as more data becomes available. For example, if an AUG codon in the 5' region of an RNA molecule were masked by the surrounding secondary structure, that AUG might not mediate initiation unless a mechanism were available to denature the RNA. Furthermore, an AUG codon located extremely close to the 5' terminus, as in Sindbis 42S virion RNA (m7GpppAUG)²¹, would probably be non-functional because of steric considerations. We suggest, based on evidence to be published elsewhere, that the methylated cap functions secondarily, in a way that does not affect where the ribosome attaches to the message but that greatly increases the yield of initiation complexes. The secondary and tertiary structure of the intervening region, between the 5' terminus and the AUG triplet, might also be expected to influence translational efficiency by determining the spatial orientation of the two putative recognition elements. Thus, although the presence of an AUG codon near the 5' end of an RNA chain might be sufficient to permit a certain low level of ribosome binding, other structural features in the RNA probably modulate the initiation event.

I thank Dr Aaron Shatkin for helpful discussions and encouragement.

Received 31 May; accepted 22 July 1977.

¹ Shine, J. & Dalgarno, L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1342-1346 (1974).

² Steitz, J. A. & Jakes, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4734-4738 (1975).

³ Dasgupta, R., Shih, D. S., Saris, C. & Kaesberg, P. *Nature* **256**, 624-628 (1975).

⁴ Baralle, F. E. *Cell* **10**, 549-558 (1977).

⁵ Elstratidis, A., Kafatos, F. C. & Maniatis, T. *Cell* **10**, 571-585 (1977).

⁶ Kozak, M. & Shatkin, A. J. *J. molec. Biol.* **112**, 75-96 (1977).

⁷ Kozak, M. & Shatkin, A. J. *J. biol. Chem.* (in the press).

⁸ Shatkin, A. J. *Cell* **9**, 645-653 (1976).

- ⁹ Banerjee, A. K. & Shatkin, A. J. *J. Virol.* **6**, 1-11 (1970).
¹⁰ Kozak, M. & Shatkin, A. J. *J. biol. Chem.* **251**, 4259-4266 (1976).
¹¹ Pieczek, G., Model, P. & Robertson, H. D. *J. molec. Biol.* **90**, 191-214 (1974).
¹² Barrall, B. G. in *Procedures in Nucleic Acid Research* (eds Cantoni, G. L. & Davies, D. R.) **2**, 751-779 (Harper and Row, New York, 1971).
¹³ Hains, J. A., Reese, C. B. & Todd, A. R. *J. chem. Soc.*, 5281 (1967).
¹⁴ Pett, D. M., Vanaman, T. C. & Joklik, W. K. *Virology* **52**, 174-186 (1973).
¹⁵ Legon, S. *J. molec. Biol.* **106**, 37-53 (1976).
¹⁶ Rhodes, D. P. & Banerjee, A. K. *J. Virol.* **17**, 33-42 (1976).
¹⁷ Yang, N.-S., Manning, R. F. & Gage, L. P. *Cell* **7**, 339-347 (1976).
¹⁸ Lockard, R. E. & RajBhandary, U. L. *Cell* **9**, 747-760 (1976).
¹⁹ Shine, J. & Dalgarno, L. *Biochem. J.* **141**, 609-615 (1974).
²⁰ Van de Voorde, A., Contreras, R., Rogiers, R. & Fiers, W. *Cell* **9**, 117-120 (1976).
²¹ Hefli, E., Bishop, D. H. L., Dubin, D. T. & Stollar, V. *J. Virol.* **17**, 149-159 (1976).
²² Piper, P. W. & Clark, B. F. C. *Nature* **247**, 516-520 (1974).

letters to nature

Identification of cosmic γ -ray sources CG135+1 and CG189+1 with HII regions

ELEVEN unidentified γ -ray sources have been reported by the COS B group¹ one of which was previously known from the SAS 2 satellite². Here, I point out two likely associations of the new sources with giant HII regions. CG135+1 lies in the direction of the complex of HII regions, OB associations, molecular clouds and dust in the Cas OB6 region of the Perseus arm. An H α photograph and identification chart for this region is given in ref. 3. The HII region W3 (IC1795) is located at ($l = 133.7^\circ$, $b = +1.1^\circ$), which is only 0.7° outside the one standard deviation error box for CG135+1 ($l = 135.5^\circ \pm 1.0^\circ$, $b = 1.5^\circ \pm 1.0^\circ$)¹. The SAS 2 longitude distribution⁴ is consistent with the existence of this source.

W3 lies at a distance of ~ 3.1 kpc in the Perseus arm⁵, and contains 10 infrared sources⁶⁻⁷, four condensed centimetre sources W3(A,B,C,D) (ref. 8) and an OH maser source⁸. A spectrum of W3(A) from 408 MHz– 10^{14} Hz is given in ref. 9. Krügel and Mezger¹⁰ suggest that W3 is an O-star association in a very early evolutionary stage, the component W3(A) consisting of a compact dense shell of ionised gas surrounded by a dense shell of neutral gas (density $\sim 10^4$ cm⁻³). The total infrared luminosity is $\sim 10^6 L_\odot$ (ref. 11); 1-mm observations¹² suggest a column density for W3 of 10^{23} H₂ molecules cm⁻², and an H₂ mass of $10^3 M_\odot$.

The reported intensity of CG135+1 is $\sim 2 \times 10^{-6}$ photons (> 70 MeV) cm⁻² s⁻¹ (ref. 13). If the cosmic-ray proton intensity is the same in W3 as locally, we can estimate the intensity of π^0 -decay γ rays using the source function given by Stecker¹⁴ to be $\sim 1.6 \times 10^{-10}$ photons (> 70 MeV) cm⁻² s⁻¹. Bremsstrahlung interactions may increase this by up to a factor ~ 2 (ref. 15) if the e/p ratio is the same in the cosmic-radiation flux near W3 as locally. Thus if the identification is correct, a density of cosmic radiation $\sim 10^4 \times$ the local density is required; this might be plausible if the sources of cosmic rays were associated with the young stars in the HII regions. The particles could either have been accelerated in the stars themselves, or in an associated supernova or SNR (the SNR HB3 is nearby and may be physically connected with W3^{16,17}). The association of W3 with the γ -ray source, however, could be attributed to an unseen γ -ray pulsar (there is no known radio pulsar in this direction) or an unknown γ -ray emitter connected with young stellar populations.

I also suggest the identification of the source CG189+1 ($l = 189.0^\circ \pm 1.5^\circ$, $b = 1.0^\circ \pm 1.5^\circ$)¹ with the HII region NGC2175 at $l = 190^\circ$, $b = +0.5^\circ$. Aperture synthesis observations of this object, and a report of an associated CO cloud 20 pc in diameter, have been published¹⁸.

NGC2175 has the same 6-cm flux density (30 Jy) as W3(A)¹⁹ and should have a similar infrared intensity to W3(A) if the proportionality of infrared to centimetre continuum applies¹¹. The positional coincidence here is in fact better than for W3.

The γ -ray source is extended in latitude¹, and also includes the direction to the SNR IC 443 ($l = 189^\circ$, $b = -3.2^\circ$)²⁰ which is an X-ray source²¹. The distance to both IC 443 and NGC 1275 is about 1.5 kpc, so that the possibility of a physical connection is not ruled out, in which case the situation may provide a case similar to that for W3 and HB3 discussed above.

If γ -ray emission is a common feature of objects like W3, other objects with similar radio and infrared fluxes should be γ -ray sources. Of the objects listed in refs 7 and 11, only W75 lies within the four areas examined by the COS B group¹. W75 ($l = 81.9^\circ$, $b = 0.8^\circ$) lies in the very densely populated Cygnus region in which the visibility of individual sources is rather low. The nearest γ -ray source is CG78.1 ($l = 78.5^\circ \pm 0.5^\circ$, $b = 1.5^\circ \pm 0.5^\circ$)¹.

The Orion complex (M42) should be easily observable if the W3 identification is correct, since it is about five times as intense both at 30–300 μ m and at 2 cm (ref. 11), and is in a region away from the Galactic plane where point-source visibility should be good. But it lies outside the four regions examined by Hermesen *et al.*¹, M17 is also a good candidate, being brighter than W3 in both infrared and radio; however, it is in the galactic plane at longitude 14.7° , and hence harder to distinguish from the galactic background.

I thank Professor A. W. Wolfendale for useful discussions and the Royal Commission for the Exhibition of 1851 for the provision of a Fellowship.

A. W. STRONG

Physics Department,
University of Durham,
Durham, UK

Received 20 June; accepted 15 August 1977.

- Hermesen, W. *et al.* *12th ESLAB Symp.* (ed. Battick, B.) (ESTEC, Noordwijk, 1977).
- Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. *Astrophys. J.* **213**, 252-262 (1977).
- Georgelin, Y. M. & Georgelin, Y. P. *Astr. Astrophys.* **49**, 57-79 (1976).
- Fichtel, C. E., Kniffen, D. A. & Thompson, D. J. *12th ESLAB Symp.* (ed. Battick, B.) (ESTEC, Noordwijk, 1977).
- Reifenstein III, E. C., Wilson, T. L., Burke, B. F., Mezger, P. G. & Altenhoff, W. J. *Astr. Astrophys.* **4**, 357-377 (1970).
- Wynn-Williams, C. G., Becklin, E. E. & Neugebauer, G. *Mon. Not. R. astr. Soc.* **160**, 1-14 (1972).
- Dyck, H. M. & Simon, T. *Astrophys. J.* **211**, 421-428 (1977).
- Wynn-Williams, C. G. *Mon. Not. R. astr. Soc.* **151**, 397 (1971).
- Mezger, P. G. in *Far Infra-red Astronomy* (ed. Rowan-Robinson, M.) (Pergamon, Oxford, 1976).
- Krügel, E. & Mezger, P. G. *Astr. Astrophys.* **42**, 441-448 (1975).
- Harper, D. A. *Astrophys. J.* **192**, 557-571 (1974).
- Westbrook, W. E. *et al.* *Astrophys. J.* **209**, 94-101 (1976).
- Wills, R. D. *IAU Circ.* No. 2992 (1976).
- Stecker, F. W. *Astrophys. J.* **185**, 499-504 (1973).
- Schlickeiser, R. & Thielheim, K. O. *12th ESLAB Symp.* (ed. Battick, B.) (ESTEC, Noordwijk, 1977).
- Caswell, J. L. *Mon. Not. R. astr. Soc.* **136**, 11-18 (1967).
- Caswell, J. L. *Mon. Not. R. astr. Soc.* **177**, 601-616 (1976).
- Felli, M., Habing, H. J. & Israel, F. P. *Astr. Astrophys.* **59**, 43-52 (1977).
- Gebel, W. L. *Astrophys. J.* **153**, 743-760 (1968).
- Hill, I. E. *Mon. Not. R. astr. Soc.* **157**, 419-431 (1972).
- Winkler, P. F. & Clark, G. W. *Astrophys. J. Lett.* **L67-L69** (1974).

Tidal interactions and the massive halo hypothesis

THE idea that galaxies may be surrounded by large amounts of unseen material has become very popular. The arguments favouring such massive haloes are almost all based on the dynamical analysis of galaxies either individually or in groups, and they have been cogently assembled by Einasto *et al.*¹ and by Ostriker *et al.*². They concluded that galaxies may well be surrounded by large (~ 100 kpc) or very large (~ 0.5 Mpc) 'isothermal haloes' whose mass varies as the radius within which it is measured. (We take $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$). Their views have subsequently received support from Arecibo observations which found flat HI rotation curves to very large radii for several spiral galaxies³, and from analyses of the dynamics of new samples of binary galaxies⁴ and of groups of galaxies⁵. The most extreme form of the massive halo hypothesis postulates very large haloes which are the dominant constituent of the Universe, and in which the observed galaxy is little more than contaminating debris left over from the formation of the unseen object⁶. It is disquieting that there seems to be little chance of direct observation of this dark material, and this letter points out that dynamical constraints can be put on either the extent or the universality of galactic haloes.

When a cluster of galaxies collapses and comes to equilibrium, the structure of the subunits which were its progenitors is rapidly broken up by the violent relaxation of the cluster as a whole⁷; similarly, if the individual galaxies in a group are too large, they may interact strongly enough that the whole group is soon smoothed into a single unit. Richstone showed that even if the galaxies in the core of the Coma cluster had once had sufficiently large haloes to contain the missing mass, their mutual interactions would by now have consigned 90% of the material to an intergalactic pool⁸. Galaxy-galaxy interactions are weakened by high encounter velocities in Coma, however, and should be much stronger in small groups where the velocity dispersion is of the same order as the internal dispersion of the individual galaxies: if the tidal destruction of galaxy-halo units has gone most of the way to completion in Coma, it may have gone entirely to completion in many groups.

The consequences of tidal interactions for the massive halo hypothesis are most easily discussed for binary galaxies. If haloes of almost unlimited extent form around most galaxies, the mass distributions of the members of a binary will overlap, and they may well orbit within a common envelope. Tidal effects will then cause the loss of orbital energy into internal energy and the merging of the observed galaxies. S.D.M.W. has recently carried out full N-body simulations of the interaction between pairs of 250-particle 'galaxies', and has found that if two equilibrium 'galaxies' are placed in a bound orbit in which their mass distributions significantly overlap, they merge very rapidly (compare ref. 9). The details of these calculations will be published elsewhere, but this statement may be quantified by noting that, for the near isothermal ($\rho \sim r^{-2}$) 'galaxies' studied, the condition $r_{\text{peri}} \lesssim 3r_i$ was sufficient for the binary to merge completely within one initial orbital time of its first close approach, where r_{peri} is the pericentric distance of the initial orbit and r_i is the half-mass radius of the initial 'galaxies'.

The dire implications of this result for the hypothesis of universal massive haloes can be seen from Fig. 1 which plots the quantity $N = \Delta v / (2)^{1/2} \pi H_0 r_p$ against magnitude difference for the 59 statistically selected binaries in Turner's sample⁴ (Δv and r_p are the observed velocity difference and projected separation); note that the galaxies in most of the pairs are of comparable magnitude. On the assumption of similar randomly-oriented circular orbits the median value of N is an unbiased estimator of the number of orbits the binaries have executed in a Hubble time (taken as H_0^{-1}); it should be close to the median number of actual orbits for the observed sample. The median pair has apparently execu-

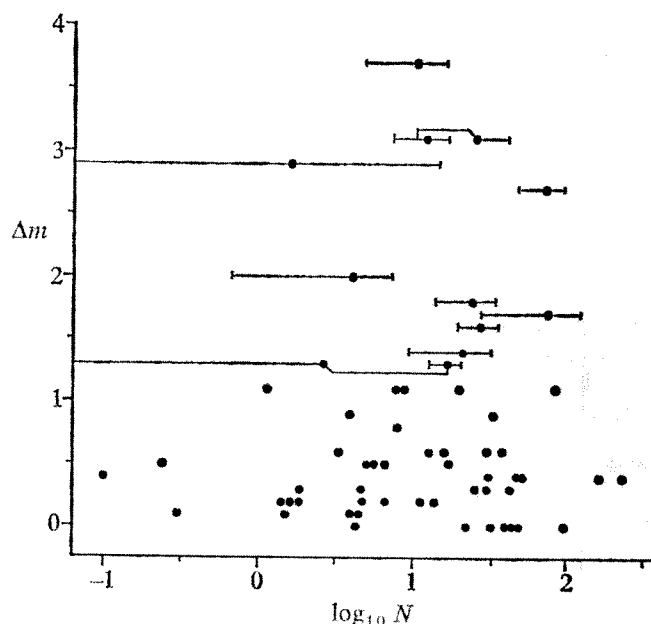


Fig. 1 The Δm - $\log N$ distribution for the 59 binaries in Turner's sample. Δm is the magnitude difference between the pair members and N is the estimated number of orbits completed in a Hubble time. Errors in $\log N$ have been calculated for representative points using Turner's quoted uncertainties in Δv and r_p .

ted 13 revolutions since the beginning of the Universe: either Turner's binaries do not overlap substantially anywhere in their orbits, or they will spiral together in the next few billion years. Discounting optical pairs there are about 139 true binaries in the Zwicky catalogue satisfying all Turner's selection criteria; if their median remaining lifetime is equal to their median apparent orbital time, then roughly $13 \times \frac{1}{2} \times 139 \approx 900$ similar pairs should have merged in the past. This number is 20% of all the galaxies in the correct magnitude range in the part of the sky surveyed; it is likely to be a severe underestimate of the total number of binaries which have merged in the past both because of the stringency of the sample selection criteria and because the initial distribution of separations may have been more strongly peaked at small radii than the distribution implicitly assumed above. We estimate that these effects could increase the percentage of merged binaries by a factor between two and five.

If one is unwilling to accept that most of Turner's pairs will merge during their next orbital period, one can ask how small their haloes need to be to avoid strong tidal interactions. The mean projected separation of the 59 pairs in the sample is 79 kpc. Under the assumption that the distribution of pair separations is a power law and is independent of the magnitudes of the constituent galaxies, the correction factor from mean projected separation to mean true separation is easily calculated. For all pairs with given magnitudes at a given distance,

$$\frac{\bar{r}_p}{r} = \frac{\int_0^\infty \int_0^{\pi/2} dr d\theta \sin\theta f(r \sin\theta, D) n(r) r \sin\theta}{\int_0^\infty \int_0^{\pi/2} dr d\theta \sin\theta f(r \sin\theta, D) n(r) r}$$

where θ is the angle between the separation vector and the line of sight, $n(r)$ is the distribution of separations, and $f(r \sin\theta, D)$ is the probability of inclusion as a function of projected separation at distance D . If $n(r) \propto r^{-\gamma}$ each integral can be separated into

an integral over $r \sin \theta$ which cancels out of the fraction, and an integral over θ which remains to give

$$\frac{\bar{r}_p}{\bar{r}} = \frac{\int_0^{\pi/2} d\theta \sin^\gamma \theta}{\int_0^{\pi/2} d\theta \sin^\gamma \theta} \quad (1)$$

This result can be expressed in terms of Γ -functions for all $\gamma > 0$. Since equation (1) does not involve D it holds for the sample as a whole, and the correction factor is independent of whether the averages are number or luminosity weighted. Turner obtains $\gamma = \frac{1}{2}$ for his sample, giving $\bar{r}_p/\bar{r} = 0.46$; the mean true separation of his pairs is, therefore, 173 kpc, and if they are not to be strongly interacting we require $r_i < 58$ kpc on average for the haloes of the constituent galaxies. This limit must be reduced if the orbits are sensibly eccentric since the condition for merging applies to the distance of closest approach, whereas most of the pairs will be seen near apocentre.

Although the above arguments are crude, they demonstrate that while certain dynamical considerations seem to require massive haloes, there are others which can severely constrain them.

We thank Jerry Ostriker, Martin Rees and Ed Turner for helpful discussions.

SIMON D. M. WHITE
NIGEL A. SHARP

*Institute of Astronomy,
Madingley Road,
Cambridge, UK*

Received 14 July; accepted 15 August 1977.

1. Einasto, J., Saar, E., Kaasik, A. & Chernin, A. *Nature* **252**, 111–112 (1974).
2. Ostriker, J. P., Peebles, P. J. E. & Yahil, A. *Astrophys. J.* **193**, L1–L4 (1974).
3. Krumm, N. & Salpeter, E. E. *Astr. & Astrophys.* **56**, 465–468 (1977).
4. Turner, E. L. *Astrophys. J.* **208**, 20–29 (1976); **208**, 304–316 (1976).
5. Gott, J. R. & Turner, E. L. *Astrophys. J.* **213**, 309–322 (1977).
6. Yahil, A. *Astrophys. J.* (in the press).
7. White, S. D. M. *Mon. Not. R. astr. Soc.* **177**, 717–733 (1976).
8. Richstone, D. O. *Astrophys. J.* **200**, 535–547 (1975); **204**, 642–648 (1976).
9. van Albada, T. S. & van Gorkom, J. H. *Astr. Astrophys.* **54**, 121–126 (1977).

Surface area and secondary nucleation theory

PRIMARY nucleation theory involves estimating the maximum free energy necessary to form a crystal of sufficient size so that further crystallisation results in a decrease in free energy. This free energy increase is caused by the creation of surfaces with the liquid or vapour. (The number of sides will depend on crystal symmetry and crystal habit. Here we will consider only the simplest six-sided crystal.) After a crystal of this minimum size has been formed it is often necessary to consider a secondary nucleation process which involves the maximum free energy necessary to crystallise a new layer on one of the existing flat crystal surfaces. This note reports an error in the estimation of surface area in the theory of secondary nucleation processes. This error makes little difference when applied to the crystallisation of small molecules but it can have significant results in the crystallisation of polymer molecules.

The secondary nucleation process controls the growth rate even after the primary crystal has grown macroscopically large. Again the cause of this free energy increase is assumed to be the creation of new surfaces between the crystal and the liquid (or vapour). It has become traditional in such nucleation processes to count only four of the five surfaces which such a secondary nucleus has exposed to the liquid. The argument being that the fifth surface simply replaces an equivalent surface on the parent crystal and

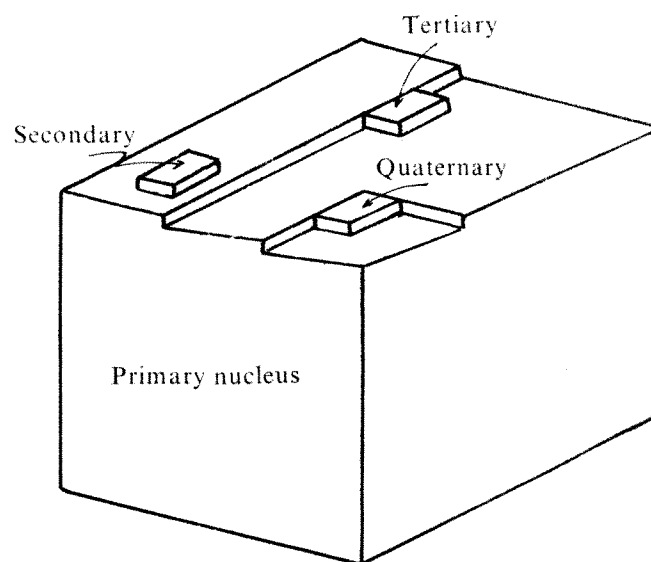


Fig. 1 Illustration of the four nucleation processes. Primary nucleation controls the number of crystals. Secondary nucleation controls the crystal growth rate. In polymer crystallisation tertiary nucleation controls the molecular fractionation process and quaternary nucleation may influence the morphology. In all of these processes the free energy of the nucleus is influenced not only by the surface energies but also by the change in conformational entropy of any attached cilia.

thus does not contribute to the increase in the total free energy. But the parent crystal may now be so large that any increase in the surface area of a secondary nucleus is insignificant in relation to the total free energy. The total free energy change reaches its maximum value at the critical size for the primary nucleus. It is not the change in total free energy which is important in secondary nucleation but rather the change in the free energy of the secondary nucleus considered as a small thermodynamic system¹ which controls secondary nucleation. A small thermodynamic system is one whose extensive properties (such as its free energy) may not be considered to be precise linear homogeneous functions of the thermodynamic variables. In such a system the surfaces have a dominant role in determining the free energy and one must consider all the surfaces which the small thermodynamic system makes with its surroundings. A coherent 'surface' which a secondary nucleus makes with the parent crystal may be considered to have zero surface energy but all surfaces which the nucleus makes with the liquid (or vapour) will contribute to the thermodynamic properties of the nucleus.

This distinction is not important when the crystallising entity is an atom or small molecule, since the secondary nucleus will always be one molecule in thickness, (or even if it is not, no difference in morphology can be distinguished) and the values of the surface energy derived from the kinetics of crystallisation have little intrinsic use except for comparison with other similarly derived data. But in considering the crystallisation of polymer molecules, the neglect of this fifth surface has led to the conclusion that such molecules should also crystallise in a monolayer of regularly folded chains^{2–4}.

Table 1 Number of crystal faces (six-sided crystal)

	Classical	Small system
Primary nucleation	6	6
Secondary nucleation	4	5
Tertiary nucleation	2	4
Quaternary nucleation	0	3

Although this assumption has led to reasonable results³ in explaining crystal growth kinetics and chain fold lengths, there are some experimental results which are incompatible. For example, large extended chain polyethylene crystals with very obvious striations parallel to the chain direction which should provide ideal secondary nuclei have been shown⁶ to be ineffective in promoting crystal growth when exposed to molten polyethylene substantially below its melting temperature. Furthermore, when the temperature is lowered sufficiently to allow crystallisation to proceed it does so by chain folding rather than extending along the existing secondary nucleation sites. These results can be explained by including all surfaces which a crystallising molecule makes with the liquid in the estimation of its free energy change⁷. Table 1 summarises the number of surfaces for each type of nucleation for both the classical and the small system approach.

Since tertiary nucleation and quaternary nucleation produce no detectable changes in atomic or small molecular crystallisation they need only be considered in polymer crystallisation where they are important in controlling morphology and molecular fractionation. In addition, in polymer crystallisation one may have surfaces exposed to the liquid which have attached portions of uncrystallised molecules. These attached molecules (sometimes called cilia) make important differences in the conformational entropy change and when added to the surface energies of the various nucleation processes make up the molecular nucleation process suggested by Wunderlich⁸.

The principal difference in applying this small system thermodynamic approach is the conclusion that secondary nucleation of polymer molecules need not result in a monolayer of regularly folded chain crystals but more likely will produce a multi-layer nucleus in which each molecule is folded into a more compact bundle with a random arrangement of folds.

P. H. LINDENMEYER

165 Lee Street,
Seattle,
Washington 98109

Received 5 July; accepted 8 August 1977.

¹ Hill, T. L. *Small Systems Thermodynamics* (Benjamin, New York, 1964).

² Lauritzen Jr, J. I. & Hoffman, J. D. *J. appl. Phys.* **44**, 4340 (1973).

³ Price, F. P. *J. Polymer Sci.* **42**, 29 (1960).

⁴ Frank, F. C. & Tosi, M. *Proc. R. Soc. A* **65**, 267 (1961).

⁵ Hoffman, J. D., Frolen, L. J., Ross, G. S. & Lauritzen, J. I. *J. Res. Nat. Bw. Std.* **79A**, 671 (1975).

⁶ Wunderlich, B., Mielello, L., Cormier, Davidson, T. & Snyder, G. J. *Macromol. Sci-Phys B1*, 485 (1967).

⁷ Lindenmeyer, P. H., Beumer, H. & Hosemann, R. (in preparation).

⁸ Wunderlich, B. *Macromolecular Physics—Crystallization, Nucleation, Growth and Annealing* (Academic, New York, 1976).

Crystal structure of alinite

ALITE, a major constituent¹ of Portland cement, is essentially tricalcium silicate $\text{Ca}_3(\text{SiO}_4)\text{O}$, which contains both $(\text{SiO}_4)^{4-}$ and free O^{2-} ions^{2,3}. It is of interest to cement technology to examine how chloride contamination of the raw materials affects the phases present in the resulting cement, and the extent to which the free oxide ions of tricalcium silicate are replaceable by Cl^- . While this replacement can be regarded as complete in the compound^{4,5} $\text{Ca}_3\text{SiO}_4\text{Cl}_2$, it is only partially accomplished in the new compound alinite, which is described in this note.

Single crystals of the new compound alinite were obtained from cement clinker containing additional Mg, Al and Cl. The crystals have diffraction symmetry $I4/mmm$, with $a = 10.4714$ and $c = 8.6171$ (16) Å. In the completed structure, in the space group $I4_2m$ (no. 121), with $R = 0.034$, based on 401 independent reflections, the unit cell contains two structural units of ideal formula $\text{Ca}_{11}(\text{Si}_{0.75}, \text{Al}_{0.25})_4\text{O}_{18}\text{Cl}$. The structure exhibits

Table 1 Alinite—atomic parameters

Atom	No. per cell	Fractional coords ($\times 10^4$) x/a y/b z/c	B_{iso} ($\text{\AA}^{-1} \times 10^3$)	Occupancy
Ca1	16	-6(4) 2609(1) 2914(1)	96	1.09
Ca2	2	0 0 0	39	1.12
Ca3	4	0 1/2 0	92	0.96
Si	8	2107(1) 2107(1) 5(6)	32	1.11
O1	16	1782(3) 3652(3) -101(9)	88	1.10
O2	8	1439(4) 1439(4) 1561(5)	48	1.10
O3	8	1450(5) 1450(5) 8489(6)	81	1.11
O4	4	0 1/2 1/4	82	1.09
Cl	2	0 0 1/2	168	1.05

Estimated standard deviations are given in parentheses.

isolated aluminosilicate tetrahedra within a framework consisting of three distinct types of Ca coordination polyhedra.

Intensity data ($\sin^2\theta \leq 0.28$) was obtained with an Enraf-Nonius CAD-4 diffractometer and $\text{MoK}\alpha$ radiation. Solution of the three-dimensional Patterson (vector) map by multiple superposition (the rhombus method⁶) yielded positions for five atoms, one of which was taken to be Si and assigned the f -curve for Si^{4+} , while the remainder were treated as Ca^{2+} . Three cycles of structure factor calculation (in $I4/mmm$) followed by the preparation of electron density maps not only revealed the positions of all of the remaining (oxygen) atoms, but also identified the

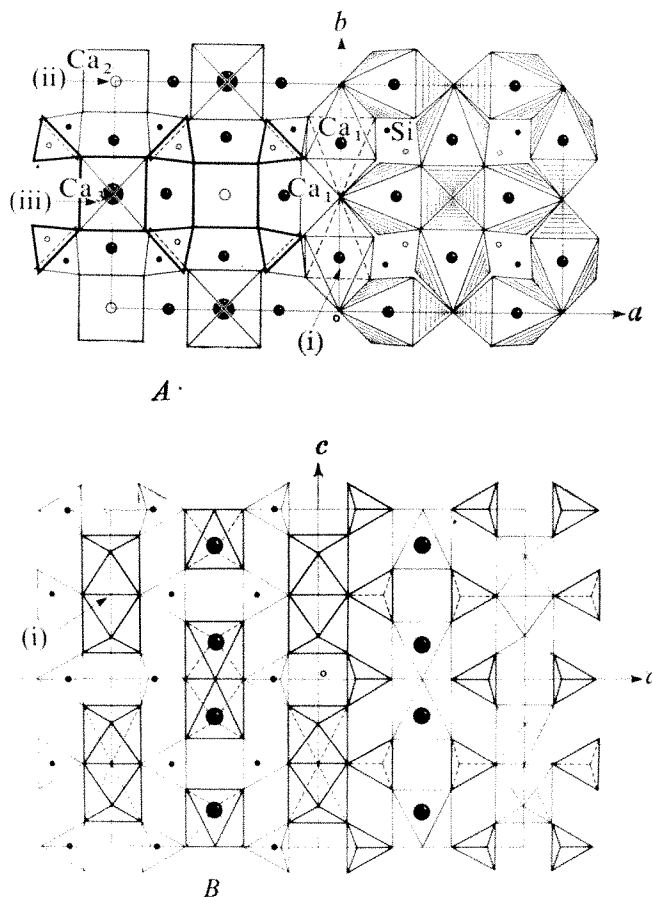


Fig. 1 The structure of alinite—general features. For clarity, representative Cl and O positions are indicated, but only as vertices of polyhedra. A, (001) projection, showing the three types of Ca coordination polyhedra and (Si, Al) tetrahedra. The smallest circles are (Si, Al) positions—● and ○ indicate $z \approx 0$ and $z \approx 0.5$ respectively. Intermediate ○ are Ca2. Intermediate ● represent overlapping pairs of Ca1 atoms ($z \approx 0.29$ and 0.71 at the cell edges; $z \approx 0.79$ and 0.21 elsewhere). The largest ● represent overlapping pairs of Ca3 atoms ($z = 0$ and 0.5). B, One layer of the structure parallel to (010) at $y \approx \frac{1}{4}$ showing pairs of face-sharing Ca1 octahedra and (Si, Al) tetrahedra—large and small circles represent Ca1 and (Si, Al) atoms respectively.

original atoms as 3 Ca, 1 Si and 1 Cl, completely independently of any analytical data. Block diagonal least squares refinement of atomic positions (throughout) with alternate refinement of occupancies and B_{150} led to $R = 0.09$. The space group $I\bar{4}2m$, however, at a similar stage of refinement gave $R = 0.08$, and finally after refinement of anisotropic temperature factors $R = 0.034$. The final parameters are given in Table 1.

Although the completed structure indicates the contents of the unit-cell as $\text{Ca}_{11}\text{Si}_4\text{O}_{18}\text{Cl}$, charge balance requires partial occupancy of the Si position by Al ($\text{Si}_{0.75}$, $\text{Al}_{0.25}$), to give the overall composition given above. The conditions in which the phase is obtained, and preliminary analytical data support this contention.

The most frequently occurring Ca coordination is that of Cal. For example, indicated by i in Fig. 1A, Cal (at 0.0, 0.26, 0.29) has as nearest neighbours: Cl(0,0, $\frac{1}{2}$); O1(0.13, 0.32, 0.49); O1' (-0.13, 0.32, 0.51); O2(0.14, 0.14, 0.16); O3(-0.15, 0.15, 0.15) and O4(0, $\frac{1}{2}$, $\frac{1}{2}$). Each such octahedron shares its Cl-O1-O1' face with a second similar octahedron, related to the first by a two-fold axis passing through the Cl atom and the mid-point of the common O1-O1' edge ((i)—Fig. 1B). Such pairs of Cal octahedra occur in four-fold clusters (eight octahedra in all) round the 0,0,z lattice row, sharing Cl-O2 and Cl-O3 edges. The O2 and O3 vertices of two such clusters provide the cubic coordination of Ca2 ((ii)—Fig. 1A). The Cal octahedron pair units are also further associated in pairs (four octahedra in all) centred on the 0, $\frac{1}{2}$, z lattice rows. In this case the O4(0, $\frac{1}{2}$, $\frac{1}{2}$) and O4'(0, $\frac{1}{2}$, $\frac{1}{2}$) common to both Cal octahedron pairs, together with the two shared O1-O1' edges (one from each pair) complete the octahedral coordination of Ca3(0, $\frac{1}{2}$, $\frac{1}{2}$) ((iii)—Fig. 1A). A similar arrangement of four Cal octahedra, but with the octahedron pair interaction at right angles to that given immediately above applies to Ca3 at 0, $\frac{1}{2}$, 0. Figure 1A and B also indicates the distribution of the alumino-silicate tetrahedra.

We thank H. F. W. Taylor and L. S. Dent Glasser for helpful discussions and the SRC for financial support (for V.V.I.).

V. V. ILYUKHIN*
N. N. NEVSKY
M. J. BICKBAU

*Institute of Crystallography,
Acad. Sci., Moscow, USSR*

R. A. HOWIE

*Department of Chemistry,
University of Aberdeen,
Meston Walk,
Old Aberdeen, UK*

Received 26 April; accepted 4 August 1977.

*Present address: Department of Chemistry, University of Aberdeen, UK.

¹ Taylor, H. F. W. *The Chemistry of Cements* (Academic, London and New York, 1964).

² Wyckoff, R. W. G. *Crystal Structures* 2nd edn, 4, 194 (Interscience, New York, London, Sydney and Toronto, 1968).

³ Jeffery, J. W. *Acta crystallogr.* 5, 26-35 (1952).

⁴ Czaya, R. Z. *anorg. allg. Chem.* 375, 124-7 (1970).

⁵ Treshnikov, E. N., Ilyukhin, V. V. & Belov, N. V. *Doklady Acad. Nauk. U.S.S.R.* 193, 1048-51 (1950).

⁶ Kuz'min, E. A. *et al. Systematic Analysis of the Patterson Function on the Basis of Crystal Symmetry* (Chabarovsk, 1974).

The nature of metastable phases of carbon

THE following forms of carbon are already known: rhombohedral and hexagonal graphite, diamond, lonsdailite, carbines, Ries crater carbon, and metallic carbon. We describe here new metastable phases in the carbon system^{1,2} which we detected while sintering highly dispersed diamond powders. Similar structures were also observed during explosive transformations of pyrographite³ and in natural diamonds⁴ that had undergone shock metamorphism. In all cases, the observed structures are close to those of the Ries crater carbon⁵.

Under pressures > 600 kbar, carbon passes into the metal phase (C3)^{6,7}. When sintering diamond powders, it may be shown that the pressures set up between particles within the

contact interaction zone, substantially exceed the mean hydrostatic pressures.

By using the Hertz solution on the interaction of two balls², it is possible to find the radius of the zone of contact of two balls⁸ (that approximate the diamond grains) of radius R

$$a = \left(\mathcal{F}_1 \frac{\mathcal{D}R}{2} \right)^{1/3} \quad (1)$$

where

$$\mathcal{D} = \frac{3}{2} \left(\frac{1 - \sigma^2}{E} \right)$$

Here \mathcal{F}_1 is the mean force applied to one sphere, σ is the Poisson coefficient, E is the Young modulus. The mean pressure set up on the area does not depend on the particle radius and is equal to

$$P = P_0^{1/3} \left(\frac{2}{\pi \mathcal{D}} \right)^{2/3} \quad (2)$$

where P_0 is the mean pressure applied to the chamber in sintering. In the case of diamond $\mathcal{D} \approx 1.5 \times 10^{-7} \text{ bar}^{-1}$, if we assume $E = 9.5 \cdot 10^{12} \text{ dyn cm}^{-2}$, $\sigma = 0.25$ (ref. 9). Then,

$$P = 2.7 \times 10^4 P_0^{1/3} \quad (3)$$

Thus, if the pressure is applied such that $P_0 = 30 \text{ kbar}$, the mean pressure within the contact zone will exceed 800 kbar. Therefore, when sintering the diamond powders, the appearance

Table 1 Calculation of interplanar spacing (d) of the sintered diamond powder

Starting $d(\text{\AA})$	600 °C $d(\text{\AA})$	800 °C $d(\text{\AA})$	Notes
3.326	3.43	3.338	Graphite
3.010	3.06	—	Ries carbon
—	2.29	—	Ries carbon
—	2.12	—	Graphite
2.045	2.07	2.062	Diamond
1.965	—	—	Lonsdailite
—	1.52	—	Lonsdailite
1.291	—	—	Ries carbon
1.258	1.27	1.27	Diamond
1.234	—	1.23	Graphite
1.074	1.08	1.078	Diamond
0.900	0.894	0.894	Diamond
0.818	0.818	0.820	Diamond

of metallic carbon may be expected. As the metallic carbon may prove to be a metastable phase, it can pass into other forms of carbon. The additional phases that are observed in sintering diamond powders may turn out to be intermediate states between the metallic carbon, on one side, and the diamond and graphite, on the other. With this in mind, an experiment was carried out, in which the diamond powder having the mean radius of particles of about 2 μm was subjected to compression at the mean pressure of 59 kbar and the temperature of 1,200 °C. Then the diamond powder was annealed in a hydrogen medium at temperatures of 600 °C and 800 °C for 1 h. The initial and the annealed powder were subjected to X-ray examination, according to the Debye method. The examination results are presented in Table 1.

From Table 1, it seems that many interplanar spacings (d) are recorded in the initial powder, this may be attributed to different forms of carbon. In annealing, the system passes to a state where only diamond and graphite are present.

Table 2 Results from the pulse-heated diamond powder

Intensity (relative units)	<i>d</i> (Å)	<i>d</i> diamond (Å)	Note
100	2.05	2.06	
10	1.746		β-carbine (1.750)
5	1.523		β-carbine (1.550)
80	1.260	1.261	
70	1.073	1.075	
60	0.891	0.892	
70	0.818	0.818	

In the case of the pulse radiant heating of the diamond powder up to 2,000 °C and its rapid cooling (which obviates the annealing of metastable states), additional phases also appear, as shown by Table 2.

From Table 2, it seems that the carbines should occupy the intermediate place between diamond and graphite.

One possibility is that, aside from the known crystallographic forms in the carbon system (like the boron nitride system), there exist transient metastable states whose stability is determined by the potential barrier height. Transition from one form of carbon to another may occur, either via those metastable states or, possibly, directly.

The following seems probable: the Ries crater carbon, which has formed on the place where a gigantic meteorite has fallen, is a product of the inverse transition of metallic carbon (or the phases that are close to it) into a state which is more stable under atmospheric pressure.

B. V. DERJAGUIN
D. V. FEDOSEEV
V. P. VARNIN
S. P. VNUKOV

*The Institute of Physical Chemistry of the USSR,
Academy of Sciences,
Leninsky prospekt 31
Moscow V-71, USSR*

Received 12 July; accepted 26 July 1977.

- Fedoseev, D. V., Varnin, V. P., Vnukov, S. P. & Derjaguin, B. V. *Dokl. Akad. Nauk SSSR* 227, 639 (1976).
- Fedoseev, D. V., Derjaguin, B. V., Varnin, S. P., Vnukov, S. P., Teremetskaya, I. G. & Polyanskaya, N. D. *Dokl. Akad. Nauk SSSR* 227, 371 (1976).
- Litvinova, V. A. & Cherkinskaya, K. T. *Chem. Solid.* (in Russian) N3, 159 (1976).
- Vishnevskij, S. A. & Polchik, N. A. *Geol. Geophys.* 1, 67 (1975).
- Goresy, A. & Donney, G. *Science* 161, 363 (1968).
- Bundy, F. J. *chem. Phys.* 41, 3809 (1964).
- Vereshchyagin, L. F., Yakovlev, E. N., Vinogradov, E. V., Saput, V. T. & Stepanova, G. N. *Zhur. eksp. teor. Fiz. Lett.* 17, 433 (1973).
- Landau, L. D. & Lifshits, E. M. *Theory of elasticity* (in Russian) (Nauka, Moscow, 1965).

Etching on large single crystals of stearic acid

ETCHING is one of the most useful ways of directly observing dislocations in a crystal¹. Many studies have been made of dislocations in organic crystals, such as aromatic hydrocarbons², but no reports on the dislocations in fatty acid crystals have been published because no large single crystal could be obtained. Recently we have succeeded in obtaining large single crystals of the saturated fatty acid stearic acid from solutions of organic solvents³. We describe here attempts to etch single stearic acid crystals using organic solvents. Optical and transmission electron micrographs of etch pits reveal several novel features and give an apparent correlation between the pits and the dislocations in stearic acid crystals.

Crystals of stearic acid were grown from a solution in benzene; the average size was about 5 × 5 mm² in area and 1 mm thick. The crystal form was monoclinic (B-form)³. The long chain of the stearic acid molecule was directed towards the (001) direction.

Etch pits were optically observed on the as-grown (001), *ab*, surfaces (Fig. 1). Attempts to etch other crystal surfaces were

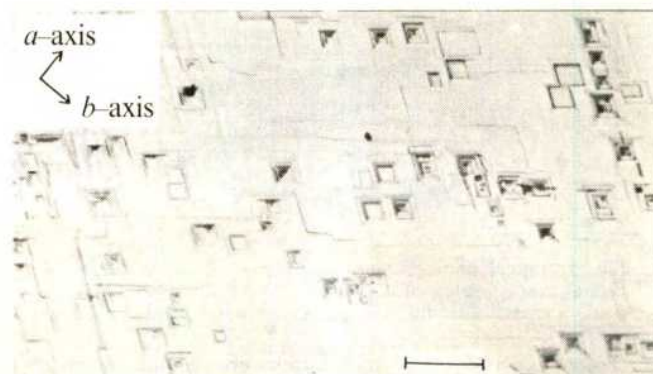
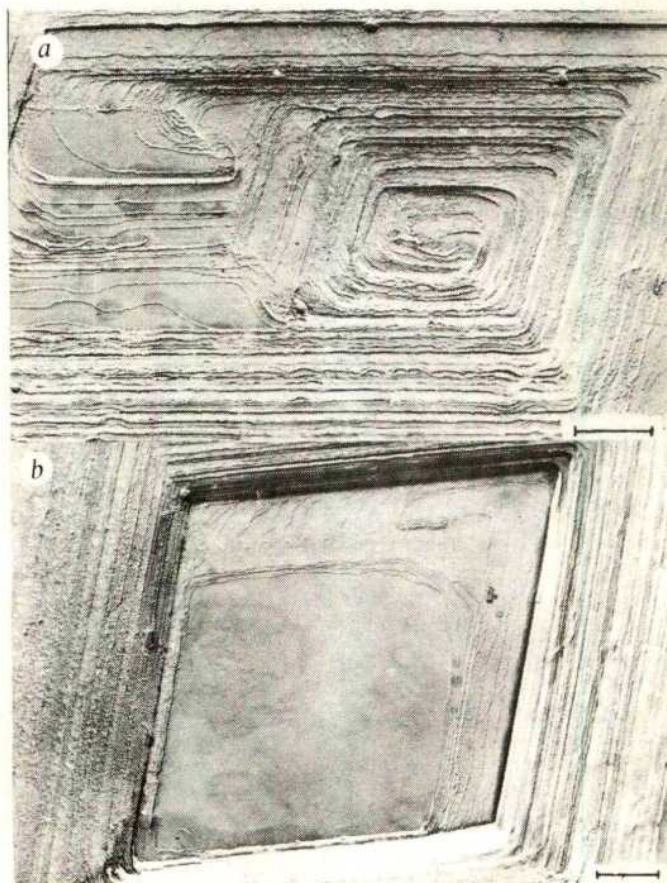


Fig. 1 Optical micrograph of etch pits. Each direction was determined by the X-ray analysis. Etching for 5 s in acetone at 18 °C. Scale bar, 30 μm.

unsuccessful. A variety of organic solvents were examined as etchants; acetic acid produced ill-defined patterns, while acetone, ethanol, benzene, carbon tetrachloride, and chloroform produced well defined etch pit patterns of lozenge shape. In every case, except for acetic acid, pits of about 10 × 10 μm² in area were easily produced within a few seconds at 18 °C all over the surface. As shown in Fig. 1, almost all the pits have a common crystallographic direction. The two angles of lozenge-shaped pits were 75° and 105°; the values were the same as those of the crystal habit. The density of the pits was of the order of 10⁴ cm⁻². Two types of pits can be seen from Fig. 1. One is of the inverted pyramidal shape, and the other is of the inverted truncated pyramidal shape.

Fig. 2 Electron micrographs of two typical pits. Etchant, acetone. The first stage of replica was made by shadowing at 30° with germanium and by supporting normally with carbon. *a*, Two adjacent pyramidal pits; *b*, a flat-bottomed pit. Scale bar, 2 μm.



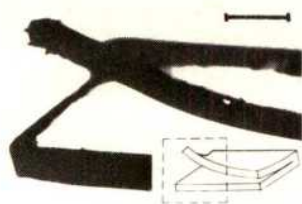
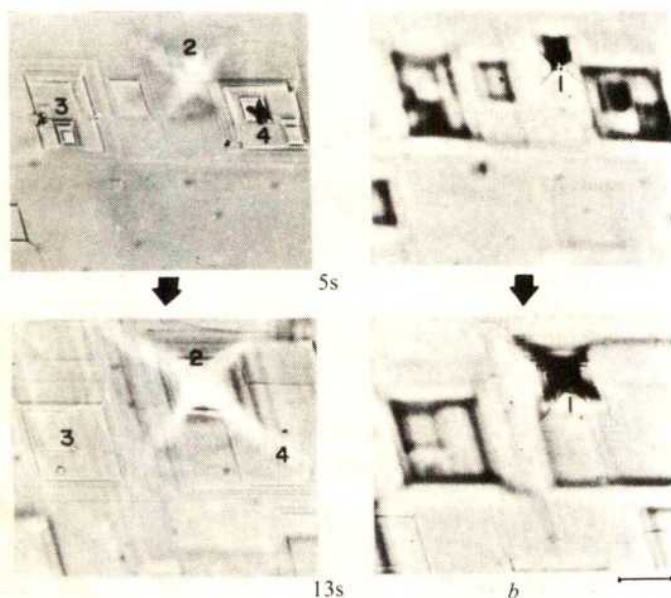


Fig. 3 Optical photograph showing part of the whole cleaved crystal and a sketch of a cleaved crystal. The lower plate, not bent, was used for simultaneous observation. Scale bar, 1 mm.

Transmission electron micrographs around the centre of an etch pit give molecular features of the etching process in detail. As shown in Fig. 2a the pit has several spiral steps whose terminations can be distinguished clearly at the specific points on the pit. These spiral steps also indicate a possible singular centre of the dissolution. Thus the pyramidal pit takes its position at the emergent end of the dislocation perpendicular to the (001) plane. Similar dislocations in stearic acid crystals have already been predicted from the observation of growth spirals on the as-grown (001) surfaces¹. In Fig. 2b, the flat area, without any etching step, of the pit-bottom can be thought to be molecularly flat. The molecularly flat-bottomed pit can be interpreted by the movement of the dislocation during the etching process; if the dislocation moves out of a pit formed during the first etching, further etching must enlarge the pit in the lateral direction so that the initial pit becomes flat-bottomed. At the same time, the movement of the dislocation would produce a new pyramidal pit at the final position of the dislocation.

If a dislocation, penetrating through the crystal from one surface to another, does not move during the etching process, the pit in direct correlation to such dislocation would grow to be a deeper and larger pyramidal one, while the flat-bottomed pit becomes wider but not deeper, as the etching proceeds. The penetrating dislocation is expected to appear at the cleavage surface. To verify this, the etch pits on the as-grown surface as well as the cleavage surface, (001) plane, of a single crystal were observed simultaneously (Figs 3 and 4). Figure 4 shows the

Fig. 4 Optical micrographs of the etch pits of pyramidal shape, no. 1 and 2, and of flat-bottomed shape, no. 3 and 4. *a*, taken in focus on the as-grown surface. *b*, taken in focus on the cleaved surface. Etchant, acetone at 18 °C. Scale bar 100 μ m.



developments of the two types of pits during their etching process. Two pits of no. 3 and 4, with a rather small-flat bottom at the etching time of 5 s, become large typical flat-bottomed pits in 13 s after the start of etching. On the other hand, each pyramidal pit on the as-grown and cleavage surface becomes deeper and larger, and then each centre of the two pits approaches each other in the projected *ab* plane. This approach can be easily understood because the dislocation line is inclined to the direction of the illumination. Thus it is obvious that two pyramidal pits observed in Fig. 4 lie at the two ends of the dislocation penetrating through the crystal.

KIYOTAKA SATO
MASAKAZU OKADA

Department of Food Technology and Chemistry,
Faculty of Fisheries and Animal Husbandry,
Hiroshima University,
Fukuyama, Japan

Received 4 July; accepted 25 July 1977.

¹ Amelinckx, S. in *The Direct Observations of Dislocations*, Solid State Physics suppl. 6 (eds Ehrenreich, H., Seitz, F. & Turnbull, D.) 15-50 (Academic, New York and London, 1964).

² Corke, N. T., Kawada, A. A. & Sherwood, J. N. *Nature* 213, 62-63 (1967).

³ Sato, K. & Okada, M. *J. Crystal Growth* (in the press).

⁴ Verma, A. R. & Reynolds, P. *Proc. Phys. Soc.* B66, 414-420 (1953).

Can sunspots influence our weather?

THE discovery of sunspots, in particular the 11-yr cycle, has led to many attempts to detect correlations between the sunspot number and weather changes in the Earth's troposphere¹. The past decade has seen a renewed interest based on modern data and the concepts of solar-terrestrial physics².

Claims have been made about correlations between H α -flares, crossings of the solar magnetic sector boundaries or sunspots, and tropospheric effects such as low pressure troughs at the 500-mbar level³, the influx of stratospheric air masses⁴, thunderstorm activity⁵, geoelectric potential⁶ or rainfall⁷. There is no general agreement about the reality of these effects because it is difficult to prove their significance and no acceptable cause and event mechanism has been proposed. Many authors have considered the invisible part of the active Sun as a possible source of these effects because the electromagnetic and corpuscular components of the radiation from the active Sun generate spectacular disturbances in the atmosphere above 60 km. The energies involved in bringing these disturbances about, are only a minute fraction of the solar constant and are, therefore, unlikely to have any direct influence on the troposphere⁸.

King *et al.*⁹ have shown that during the last 11-yr solar cycle, the sunspots tended to appear on preferred longitudes on the Sun so that a period of about 27.5 d, the mean period of the solar rotation, is clearly visible in the sunspot number. This is in accord with observations of coronal transients¹⁰, high-speed solar wind flows¹¹ and the sector structure of the interplanetary magnetic field¹². King *et al.*⁹ claimed furthermore, that the solar rotation effect in sunspots is correlated with changes in the height of the tropospheric 500-mbar level. The global pressure field displays a wave-like pattern with waves of zonal wave numbers 0, 1 and 2 having maximum amplitudes at higher latitudes⁹.

Although this observation has not been confirmed it suggests a mechanism which may be associated with the visible spectrum of the solar radiation. Radiation from the active Sun, which behaves like a rotating beacon, may influence the transmission of the Earth's atmosphere so that the radiation reaching the ground varies with the same period of ~ 27 d. At least one piece of evidence indicates that the solar constant *S* observed on the ground exhibits such a periodicity with an amplitude of about 0.1% (ref. 13).

In order to estimate the reaction of the atmosphere to such a small variation of S , consider an isothermal atmosphere with scale height $H = 8$ km. The mean energy input per unit mass within the atmosphere due to a change $\Delta S = 10^{-3} (1-A) S \simeq 1 \text{ W m}^{-2}$ is given by

$$\Delta Q = \frac{\Delta S}{4\rho_0 H} = 2.3 \times 10^{-5} \text{ W kg}^{-1} \quad (1)$$

where $A = 0.3$ (the albedo), $\rho_0 = 1.3 \text{ kg m}^{-3}$ (the density at the ground) and $S = 1.37 \text{ kW m}^{-2}$ (the solar constant).

From the first law of thermodynamics and the hydrostatic law, I estimate a pressure amplitude of

$$|\Delta p| = \frac{\Delta S}{4\omega H} = 10 \text{ N m}^{-2} = 0.1 \text{ mbar} \quad (2)$$

if $\omega = 2\pi/27 \text{ d} = 2.7 \times 10^{-6} \text{ s}^{-1}$. The solar radiation primarily heats the ground, from where it is transported into the atmosphere through turbulent heat exchange. That transport mechanism is mainly effective within the lowest part of the troposphere so that ΔQ from equation (1) is probably an underestimation for that part of the atmosphere. The land-ocean coverage is such that global-scale waves including mainly those of zonal wave numbers 0, 1 and 2 are generated and propagate upwards. The prevailing, but fluctuating, westerly wind modifies the horizontal and vertical structure of the planetary waves, and it is clear that the only waves that will be detectable within the meteorological noise by a statistical analysis are those which have vertical wave lengths of the order of, or greater than, the scale height. It can be shown that these waves are of the Rossby-Haurwitz type having maximum amplitudes at higher latitudes.

Detailed calculations (to be published elsewhere) assume a heat source distribution on the ground which reflects the land-ocean distribution¹⁴. The heat input into the atmosphere by turbulent transport was derived from a diffusion equation¹⁴. Linear tidal theory was then used¹⁵ to determine the meridional and the height structure of the individual wave modes in the presence of a constant prevailing westerly wind of the order 10 m s^{-1} . Only those wave modes were considered which have vertical wave lengths of the order or greater than one scale height. It was shown that for zonal wave number $m = 2$, these waves consist of eastward- and westward propagating waves of the Rossby-Haurwitz type. For waves of wavenumber $m = 1$, only westward propagating waves of that type exist, so that the waves of zonal wavenumber two probably will dominate. Maximum pressure amplitudes of $|\Delta p| = 0.3 \text{ mbar}$ near $\pm 60^\circ$ latitude and on the ground are predicted for waves of wavenumber two. Waves of such small amplitude are at the limit of detectability. But they may be found in analyses such as that carried out by King *et al.*⁹.

The mechanism suggested here may be the primary source of different types of Sun-weather relationship. If short-lived meteorological phenomena such as those associated with solar flares or sector boundary crossings are generated by active longitudes on the Sun, the 11-yr solar-cycle effects may be the result of an 11-yr variation of the amplitude of the 27-d forcing function.

H. VOLLAND

Radioastronomical Institute,
University of Bonn, 5300 Bonn, West Germany

Received 30 May; accepted 10 August 1977.

- 1 Flohn, H. *Arch. Met. Geophys. Bioklim.* A111, 303-329 (1951).
- 2 Meadows, A. J. *Nature* 256, 95-97 (1975).
- 3 Wilcox, J. M. *J. Atmos. Sci.* 31, 581-588 (1974).
- 4 Reiter, R. *J. Geophys. Res.* 78, 6167-6172 (1973).
- 5 Markson, R. *Pure appl. Geophys.* 84, 161-202 (1971).
- 6 Park, C. G. *Geophys. Res. Lett.* 3, 475-478 (1976).
- 7 King, J. W. *Nature* 245, 443-446 (1973).
- 8 Willis, D. M. *J. Atmos. terr. Phys.* 38, 685-698 (1976).
- 9 King, J. W., Slater, A. J., Stevens, A. D., Smith, P. A. & Willis, D. M. *J. Atmos. terr. Phys.* (in the press).

- 10 Hildner, E. *et al. Solar Phys.* 48, 127-135 (1976).
- 11 Gosling, J. T., Ashridge, J. R., Bame, S. J. & Feldman, W. C. *J. Geophys. Res.* (in the press).
- 12 Svalgaard, L. & Wilcox, J. M. *Solar Phys.* 41, 461-475 (1975).
- 13 Foukal, P. V., Mack, P. E. & Vernazza, J. E. *Astrophys. J.* 215, 952-959 (1977).
- 14 Kertiz, W. *Nachr. Akad. Wiss. Math.-Phys.* 6, 145-166 (1956).
- 15 Volland, H. & Mayr, H. G. *Rev. Geophys. Space Phys.* 15, 203-226 (1977).

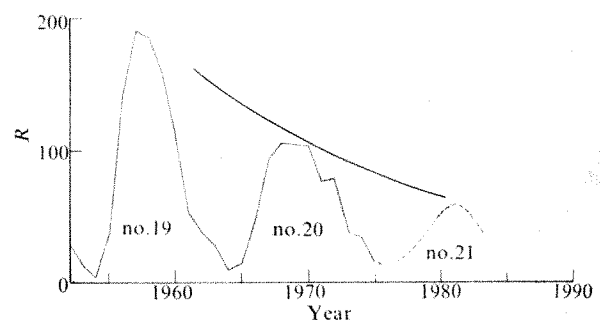
Equatorial solar rotation and its relation to climatic changes

THE equatorial solar rotation speed, estimated from observations made over the past 10 years of the sunspot longitudinal motions over the solar disk, has shown a tendency to increase as relative sunspot numbers decreased. During these years, covering most of solar cycle no. 20 (1965-76), the magnitude of the solar rotation speed averaged annually showed a good inverse correlation with the annual relative sunspot numbers. I suggest here that this variation of the equatorial solar rotation speed may be responsible for the Earth's present unusual climatic conditions.

The highest speed was observed for 1976, when the observed sunspot activity was at its lowest since 1967, except for 1974, and was approximately equal to that estimated for the year 1643, just before the beginning of the Maunder Minimum (1645-1715), during which very few sunspots were reported. According to Eddy *et al.*¹, there is evidence that, during the Maunder Minimum, the speed of the equatorial solar rotation, averaged annually, was larger by 4% or more than that now observed². They have based this assumption on analysis of the sunspot observations made by Scheiner, Hevelius and others in the seventeenth century (see ref. 3 for details). Although the causal relationship between the degree of the development of solar activity and the pattern of this solar rotation speed was not known, their result suggested that, during the period when this solar activity was higher relative to the averaged one, this degree of development, as indicated by the relative sunspot numbers, for instance, must be lower than that observed for any other period when this speed is relatively low². Furthermore, from their results, it could be predicted that the severe climatic conditions experienced during the Maunder Minimum might have been related to the strong depression of solar activity and hence with the increase of the solar rotation speed.

Based on observations of the motion of sunspot groups over the solar disk, Howard⁴ has measured the yearly variation of the mean speed of the solar rotation as a

Fig. 1 The relative sunspot numbers R since 1952. These numbers, often called the Wolf numbers R , are published annually by the Zurich observatory, based on the reports on sunspot numbers from observatories throughout the world. These numbers are counted by using the formula, $R = k(10g + f)$, where k , g and f are, respectively, the seeing factor, the numbers of sunspot groups and of individual sunspots. A curve predicting these numbers for the cycle no. 21 is indicated by a dotted line (see text). A heavy line shows the general trend of the sunspot activity for the three cycles nos 19-21.



function of the solar latitude for 1967–76. He found that this speed has gradually increased throughout this period until 1976, when the sunspot activity was at minimum. Furthermore, it should be noted that the speeds observed during the above period were all relatively greater than those estimated by Ward² for the earlier period.

Although it is not clear whether this tendency will continue for the coming cycle no. 21, it seems likely that the whole pattern will still show the same tendency as observed in the last cycle no. 20 (1965–76), even though it might slow down slightly during the rising portion of this cycle (1977–79). As suggested in Fig. 1, the relative sunspot number for the next maximum is expected to be 20 or so less than that observed in the last cycle, because the solar activity as a whole has tended to decrease since cycle no. 19 as predicted from both the observed progression of the 80-year quasi-periodic variation of the relative sunspot numbers and the relatively slower start of the present cycle no. 21.

In order to discover whether there is a causal relationship between the sunspot activity and the solar rotation speed, the values of this speed at the equator observed by Howard⁴ have been plotted as a function of the relative sunspot numbers for the years from 1967 to 1976. Figure 2 clearly shows that the magnitude of this equatorial rotation speed has an inverse relationship with these sunspot numbers. Thus, the solar activity as a whole tends to decrease with the increase of the speed of the equatorial solar rotation during the above period.

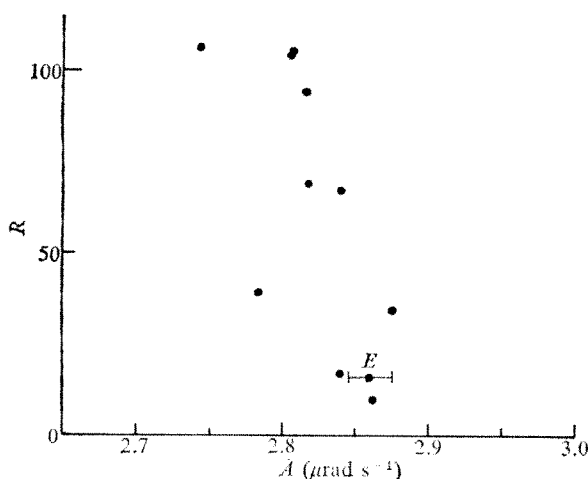


Fig. 2 The equatorial angular velocity A of the solar rotation as a function of the relative sunspot numbers R for the years from 1967 to 1976 (●, average velocity). (The data are from ref. 4.) E with error bar shows the velocity for 1643, two years before the beginning of the Maunder Minimum¹.

If the sunspot numbers during cycle no. 21 vary as predicted in Fig. 1, the tendency shown in Fig. 2 would continue for this cycle because of the lower sunspot activity; that is, it is possible that this speed will increase to the values observed during the Maunder Minimum.

In Fig. 2, the equatorial rotation speed for 1643, estimated by Eddy *et al.*¹, is also plotted as a comparison with the present trend. By examining the observed speed for 1643, the range of this speed has been estimated and shown with an error bar in Fig. 2. The mean value of this speed is almost the same as that observed in 1976, though the relative sunspot numbers are slightly different. Hence, it seems possible that the general trend of the variation of the equatorial rotation speed will be maintained during the new cycle if the relative sunspot numbers for these years behave as predicted in Fig. 1.

Climatic conditions were very severe during the Maunder Minimum as recorded in London, for instance^{3,5}. When the observations from this period are compared with the corresponding climatic conditions, it becomes possible to conclude that the rare severe depression of the solar activity (and the consequent speeding up of the equatorial solar rotation) was responsible for this little ice age for 70 years or more⁶.

Figures 1 and 2 and the discussion on the Maunder Minimum, indicate that the Earth's present unusual climate might be associated with the decreasing trend of the sunspot activity (Fig. 1) and the related increase in the equatorial solar rotation speed—the speed at the equator of the solar differential rotation. It therefore seems likely that the present unusual climatic conditions will remain as long as the solar activity continues to decrease.

When we consider both the photospheric magnetic field and the high-speed flow of the solar wind during the past few years, it is clear that both the large-scale coronal hole and the apparent width of this high-speed flow region have been steadily growing^{6,7}. Their steady growth strongly indicates that the decrease of the solar activity is likely to continue for the coming cycles, continued observations would give us important clues as to the Earth's future, in particular, to its climate. It may also be possible to study possible causal relationship between the Earth's climate and the solar activity variations in the long term.

KUNITOMO SAKURAI

Institute of Physics,
Kanagawa University,
Yokohama 221, Japan

Received 26 April; accepted 2 August 1977.

¹ Eddy, J. A., Gilman, P. A. & Trotter, D. E. *Solar Phys.* **46**, 3–14 (1976).

² Ward, F. *Astrophys. J.* **145**, 416–425 (1966).

³ Eddy, J. A. *Scient. Am.* **230**, No. 5, 80–92 (1977).

⁴ Howard, R. *Astrophys. J. Lett.* **210**, L159–L161 (1976).

⁵ Eddy, J. A. *Science* **192**, 1189–1202 (1976).

⁶ Bame, S. J., Asbridge, J. R., Feldman, W. C. & Gosling, J. T. *Astrophys. J.* **207**, 977–980 (1976).

⁷ Levine, R. H., Altschuler, M. D. & Harvey, J. W. *J. geophys. Res.* **82**, 1061–1065 (1977).

Reproductive effort and life history strategy of the Aldabran giant tortoise

THE Aldabra giant tortoise, *Geochelone gigantea* Schweigger, has a large body size (19–120 kg)¹, long life span (65–90 yr)², late maturity (females 17–23 yr)^{3,4} and iteroparity³. The three isolated island populations of different densities on Aldabra show dissimilarity in their reproductive parameters (for example mean clutch size, mean egg weight, proportion of mature females breeding and the number of clutches per female per year) and in the variation of these parameters from year to year through annual changes in rainfall³. Moreover, their reproductive response to these changes in rainfall is rapid; they have a relatively high maximum (potential) rate of natural increase (maximum clutch size 28)³ and mature females in the high density population are half the weight of those in the low density area^{2,4}. I have postulated that the reproductive effort would differ between the different density populations and that this effort would change in relation to changes in food availability. This contention is supported by the results reported here.

I examine here the apportionment of the assimilated resources towards reproduction, growth and respiration and the selective pressures which give rise to this allocation of energy. Assimilation (A) is defined as the sum of production due to reproduction (P_r), production due to growth (P_g) and respiration (R). After the onset of sexual maturity, growth is very slow (ref. 2 and my unpublished results) and for the purposes of this work is taken to be zero. Thus $P_r/A \approx P_r/(R + P_r)$ and this ratio will be used to measure reproductive effort.

Table 1 Reproductive effort compared with population density and years

	Very low density (5 per ha)* 1976	Low density (7 per ha)* 1975	High density (27 per ha)* 1975	1976
Mean weight of mature female (g)†	48,300	46,700	19,300	
Reproductive output (g per ♀ per yr)‡	4,204	2,303	91	303
Reproductive output (kJ per ♀ per yr) §	29,786	16,317	645	2,147
Annual respiration (kJ per ♀ per yr) ¶	470,323	455,905	201,864	
Reproductive effort (%)	6.0	3.4	0.3	1.0

*Figures taken from ref. 1.

†Maturity was determined by dissection³.‡Mean clutch size × mean egg weight × mean clutches per ♀ per yr³.

§Calculated using energy value per g dry weight of eggs excluding the shell (my work with N. K. Jenkins, in preparation).

¶Assuming 7 h activity (feeding) and 17 h inactivity (sleeping) per 24 h (from observation) (my unpublished results). Oxygen consumption in ml per animal per h (ref. 11) is 140.8 (individual weight^{0.97}) for active animals and 45.47 (individual weight^{0.82}) for inactive animals. Calorific equivalent for 1 ml of oxygen is 5.05 where respiratory quotient is unity¹¹.

||Mean reproductive output/(mean reproductive output + mean annual respiration).

This index is subject to several objections as it does not take into account the energy expended in growth in the annual energy budget (albeit low), finding or digging a suitable place for egg deposition, possible differences in metabolic rates between individuals from the three populations or possible variations in the energy costs of egg production. The care invested in a single offspring was estimated by the average egg weight as there is no parental care after oviposition. Recognising these difficulties, I feel that the methodology is adequate to allow discussion of the differences in reproductive effort and the life history adaptations of this large terrestrial herbivore.

Table 1 shows the reproductive effort compared with population density during the 2 yr of study. Reproductive effort increased as the population density decreased. The rainfall increased by approximately 40% in 1976 compared with 1975 and there was a greater proportional increase in reproductive effort in the high density population than in the low density population through an increase in food resource availability.

One would not expect any increase in reproductive effort by the low density population if it was food resource limited. But both the very low density and low density populations are expanding^{1,3}. The apparently greater sensitivity of the high density population to changes in food resource availability (as represented by rainfall) suggests they are closer to the carrying capacity (that is available food) than in the low density population. The low density population is mainly limited, probably much below the level set by food availability, by high levels of nest destruction by nesting females caused by the severe lack of suitable nesting areas³. Indeed the increase in reproductive effort by the low density area females occurred through increasing mean egg weight, whereas in the high density area mean clutch size, mean egg weight and the proportion of mature females breeding all increased (ref. 3 and my work with M. Coe, in preparation). This was the only available 'tactic' left to the low density area females in order to increase their effort, as any increase in nest numbers would involve the destruction of an equivalent number of existing nests and an increase in clutch size would have a similar effect. In both years of study the number of nests remained the same³.

In the high density population mortality is caused mainly by thermal stress¹. Individual tortoises must spend the middle of each day (approximately 1000–1600 h) shading⁴ and, as the distance between shade points particularly on the coast (with a high density population during the rains (ref. 4 and my unpublished results)) prohibits movement by exposing the animal to probable death, their feeding activities are confined to a specific area. Only during short (2–3 d) and infrequent periods of dull cloudy weather can the tortoise move more freely. When the Sun abruptly returns after these periods large numbers of recently dead animals are found well away from any available shade (my unpublished results). It can be predicted that as the rainy season gives way to the dry season (with far fewer dull weather periods) food availability will decrease. Eventually the local population within these shade-focussed feeding areas must exceed the food availability and it is at this time of year that tortoises will move away from these areas in large numbers, presumably risking death through thermal stress rather than slow but inevitable malnutrition. This, mainly circum-

stantial, argument together with other observations on reduced body size^{1,2}, retarded growth^{2,3}, very low recruitment³, pre-ovulatory follicular atresia³ and the ratio of estimated to predicted production⁵ supports the suggestion that the population in the high density area is at the carrying capacity determined by food.

The low density population is in a relatively less food-limited environment and shows early maturity and large clutches of large eggs; whereas the high density population, living in a more food-limited environment, shows delayed maturity and small clutches of small eggs. Most of these parameters could be predicted if the position of the populations with respect to resources were the primary determinants of the evolution of life history characters. The advent of large eggs in the low density population, however, was not predicted as the eggs in the very low density and high density populations were both small³. Large eggs produce large hatchlings which survive better than small hatchlings³. Giant tortoise hatchlings for the first 2 or 3 yr of life feed in areas where, because of the terrain, animals older than 4–5 yr cannot feed (my unpublished results). It was argued³ that these very young individuals had greater difficulty in finding suitable food in the low density area than in the other two areas and therefore the larger eggs were incorporated into the life history adaptation. Evidence for this hypothesis was inferred from observing that hatchlings in the high density area grew more than those in the low density area during the first year³.

It becomes apparent when considering the high maximum rate of natural increase, the quick response to changes in food availability, the larger hatchlings in the less-limited low density area and the lower mean weight of mature adults in the high density area that the Aldabran giant tortoise populations highlight the inadequate and over-simple concept of *r* and *K* selection⁶. The low mean weight of mature females in the high density area together with the ability to make a rapid and potentially large increase in reproduction may have been selected in order to effect a compromise between parental risk (in making resources available to the offspring) and food availability. By having a smaller body size, a greater proportion of any increase in food availability can be used in reproduction without increasing, unacceptably, the risk to a parent's lifetime reproductive success.

A recent paper⁷ on the problem of 'temporally dynamic reproductive strategies' viewed in terms of the conceptual framework of *r* and *K* selection concluded that mean *r*-*K* continuum⁸ positions have relatively little explanatory value where a measure of year-to-year variation in reproductive effort is more relevant in considering life history strategies and selective pressures. Moreover it was suggested that the proportion of mature females breeding can be used to infer variation in population reproductive effort. In the case of the giant tortoise the increase in reproductive effort in the low density population would be overlooked using this index because it occurred through an increase in mean egg weight and not an increase in breeding females. Additionally in the high density population not only did the proportion of mature females breeding increase but mean clutch size and mean egg weight also increased. Other work^{9,10} has emphasised the need to consider the environmental dimensions essential to provide complete understanding of the evolution of life histories. It is hoped that this

example within a species clearly demonstrates some of these dimensions.

I would like to thank Nigel Jenkins, Sarah Randolph, Tony Russo, Ken Simkiss and Peter Woodall for comments; Ilkka Hanski for critical dialogue, the Natural Environment Research Council for funds, the Royal Society for facilities and Linda Wallan for typing

IAN R. SWINGLAND

*Animal Ecology Research Group,
Department of Zoology,
University of Oxford,
South Parks Road, Oxford, UK*

Received 18 May, accepted 27 July 1977

- ¹ Bourn, D. & Coe, M. *Phil Trans R Soc, Lond B* (in the press)
- ² Grubb, P. *Phil Trans R Soc, Lond B* 260 327-372 (1971)
- ³ Swingland, I. R. & Coe, M. *Phil Trans R Soc, Lond B* (in the press)
- ⁴ Frazer, J. thesis, Oxford Univ (1971)
- ⁵ Coe, M., Bourn, D. & Swingland, I. R. *Phil Trans R Soc, B* (in the press)
- ⁶ MacArthur, R. H. & Wilson, E. O. *The Theory of Island Biogeography* (Princeton University Press, Princeton, 1976)
- ⁷ Nichols, H. D., Conley, W., Batt, B. & Tipton, A. R. *Am Nat* 110, 995-1005 (1976)
- ⁸ Pianka, E. R. *Am Nat* 104 592-597 (1970)
- ⁹ Wilbur, H. M., Tinkle, D. W. & Collins, J. P. *Am Nat* 108, 805-817 (1974)
- ¹⁰ Wilbur, H. M. *Am Nat* 111, 43-68 (1977)
- ¹¹ Hughes, G. M., Gaymer, R., Moore, M. & Wonkes, A. J. *J exp Biol* 55, 651-665 (1971)

Behaviour of adult and immature male baboons during inter-group encounters

SOCIAL interaction between groups of Old World monkeys varies from peaceful mingling to marked aggression¹⁻³. One constant feature, however, concerns the differing behaviour of immature and adult males; and these differences have been related to differences in male reproductive success at different stages of the life cycle. Immature and young adult males in multi-male groups of baboons and macaques frequently approach and interact with individuals outside their natal group, sometimes transferring from one group to another⁴⁻⁷. Two suggested causes of such group transfer are inability to gain access to oestrous females in the natal group and attraction to oestrous females in other groups⁸. In contrast, adult males—both in species with one-male social units and in species with multi-male groups—are frequently aggressive during intergroup encounters, often chasing or 'herding' members of their own group away from other groups^{7,9,10}. Herding usually involves high-ranking adult males who are also the individuals who copulate most often^{10,11}. Males are most likely to herd adult females, but rarely herd females who are lactating¹⁰⁻¹². (Although occasional herding in gelada baboon one-male units has been reported¹³) Herding has been suggested both to mobilise individuals before group movement⁷ and to maintain a male's access to oestrous females over time¹⁰. To investigate the function of herding we observed the intergroup encounters of one multi-male group of baboons for 15 months. Our observations suggest not only that herding is correlated with differences in male reproductive success but also that it is related to changes in the female reproductive cycle and to long-term bonds between particular males and females.

We studied the intergroup encounters of a group of southern African baboons (*Papio cynocephalus ursinus*) consisting of two adult males, eight adult females, and between 14 and 20 immatures. During 15 months' observation, this group came to within 500 m of another group on 174 occasions, with such 'encounters' lasting from 15 min to 16 h (the latter occurred when two groups occupied adjacent sleeping sites). Although the study group regularly came into contact with three different groups, and behaved similarly during encounters with each, 77% of all encounters involved one group of approximately 50 individuals.

During 44% (77) of all encounters, one or more of the group's five subadult males (estimated age 44-58 months)

sat at the boundary between groups. In addition, during the 4 months preceding their emigration, at least one of the subadults exchanged affiliative gestures with the other group's subadult males and/or copulated with the other group's females on 59% (13) of all encounters. Copulation occurred when oestrous females from the neighbouring group approached the study group and presented to both adult and immature males. Although the females were occasionally herded away by the adult males of their group, such males never threatened males in the study group^{10,14}. More often, males in the study group were able to respond to the females' presents. Immature males and the subordinate adult male were more likely than the alpha male to receive presents, and subadult males were the most likely to respond to them (Table 1). When four of the subadult males emigrated from the study group, they did so following an encounter which included copulations, and they left in the company of oestrous females from the neighbouring group.

Concurrently, more than 90% (159) of all encounters were accompanied by at least one 'bout' of herding by an adult male: separate bouts were defined as any instance of herding separated by an interval of at least 1 min when herding did not occur. The alpha male, who accounted for the largest single proportion of copulations¹⁵ and fertilised six of the eight females who became pregnant¹⁶, was responsible for 85% of all herding bouts. Immature males, in contrast, accounted for less than 1% of all observed herding bouts.

Table 1 Proportion of presents by oestrous females from neighbouring groups to which males responded, either by touching, grooming, or copulating with the presenting female

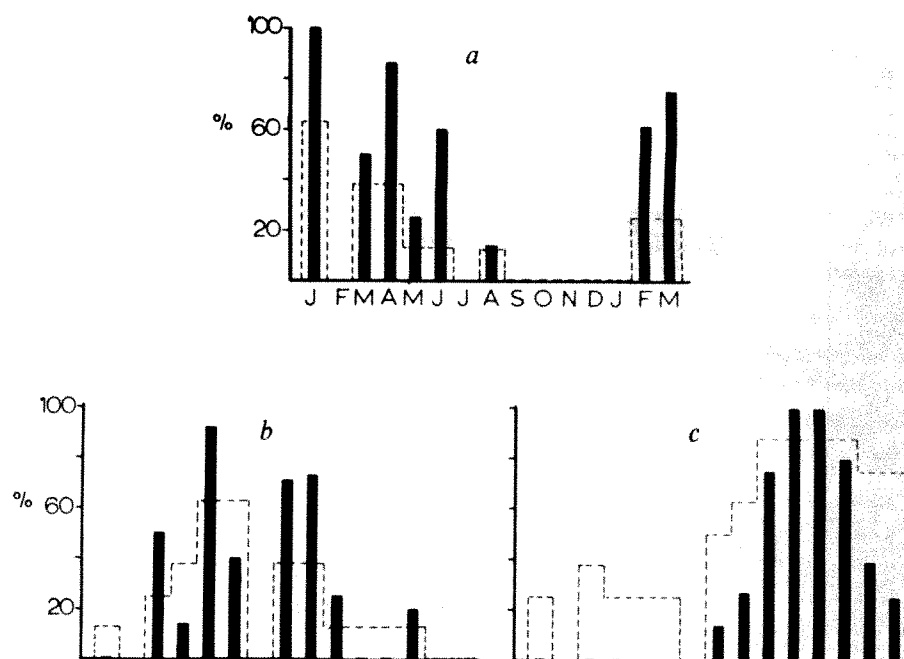
		No. of presents received	% Presents responded to
Adult ♂♂	Alpha	0	—
	subordinate	20	10
Subadult ♂♂	A	10	40
	C	8	50
	K	4	50
	S	3	67
	L	17	59

During herding, the male involved chased one or more female around and through the group, repeatedly giving loud double-barks^{10,17}. Of 200 herding bouts, 81% (161) began with the chasing of one adult female. During the course of herding, however, other animals were often supplanted by the male's approach and they, too, could be herded. Herding seemed to cause great anxiety among females and immatures, who screamed, raised their tails, and fear-grinned as they were herded. The net effect of herding was to drive most adult females, and many immatures near them, away from the other group. Herding was therefore both an aggressive, disruptive behaviour and a behaviour which tended to promote intragroup cohesion by maintaining spatial separation between groups.

Throughout the study, there was a variable number of females each month either sexually cycling, pregnant, or lactating. Taking data from the 150 occasions when the alpha male 'chose' only one female for herding, Fig. 1 shows that this male herded sexually cycling females more often, and lactating females less often, than would have been expected had females been 'chosen' at random.

The amount of herding received by each female thus depended on both the ranging behaviour of her own and other groups, and her reproductive state when intergroup encounters occurred. It was also possible to test for individual preferences on the part of each adult male and to

Fig. 1 Proportion of all adult females who were either (a) sexually cycling, (b) pregnant or (c) lactating each month (broken lines), compared with the proportion of the alpha male's 'choices' for herding each month that involved females in each of these reproductive states (solid histograms). Sexually cycling females were chosen more often than expected in eight out of eight possible months, pregnant females more often in six out of 11 months, and lactating females more often in 2 out of 13 months



compare these preferences with the males' long-term bonds with particular adult females.

Examination of social interactions between the two adult males and eight adult females throughout the reproductive cycle indicated that, whereas each male formed sexual consortships with a number of females at the height of oestrus¹⁶, males and females also showed individual preferences which persisted during pregnancy and lactation¹⁸. Table 2 presents data on the relative frequency with which each adult male herded each female in different reproductive states. Results indicate that, in all reproductive states, males were most likely to herd females with whom they had the closest long-term social bonds. Each male's preference for one specific individual often overrode preference for females in particular reproductive states, as, for example, when the alpha male repeatedly herded his preferred partner even though she was lactating and there were other, sexually cycling females in the group.

Disparities in the behaviour of adult and immature males may be related to the males' differing access to oestrous females at different times during their life cycle. Adult males in the study group accounted for the great majority of copulations—particularly around the time of ovulation—

and seemed to limit the access of immature males to sexually receptive females¹⁵. As a result, immature males may have been more attracted than adult males to sexually receptive females in neighbouring groups.

The alpha male's 'choice' of particular females for herding had two important consequences. First, in both the short and the long term, it served potentially to increase his reproductive success by isolating from males in other groups (1) those females who were about to ovulate, and (2) those females in whom the male would invest the greatest direct social support in future. Male herding of sexually receptive females was therefore both behaviourally and functionally similar to the herding of oestrous females in intragroup interactions observed in a previous study of a multi-male group of baboons¹⁴. Second, male choice of particular females seemed to minimise the potentially disruptive effect of herding. As noted earlier, each male was most likely to herd the female with whom he had the closest long-term social bond. Consequently, herding most often involved those individuals who were most likely to follow agonistic behaviour with relaxed proximity or grooming¹⁸. Males therefore seemed to be able to maintain separation between groups with a minimum of social disruption.

Table 2 Ranked frequencies with which each female was 'chosen' for herding by each adult male in each reproductive state

♀	Alpha male (n = 150)				♀	Subordinate male (n = 11)			
	Sexually cycling	Pregnant	Lactating	Sum of ranks		Sexually cycling	Pregnant	Lactating	Sum of ranks
H	2	7	6.5	15.5	H	5	5.5	5.5	16.0
W	6.5	6	4	16.5	W*	1	1	1.5	3.5
L*	1	1	2	4.0	L	5	5.5	5.5	16.0
S	6.5	5	6.5	18.0	S	5	5.5	5.5	16.0
LP	6.5	4	3	13.5	LP	5	5.5	5.5	16.0
PM	4	8	1	13.0	PM	5	5.5	1.5	12.0
M	3	2	5	10.0	M	5	2	5.5	12.5
P	6.5	3	8	17.5	P	5	5.5	5.5	16.0

n, Number of herdings involving each male and one female.

*Each male's 'preferred' female^{16,18}.

Relative frequencies with which each female was chosen by each male in each reproductive state calculated as follows: for example, for the alpha male and female H during H's sexual cycle

$$\frac{\text{total times alpha male 'chose' } \text{♀H during } \text{♀H's sex. cyc.}}{\text{total times alpha male 'chose' any female during } \text{♀H's sex. cyc.}}$$

For every female, at least five bouts of herding occurred during each reproductive state.

Finally, the pattern of male herding reported here for a multi-male group of baboons bears several similarities to herding by hamadryas baboon males in one-male units¹⁰. Results thus support the suggestion^{10,11} that there may be marked similarities in the pattern of social relationships within one-male and multi-male social groups despite the more obvious differences in group size and composition.

We thank A. H. Harcourt, C. Packer, and especially Professor R. A. Hinde for advice and criticism during the preparation of this paper.

D. L. CHENEY*
R. M. SEYFARTH*

Sub-department of Animal Behaviour,
Madingley, Cambridge, UK

Received 9 March; accepted 26 July 1977.

*Present address Rockefeller University Field Research Center, Millbrook, New York 12545.

- 1 Altmann, S. A. & Altmann, J. *Baboon Ecology* (University of Chicago Press, Chicago, 1970).
- 2 Kawanaka, K. *Primates* 14, 113–159 (1973).
- 3 Struhsaker, T. T. *Behaviour* 29, 83–121 (1967).
- 4 Hausfater, G. *Folia primat.* 18, 78–107 (1972).
- 5 Vessey, S. H. *Folia primat.* 8, 228–239 (1968).
- 6 Boelkins, R. C. & Wilson, A. P. *Primates* 13, 125–140 (1972).
- 7 Buskirk, W. H., Buskirk, R. E. & Hamilton, W. J. *Folia primat.* 22, 9–18 (1974).
- 8 Packer, C., thesis, Univ. Sussex (1977).
- 9 Saayman, G. S. *Folia primat.* 15, 36–57 (1971).
- 10 Kummer, H. *Social Organization of Hamadryas Baboons* (University of Chicago Press, Chicago, 1968).
- 11 Stoltz, L. P. & Saayman, G. S. *Ann. Transvaal Mus.* 26, 99–143 (1970).
- 12 Boese, G. K. In *Socioecology and Psychology of Primates* (ed. R. Tuttle) 205–230 (Mouton, The Hague, 1975).
- 13 Dunbar, R. & Dunbar, P. *Contrib. primat.* 6, (S. Karger, Basel, 1975).
- 14 Hausfater, G. *Contrib. primat.* 7 (S. Karger, Basel, 1975).
- 15 Cheney, D. L. *Anim. Behav.* (in the press).
- 16 Seyfarth, R. M. *Behaviour* (in the press).
- 17 Hall, K. R. L. *Proc. zool. soc. Lond.* 139, 283–327 (1962).
- 18 Seyfarth, R. M., *Behaviour* (in the press).
- 19 Seyfarth, R. M. *J. theor. Biol.* 65, 671–698 (1977).

Nitrate reductase-deficient mutants in barley

MUTANTS have long been used to study genetic and biochemical regulation of metabolic pathways in microorganisms. Often it is a relatively simple task to screen large bacterial populations for an individual lacking a specific enzyme or enzyme product. Defined enzyme mutants would also be valuable research tools for plant geneticists and biochemists. Unfortunately, very few biochemically defined mutants have been selected in higher plants, principally because of the genetic and biochemical complexity, and the long generation time of higher plants¹. But, these obstacles could be overcome in plants by inducing high mutation frequencies and developing effective techniques for selecting biochemically defined mutants. High mutation frequencies can be readily induced in some higher plant species by sodium azide, a potent base substitution mutagen in barley^{2,3}. Nitrate reductase, which has an important function in higher plants⁴, has been extensively studied in bacteria and fungi⁵, is highly regulated^{6,7}, and has several catalytic activities including NAD(P)H, FMN₂ and reduced methyl viologen nitrate reductase activities, and NAD(P)H cytochrome *c* reductase activity⁸. Nitrate reductase is therefore an excellent enzyme in which defined mutants in higher plants can be selected, studied, and compared with microorganisms. We describe here a procedure for the induction and selection of nitrate reductase-deficient mutants in higher plants, and the partial characterisation of three nitrate reductase-deficient mutants in barley.

Nitrate reductase-deficient mutants were induced with sodium azide in 'Steptoe' barley (*Hordeum vulgare* L.) as described by Kleinhofs *et al.*^{9,10}. Steptoe barley seeds were hydrated at 0–2 °C overnight and then germinated at 20 °C in vigorously aerated water for 8 h. The seeds were then placed in an aerated solution containing 1 mM sodium azide and 0.1 M phosphate buffer (pH 3.0) for 2 h. The treated seeds were washed for 30 min, blotted and dried in a fume hood. The dry seeds were planted in an

isolated field plot. The crop produced (M₂ seed) was harvested in bulk.

The nitrate reductase-deficient mutants were selected from the M₂ segregating population by qualitatively estimating *in vivo* nitrate reductase activity. Individual 7-d-old control or segregating M₂ seedlings with normal green appearance were screened for the presence of nitrate reductase activity by using a modification of the *in vivo* assay described by Finke *et al.*⁹. Approximately 8 cm of the primary leaf was excised, cut in half, and placed in a test tube containing 4 ml of an incubation medium containing 0.1 M potassium nitrate, 0.1 M potassium phosphate (pH 7.0) and 0.01% Tergitol NPX. The leaf tissue was then vacuum infiltrated and incubated in the dark at 30 °C; nitrite was then determined as previously described⁹. Nitrate reductase-deficient seedlings were transplanted to soil and fertilised with ammonium sulphate.

Nine nitrate reductase-deficient seedlings were selected from approximately 6,000 M₂ seedlings tested. None of the 5,000 control seedlings tested was nitrate reductase-deficient. Five of the nine nitrate reductase-deficient selections were sterile or otherwise failed to produce seed. Progeny tests of the four selections confirmed that three (Az12, Az13, Az23) are nitrate reductase-deficient (Table 1).

These mutants have low *in vivo* nitrate reductase activity and low *in vitro* nitrate reductase activity when either NADH or FMN₂ is used as an electron donor. All three mutants are also similar in that they have elevated leaf nitrate levels and nitrite reductase activities. But they differ in that Az12 and Az23 have low cytochrome *c* reductase activities whereas Az13 has cytochrome *c* reductase activity similar to the control. Similar results were obtained with field grown plants except that the nitrate content of the leaves of the nitrate reductase mutant plants was much greater than in the control (Table 2). In spite of the low nitrate reductase activity, the mutant selections seem to be as vigorous as, and morphologically indistinguishable from, the control.

The higher levels of nitrate in the mutants is presumably the result of the lowered capacity of the mutants to reduce nitrate. The high levels of nitrite reductase in the mutants (Table 1) may be caused by the high levels of nitrate inducer, although the levels of nitrate in the control are well above those generally considered necessary to give optimum induction of nitrate reductase¹⁰. Alternatively, the mutants may have lower levels of reduced nitrogen available for the repression of nitrite reductase synthesis. Ammonium and other forms of reduced nitrogen have been reported to repress nitrate reductase synthesis⁶ and would be expected to have a similar effect on nitrate reductase.

The different levels of cytochrome *c* reductase activity in the mutants seems to be the result of different muta-

Table 1 Nitrate, nitrite, cytochrome *c* reductase activities, and nitrate content of 7-d-old nitrate reductase mutant and control seedlings

Selection	NRA			NiRA	CRA	NO ₃ ⁻
	NADH	FMN ₂	<i>in vivo</i>			
Az12	0.4	0.0	0.2	315	43	1,806
Az13	0.4	0.0	0.3	355	242	1,657
Az23	0.3	0.0	0.3	370	36	2,492
Control	27.2	28.8	4.8	197	194	1,246

Nitrate reductase (NRA) was assayed *in vitro* with NADH as electron donor (ref. 15), with FMN₂ (ref. 16) and assayed *in vivo* (ref. 9). Nitrite reductase (NiRA) and nitrate content were determined as in ref. 15 and cytochrome *c* reductase (CRA) as in ref. 12. Enzyme activities are expressed as $\mu\text{mol per g of fresh weight per h}$ and nitrate content as $\mu\text{g NO}_3^- \text{N per g fresh weight}$. Plants were grown in a growth chamber at 20 °C and continuous light for 7 d as described by Warner and Kleinhofs¹⁵.

Table 2 Nitrate, nitrite and cytochrome *c* reductase activities, and nitrate content of field grown (heading stage) nitrate reductase mutants and control plants

Selection	NRA		NiRA	CRA	NO ₃ ⁻
	NADH	<i>in vivo</i>			
Az12	0.3	0.8	604	114	1,007
Az13	0.4	1.6	625	288	1,135
Control	19.6	10.0	226	174	41

tional events. It has previously been reported that nitrate reductase also has cytochrome *c* reductase activity^{11,12}. These results suggest that Az12 and Az23 mutations inactivate or prevent the synthesis of the nitrate reductase enzyme including the cytochrome *c* reductase component whereas Az13 seems to have the cytochrome *c* reductase portion of the enzyme still functional. Wray and Filner¹² reported that the cytochrome *c* reductase associated with barley nitrate reductase is induced by nitrate whereas two other cytochrome *c* reductases are constitutive and not associated with nitrate reductase. When grown on an ammonium nitrogen source, Az12, Az13 and the control have very low levels of nitrate reductase and approximately the same cytochrome *c* reductase activities (Table 3).

Table 3 Induction of nitrate and cytochrome *c* reductase activities in 12-d-old nitrate reductase mutant and control seedlings

Selection	N source	NRA	CRA	NO ₃ ⁻
Az12	NO ₃ ⁻	0.3	29	2,506
	NH ₄ ⁺	0.0	40	13
Az13	NO ₃ ⁻	0.3	343	2,579
	NH ₄ ⁺	0.1	46	19
Control	NO ₃ ⁻	30.5	300	1,545
	NH ₄ ⁺	1.2	49	9

When induced with nitrate, Az13 and control seedlings have a nitrate-inducible cytochrome *c* reductase which is presumably associated with the nitrate reductase protein. Based on work with nitrate reductase mutants in *Aspergillus nidulans*¹³ and *Neurospora crassa*¹⁴, it may be possible to predict the nature of the barley nitrate reductase mutants. Mutations in the regulator loci in fungi^{11,12}, result in the loss of all of the nitrate reductase catalytic functions and of nitrite reductase as well. Since all of the barley nitrate reductase mutants contain nitrite reductase then these mutations seem not to be in the regulator locus unless nitrate and nitrite reductases are controlled by separate regulator loci in higher plants. Fungi nitrate reductase structural mutants generally have nitrite reductase activity and in some cases nitrate-inducible cytochrome *c* reductase activity^{11,12}. Therefore, Az12 and Az13 are probably nitrate reductase structural mutants since both mutants lack the nitrate reductase catalytic functions and have nitrite reductase activity.

In addition to mutations in the nitrate reductase structural and regulator loci, mutations in the loci controlling the molybdenum component of nitrate reductase also result in the loss of nitrate reductase activity¹⁵. In *Aspergillus* the molybdenum component mutants have nitrite reductase activity and either a constitutive or an inducible cytochrome *c* reductase activity¹⁴. The mutants having the inducible cytochrome *c* reductase usually have low levels of nitrate reductase similar to Az13 (Table 3). Therefore, Az13 may be either a nitrate reductase structural mutant or a molybdenum component mutant assuming the barley and *Aspergillus* systems are analogous. Whether these nitrate reductase mutants are in the structural protein for nitrate reductase or some other

locus is not known. More mutants and subsequent analyses are needed to adequately resolve this question.

The induction and selection of defined enzyme mutants such as the nitrate reductase mutants described, should be of considerable help in resolving genetic and biochemical control mechanisms of metabolic pathways in higher plants. Nitrate reductase has received considerable attention in the past 20 years but the work in higher plants has been severely handicapped by the lack of sufficient genetic diversity. Nitrate reductase-deficient mutants have previously been identified in *Arabidopsis thaliana*¹⁷ and now in barley. These mutants are an important first step for studying the genetic regulation of nitrate reductase in higher plants.

This investigation was supported in part by the Graduate School research funds from the US NIH Biomedical Sciences support grant and College of Agriculture Research Center, Washington State University.

R. L. WARNER
C. J. LIN
A. KLEINHOF

Department of Agronomy and Soils,
Washington State University,
Pullman, Washington 99164

Received 6 May; accepted 18 July 1977.

- Rice, T. B. & Carlson, P. S. *Rev. Pl. Physiol.* 26, 279–308 (1975).
- Nilan, R. A., Sideris, E. G., Kleinhofs, A., Sander, C. & Konzak, C. F. *Mutat. Res.* 17, 142–144 (1973).
- Kleinhofs, A. & Smith, J. A. *Mutat. Res.* 41, 233–240 (1976).
- Beevers, L. & Hageman, R. H., *A. Rev. Pl. Physiol.* 20, 495–522 (1969).
- Payne, W. G. *Bact. Rev.* 37, 409–452 (1973).
- Hewitt, E. J. *A. Rev. Pl. Physiol.* 26, 73–100 (1975).
- Kleinhofs, A., Sander, C., Nilan, R. A., & Konzak, C. F., in *Polyplody and Induced Mutation in Plant Breeding* 195–199 (IAEA, Vienna, 1974).
- Kleinhofs, A., Warner, R. L., Lin, C. J., Meuhlbauer, F. J. & Nilan, R. A. *Mutat. Res.* (in the press).
- Finke, R. L., Warner, R. L. & Muzik, T. J. *Weed Sci.* 25, 18–22 (1977).
- Beevers, L., Schrader, L. E., Flesher, D. & Hageman, R. H., *Pl. Physiol.* 40, 691–698 (1965).
- Sorger, G. J. *Biochim. biophys. Acta* 118, 484–494 (1966).
- Wray, J. L. & Filner, P. *Biochem. J.* 119, 715–725 (1970).
- Pateman, J. A., Cove, D. J., Rever, B. M. & Roberts, D. B. *Nature* 201, 58–60 (1964).
- Pateman, J. A., Rever, B. M. & Cove, D. J. *Biochem. J.* 104, 103–111 (1967).
- Warner, R. L. & Kleinhofs, A. *Crop Sci.* 14, 654–658 (1974).
- Schrader, L. E., Ritenour, G. L., Eilrich, G. L. & Hageman, R. H. *Pl. Physiol.* 43, 930–940 (1968).
- Oostindier-Braaksma, F. J. & Feenstra, W. J. *Mutat. Res.* 19, 175–185 (1973).

Possible *in vitro* test for screening drugs for activity against *Babesia* and other blood protozoa

THE normal method of screening compounds for activity against *Babesia* infections of animals is based in the first instance on testing the compound in laboratory rats or mice infected with *B. rodhaini* using a regimen similar to that described for the testing of anti-malarial compounds¹. Most compounds screened against *B. rodhaini* in this way show no activity at all, therefore there would be justification for developing an *in vitro* test which would greatly reduce the time, cost and numbers of animals involved. We describe here such a test which is applicable to all species of *Babesia* and which exploits the finding that *Babesia* parasites readily incorporate hypoxanthine in an *in vitro* culture system².

The parasites used were *B. rodhaini*³ maintained in intact LACG mice, *B. divergens*⁴ and *B. major*⁵ maintained in splenectomised calves, and a *Babesia* sp. (probably *B. ovis*) recently isolated from Scotland⁶ and maintained in splenectomised sheep.

Blood from infected and similar non-infected animals was set up in a series of flasks containing culture medium and a test compound. Appropriate controls were included. Cultures were incubated for 1 h and ³H-hypoxanthine was then added to all flasks, which were incubated overnight. ³H-hypoxanthine incorporation was measured the next day by liquid scintillation counting⁷. The amount incorporated

by *Babesia* parasites was obtained by subtracting the count of non-infected blood from that of infected blood.

Various compounds were tested and those that were active against *Babesia* parasites inhibited the uptake of ^3H -hypoxanthine, whereas inactive compounds did not (Table 1). Any compound which reduced the uptake of ^3H -hypoxanthine by about 75% or more was considered to be active.

In addition to detecting active compounds, the test also indicated which drugs were most effective against particular species of parasite. Of the babesicides tested, Imidocarb and Amicarbalide were the most active *in vitro* against *B. rodhaini* and *B. divergens*, and Diminazene was the most active against *B. major* and '*B. ovis*'. These findings agree with *in vivo* studies (refs 8–10 and D. W. Brocklesby, personal communication). Diminazene, however, was ineffective *in vitro* against *B. rodhaini* and *B. divergens* at the concentration used, although in a subsequent experiment a tenfold increase in drug resulted in >95% reduction in ^3H -hypoxanthine uptake. This drug was also less effective *in vivo* against these parasites^{8,9}. *In vivo* studies with Trypan blue have shown that it is principally active against 'large' babesias¹¹ and ineffective against *B. ovis*¹⁰, *B. major*¹² and the small parasites; similar findings were recorded in the present work. The antimalarial drug, chloroquine sulphate, was ineffective *in vitro* against the three parasites screened; similar lack of activity was recorded *in vivo*¹³.

Hypoxanthine itself blocked the uptake of ^3H -hypoxanthine, and both 6- and 2-mercaptapurine (structural analogues of hypoxanthine) seemed to be active. *In vivo* tests with these two compounds showed that they delayed time to death in *B. rodhaini*-infected mice by about 14%

(our unpublished observations). Their activity is probably explained on the basis of competition with hypoxanthine for the enzyme hypoxanthine guanine phosphoribosyl transferase¹⁴. Activity of 6-thioguanine can be similarly explained. Occasionally more ^3H -hypoxanthine was incorporated by treated samples than by controls. This could be as a result of drug-induced changes, such as increased parasite or erythrocyte permeability, or because of increased parasite metabolism, but such factors have not so far been investigated.

A small amount of ^3H -hypoxanthine is incorporated by non-infected blood, principally by white cells. This amount is insignificant when compared with the massive uptake by *Babesia* parasites⁷. By subtracting counts of control blood from those of infected blood such 'background' was largely eliminated. Any difference in white cell count between control and infected blood, which might result in a possible difference in 'background', would not be detected by the present test. The counts can therefore be regarded as an accurate record of ^3H -hypoxanthine uptake by *Babesia* alone.

The level of parasitaemia, provided it was >5% and not past peak², did not seem to affect the percentage inhibition of ^3H -hypoxanthine uptake. For example, three trials with amicarbalide against *B. divergens*-infected blood of 12.5%, 18.5% and 24.0% parasitaemia gave the following respective figures for uptake of ^3H -hypoxanthine, 24.1%, 21.0% and 17.2%.

It is not yet clear how the test works, but a purine salvage pathway involving incorporation of hypoxanthine may be essential for nucleic acid synthesis in *Babesia*², just as in *Plasmodium*^{15–17}, in which case any compound which

Table 1 Percentage uptake of ^3H -hypoxanthine by *Babesia* spp. incubated *in vitro* in the presence of different drugs

Parasitaemia (%)	Parasite			
	<i>B. rodhaini</i> 18.6 (3)	<i>B. divergens</i> 18.3 (3)	<i>B. major</i> 9.3 (2)	' <i>B. ovis</i> ' 5.4
Drug and type	Uptake of ^3H -hypoxanthine (%)			
Purine				
Hypoxanthine	5.3	6.5	4.3	
Purine analogues				
2-Mercaptopurine		8.7 (2)*	16.2 (2)	7.0
6-Mercaptopurine	22.9	42.1 (2)	32.2	
8-Azaguanine	90.1 (2)	68.1	81.0	
6-Thioguanine	41.1 (2)	34.9	39.5	
8-Aza-adenine	60.8	84.1		
Allopurinol	55.7	34.3		
Pyrimidine analogues				
Fluorodeoxyuridine	82.1	102.4		
Bromodeoxyuridine	73.4	122.7		
Antifolate				
Aminopterin	86.5 (2)	120.8	72.1	
Babesicides				
Trypan blue		36.7	67.7	90.0
Quinuronium SO ₄	28.2	65.6 (2)	10.2 (2)	39.2
Imidocarb	14.6	13.5		32.7
Diminazene	39.5	72.1	9.0	16.9
Amicarbalide	10.1 (3)	20.8 (3)	21.1 (2)	35.7
Antimalarial				
Chloroquine SO ₄		81.0	113.4	75.4
Control (no drug)	100	100	100	100

*Figures followed by (2) or (3) indicate that values are the means of two or three separate readings. Each reading represents the mean from duplicate samples.

Blood from infected and control animals was collected into anticoagulant and washed thrice in PBS (pH 7.2). Aliquots of 6×10^8 erythrocytes were placed in 25-cm² Falcon flasks containing 10 ml of Eagles' MEM, supplemented with 100 IU ml⁻¹ of benzyl penicillin, 100 µg ml⁻¹ streptomycin sulphate, 25 U ml⁻¹ mycostatin, 50 mM HEPES buffer (pH 7.2) and 10% foetal calf serum. Appropriate drugs were added to cultures at the rate of 20 µg ml⁻¹. Cultures were incubated at 37 °C and after 1 h, 2 µCi of ^3H -hypoxanthine ml⁻¹ (specific activity 1.8 Ci mmol⁻¹) was added to each culture. Cultures were further incubated overnight; erythrocytes were collected after centrifugation at 1,500g and three washings in PBS. Cells were resuspended in 5 ml of PBS; 1-ml samples were transferred to 5 ml of 5% ice-cold TCA and duplicate 1-ml aliquots of this suspension were passed through separate 2.5-cm diameter 1.2-µm Millipore filters. Filter pads were washed and dried and then placed in scintillation vials containing 10 ml of toluene-based scintillation fluid. Counts per minute were recorded after 10 min of counting in a liquid scintillation analyser at 8 °C. Uptake of ^3H -hypoxanthine by parasites was recorded after counts were corrected to eliminate background and uptake by non-infected cells.

Table 2 Percentage uptake of ^3H -hypoxanthine by normal and Diminazene-resistant¹⁹ strains of *B. rodhaini* incubated *in vitro* in the presence of different babesicidal drugs

Strain of <i>B. rodhaini</i>	Control	Amicarbalide	Drug Quinuronium SO ₄	Imidocarb	Diminazene
Normal	100	17.3	28.2	14.6	39.5
Diminazene-resistant	100	11.6	32.9	5.0	77.9

Drug concentrations and experimental methods as for Table 1.

inhibits parasite multiplication, and thus nucleic acid synthesis either directly or indirectly, will result in a reduction of hypoxanthine uptake. The test may therefore be measuring the degree of drug-induced inhibition of nucleic acid synthesis. Although the mode of action needs to be determined, the test seems to be reliable, simple, quick and cheap, it does not involve the use of large numbers of animals; it can be applied to any species of *Babesia*; and the drugs do not require previous toxicity testing. Before *in vivo* tests, active compounds can be further screened at different concentrations *in vitro* and structure-activity relationships¹⁸ can be quickly evaluated.

Preliminary tests with a Diminazene-resistant strain of *B. rodhaini*¹⁹ suggest that drug resistance patterns may also be detected by the inhibition of ^3H -hypoxanthine uptake. The results shown in Table 2 agree with *in vivo* tests¹⁹ which have shown that a Diminazene-resistant strain was also resistant to Quinuronium sulphate but less resistant to Amicarbalide and Imidocarb.

In vitro tests for the detection of antimalarial drug activity, based on the inhibition of ^{32}P and ^3H -adenosine uptake by *Plasmodium*-infected blood, have been described^{20,21} but never widely adopted, possibly because of their complexity or lack of versatility. The test described here does not seem to be subject to these constraints and, since many malaria parasites are known to incorporate hypoxanthine^{15,16} readily, the test could also be considered for the primary screening of antimalarial drugs.

We thank Professor G. Urquhart, Drs R. E. Purnell and E. Beveridge for supplying parasite strains, Drs D. W. Brocklesby and D. L. Hughes for helpful discussions and Mr C. Mallinson for liquid scintillation analyses.

A. D. IRVIN
E. R. YOUNG

ARC Institute for Research on Animal
Diseases, Compton,
Newbury, Berkshire, UK

Received 29 April; accepted 11 August 1977

- Thurston, J. P. *Br J Pharmacol* 5, 409-416 (1950)
- Irvin, A. D., Young, E. R. & Purnell, R. E. *Int J Parasitol* (in the press)
- Cox, F. E. G. & Young, A. S. *Parasitology* 59, 257-268 (1969)
- Purnell, R. E., Brocklesby, D. W., Kitchenham, B. A. & Young, E. R. *J. comp. Path.* 86, 609-614 (1976)
- Morzarra, S. P., Brocklesby, D. W., Harradine, D. L. & Barnett, S. F. *Int J Parasitol* 4, 437-438 (1974)
- Reid, J. F., Armour, J., Jennings, F. W. & Urquhart, G. M. *Vet Rec* 99, 419 (1976)
- Gutteridge, W. E. & Trigg, P. I. *J Protozool* 17, 89-96 (1970)
- Beveridge, E. *Res. vet. Sci.* 10, 534-539 (1969)
- Joyner, L. P. & Brocklesby, D. W. *Adv. Pharmacol. Chemother.* 11, 321-355 (1970)
- Soulsby, E. J. L. *Helminths, Arthropods and Protozoa of Domesticated Animals* (Baillière, Tindall and Cassel, London, 1968).
- Joyner, L. P., Davies, S. F. M. & Kendall, S. B. *Expl. Chemother.* 1, 603-623 (1963)
- Zwart, D., Van Den Ende, M. C., Kouwenhoven, B. & Buys, J. *Tijdschr. Diergeneesk.* 93, 126-139 (1968).
- Taylor, A. E. R., Terry, R. J. & Godfrey, D. G. *Br J Pharmacol* 11, 71 (1956)
- Handschumacher, R. E. & Welch, A. D. *The Nucleic Acids III* (eds Chargaff, E. & Davidson, J. N.) 453-526 (Academic, London, 1960)
- Bunger, W. & Nielson, G. Z. *Tropenmed. Parasit.* 19, 185-197 (1968)
- Van Dyke, K. Z. *Tropenmed. Parasit.* 26, 232-238 (1975)
- Oelshlegel, F. J. & Brewer, G. J. *The Red Blood Cell II* (ed Surgenor, D. M.) 1263-1302 (Academic, London, 1975)
- Van Rossum, J. M. J. *Pharm. Pharmacol.* 15, 285-316 (1963)
- Beveridge, E. *Br J Pharmacol* 39, 239 (1970)
- Schellenberg, K. A. & Coatsney, G. R. *Biochem. Pharmacol.* 6, 143-152 (1961).
- Van Dyke, K., Szustkiewicz, C., Cenedella, R. & Saxe, L. H. *Chemotherapy* 15, 177-188 (1970)

Synergistic interaction of testosterone and oestradiol inhibits spermatogenesis in rats

ONE experimental approach to male contraception has been the systemic administration of androgenic steroids, but treatment has failed sometimes to induce azoospermia¹⁻⁴. To overcome this difficulty, androgen-progestin formulations have been tested, but they did not inhibit spermatogenesis completely in all treated men⁵⁻⁸. We thought that this variable response was probably due to the relatively weak anti-gonadotropic activity of both androgens and progestins and the difficulty of controlling precisely the dose of steroids administered. We reasoned that azoospermia might be achieved in every male if steroids were administered continuously and at relatively constant rates and if progestins were replaced by a more potent inhibitor of pituitary gonadotropin secretion. We describe here the results of a test of the efficacy of testosterone-oestradiol formulations administered to adult male rats by means of a subdermal sustained release device. Oestradiol was used because, like testosterone, it is a naturally occurring steroid secreted by the mammalian testis⁹⁻¹³ and because it is a potent inhibitor of pituitary gonadotropin secretion¹⁴⁻¹⁶.

Ninety adult male Sprague-Dawley rats (225-260 g) were assigned randomly to one of 15 treatment groups. Subdermal implants of Silastic of different sizes, containing either testosterone or oestradiol-17 β were used to administer steroid. These sustained release devices were constructed from Silastic (polydimethylsiloxane) medical grade tubing because studies in our laboratory have established that such devices release hormonal steroids at relatively constant rates for a long time^{17,18}. Three groups of 30 rats each were given either no oestradiol-17 β or oestradiol-17 β -filled Silastic implants measuring 0.1 cm or 0.3 cm (release rate for oestradiol was approximately 0.1 $\mu\text{g cm}^{-1} \text{d}^{-1}$). The 30 rats in each group were further subdivided into five groups of six rats each, receiving either no testosterone or testosterone-filled Silastic implants measuring 1.0, 2.5, 4.0 or 6.0 cm (release rate for testosterone was approximately 30 $\mu\text{g cm}^{-1} \text{d}^{-1}$). The implants were prepared by filling Silastic tubing (Dow Corning No 602-305) with steroid and implanting it subdermally as before¹⁷⁻¹⁸. Three months later the rats were weighed and decapitated, trunk blood was collected and serum was prepared for radioimmunoassay of oestradiol¹⁹, testosterone²⁰ and luteinising hormone (LH)²¹. The seminal vesicles and ventral prostate were dissected, freed of extraneous tissue and weighed, the fluid was expressed from seminal vesicles before weighing. Spermatids and spermatozoa were counted haemocytometrically.

Table 1 Average number of spermatozoa and spermatids in adult male rats with subdermal implants containing testosterone or oestradiol

Oestradiol implant length (cm)	Testosterone implant length (cm)				
	0	1	2.5	4	6
0	234 \pm 12	193 \pm 9	61 \pm 39	17 \pm 6	80 \pm 19
0.1	194 \pm 18	38 \pm 26	<1*	48 \pm 2	85 \pm 7
0.3	205 \pm 15	<1	<1	3 \pm 1	43 \pm 11

Values represent the mean \pm s.e.m. of six animals

*Indistinguishable from zero.

Table 2 Effect of testosterone and oestradiol in subdermal implants on serum LH, testosterone and oestradiol and on the weight of seminal vesicles and ventral prostate in adult male rats

	Treatment			
	Control	2.5 cm Testosterone	0.1 cm Oestradiol	2.5 cm Testosterone plus 0.1 cm oestradiol
LH (mg ml ⁻¹)*	8.0 ± 1.0	2.9 ± 0.3	2.8 ± 0.4	ND
Testosterone (ng ml ⁻¹)	2.3 ± 0.5	3.0 ± 0.6	0.9 ± 0.5	2.2 ± 0.2
Oestradiol (pg ml ⁻¹)	45.8 ± 11.0	47.5 ± 11.0	46.2 ± 3.0	42.7 ± 4.0
Paired seminal vesicle weight (mg)	454.0 ± 8.0	464.0 ± 26.0	258.0 ± 28.0	451.0 ± 21.0
Ventral prostate weight (mg)	577.0 ± 62.0	686.0 ± 57.0	358.0 ± 36.0	678.0 ± 34.0

*Values are expressed in terms of ng equiv of NIAMD-rat-LH-RP-1 per ml of blood serum. The rat LH standard has a relative potency of 0.03 times the NIH standard (NIH-LH-S1) in the ovarian ascorbic acid depletion assay.

Each value represents the mean ± s.e.m. of the mean of six animals.

ND, Not detectable.

from homogenates of testes from each animal²². Testicular perfusions were done as described earlier²³⁻²⁴.

Testosterone administered in the absence of oestradiol (Table 1) caused the expected biphasic changes in the combined number of spermatozoa and/or spermatids per testis²⁵. Numbers of spermatids and spermatozoa did not reach zero in any of the five treatment groups: this differs from the result reported by Berndtson *et al.*²⁵. The difference probably resulted because Berndtson *et al.* quantified spermatogenesis by counting germ cells in histological sections of testes²⁵, whereas we used a haemocytometer to count nuclei of spermatids and spermatozoa in a testicular homogenate. No significant alterations in spermatogenesis were apparent in rats receiving oestradiol without testosterone (Table 1). Surprisingly, testosterone and oestradiol doses (for example, 1.0 cm testosterone plus 0.1 cm oestradiol) that failed significantly to inhibit spermatogenesis when administered alone, markedly inhibited spermatogenesis when given simultaneously (Table 1). This interaction is apparently synergistic (supra-additive) because increasing the dose of either steroid by more than two-fold did not result in the major suppression of spermatogenesis obtained with the combination of testosterone and oestradiol.

The testes of each animal, treated with several of the testosterone-oestradiol combinations, were devoid of condensed spermatids and spermatozoa. At the lower dose of oestradiol-17β (0.1-cm implant), azoospermia was first obtained with a 2.5-cm Silastic implant of testosterone (Table 1). Consequently, further measurements were completed on animals receiving this steroid formulation.

The results in Table 2 suggest that the 2.5-cm testosterone-0.1 cm oestradiol treatment inhibited spermatogenesis indirectly by suppressing release of immunoreactive LH. We cannot, however, rule out a direct effect of oestradiol on testosterone secretion because Chowdhury *et al.*²⁶ suggested that oestrogen inhibits testicular steroidogenesis. Serum concentrations of testosterone and oestradiol were similar to control values in rats receiving the combination treatment (Table 2). Serum testosterone concentration reflected the free biologically active hormone, for the accessory sex organ weights of these rats were not significantly different from those of control rats.

These results raise two important questions about the mechanism by which testosterone-oestradiol combinations induced azoospermia, because the circulating levels of each steroid remained within the range typically noted in the sera of untreated adult male rats. First, why were concentrations of peripheral blood serum testosterone and oestradiol not increased? The probable answer is that testes of rats receiving the 2.5-cm testosterone-0.1-cm oestradiol treatment failed to produce testosterone. Testosterone secretion by *in vitro* perfused testes from control rats and rats with the 2.5-cm testosterone-0.1-cm oestradiol implants was 5.0 ± s.e.m. 0.4 (n=6) and 0.1 ± s.e.m. 0.03 (n=6) µg h⁻¹. Thus it seems reasonable to conclude that the implant was the major source of testosterone in the peripheral blood of the aspermatic rats. The steroids thus released replaced those usually

derived directly from the testis or indirectly from peripheral conversion of testicular steroids. Presumably testosterone from the implants was sufficient to maintain peripheral androgen-dependent functions but insufficient to maintain the high intratesticular concentration of testosterone^{25,27} required for spermatogenesis. The second question is: how did normal circulating titres of testosterone and oestradiol completely suppress circulating levels of immunoreactive LH? Probably because, in contrast to the episodic fluctuations in testosterone titres seen in normal males²⁸, implants release steroids at relatively constant rates^{17,29-30}. Thus it seems that sustained release of testosterone provides a more effective signal to androgen-dependent target tissues than the episodic fluctuations characteristic of intact animals^{25,31}.

Thus azoospermia can be induced in adult male rats with the appropriate testosterone-oestradiol formulation administered from a subdermal sustained release device. Preliminary results from our laboratory suggest that these azoospermic rats are sexually active but, predictably, infertile. Taken together, these results indicate that androgen-oestrogen combinations are an effective male contraceptive formulation in the rat, and thus the suggestion of Briggs and Briggs³² to test such combinations in the human male should be taken up.

We thank N. Ruiz Bravo, D. W. Carroll, N. Rugg and G. Zeller for technical assistance. Reagents used to assay serum LH and NIH-LH-S₁₉ were given by the NIAMD Hormone Distribution Program. This work was supported by a fellowship, a contract and grants from NICHD.

L. L. EWING

Division of Reproductive Biology,
Department of Population Dynamics,
Johns Hopkins University,
School of Hygiene and Public Health,
Baltimore, Maryland 21205

C. DESJARDINS

Department of Zoology,
University of Texas,
Austin, Texas 78712

D. C. IRBY

Department of Anatomy,
Monash University,
Melbourne, Australia

B. ROBAIRE

Department of Obstetrics and Gynecology and
Pharmacology and Therapeutics,
McGill University,
Montreal, Canada

Received 7 June; accepted 11 July 1977.

- Heller, G. C. *et al.* *Fert. Steril.* **1**, 415-422 (1950).
- Heller, G. C., Morse, H. C., Su, M. & Rowley, M. J. in *The Human Testis* (ed. Rosenberg, E. & Paulsen, C. A.) 249-257 (Plenum, New York, 1970).
- Mauss, J., Borsch, G., Richter, E. & Bormacher, K. *Contraception* **10**, 281-289 (1974).
- Paulsen, C. A. & Leonard, J. M. in *Regulatory Mechanisms of Male Reproductive Physiology* (eds. Spilman, C. H., Lobl, T. J. & Kirton, K. T.) 197-211 (Excerpta Medica, Amsterdam, 1976).

- ⁷ Coutinho, E. M. & Melo, J. F. *Contraception* **8**, 207-212 (1973).
⁸ Frick, J. *Contraception* **8**, 191-206 (1973).
⁹ Frick, J. in *Control of Male Fertility* (ed. Sciarra, J., Markland, C. & Speidel, J. J.) 230-239 (Harper and Row, Hagerstown, Maryland, 1975).
¹⁰ Johnson, E. D. B. & Nygren, K. G. *Contraception* **8**, 219-226 (1973).
¹¹ Baird, D. T., Galbraith, A., Fraser, I. S. & Newsam, J. E. *J. Endocr.* **57**, 285-288 (1973).
¹² DeJong, F. H., Hey, A. H. & van der Molen, H. J. *J. Endocr.* **57**, 277-284 (1973).
¹³ Kelch, R. P., Jenner, M. R., Weinstein, R., Kaplan, S. L. & Grumbach, M. M. *J. clin. Invest.* **51**, 824-830 (1972).
¹⁴ Longcope, C., Widrich, W. & Sarvin, C. T. *Steroids* **20**, 439-448 (1972).
¹⁵ Savard, K. & Goldzler, J. W. *Endocrinology* **66**, 617-624 (1966).
¹⁶ Gay, V. L. & Dever, N. W. *Endocrinology* **39**, 161-168 (1971).
¹⁷ Kinlin, H. E. & Reiter, E. O. *J. clin. Endocr. Metab.* **35**, 836-839 (1972).
¹⁸ Walsh, P. C., Swerdloff, R. S. & Odell, W. D. *Acta Endocr.* **74**, 449-460 (1973).
¹⁹ Ewing, L. L., Stratton, L. G. & Desjardins, C. J. *Reprod. Fertil.* **35**, 245-253 (1973).
²⁰ Stratton, L. G., Ewing, L. L. & Desjardins, C. J. *Reprod. Fertil.* **35**, 235-244 (1973).
²¹ England, B. G., Niswender, G. D. & Midgley, A. R., Jr. *J. clin. Endocr. Metab.* **38**, 42-50 (1974).
²² Schanbacher, B. D. & Ewing, L. L. *Endocrinology* **97**, 787-792 (1975).
²³ Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E. & Reichert, L. E., Jr. *Proc. Soc. exp. Biol. Med.* **128**, 807-811 (1968).
²⁴ Amann, R. P. & Lambiase, J. T., Jr. *J. Anim. Sci.* **28**, 369-374 (1969).
²⁵ VanDemark, N. L. & Ewing, L. L. *J. Reprod. Fert.* **6**, 1-8 (1963).
²⁶ Ewing, L. L. & Eik-Nes, K. B. *Can. J. Biochem.* **44**, 1327-1344 (1966).
²⁷ Berndtson, W. E., Desjardins, C. & Ewing, L. L. *J. Endocr.* **62**, 125-135 (1974).
²⁸ Chowdhury, M., Tcholakian, R. & Steinberger, E. *J. Endocr.* **60**, 375-376 (1974).
²⁹ Desjardins, C., Ewing, L. L. & Irby, D. C. *Endocrinology* **93**, 450-460 (1973).
³⁰ Katangole, C. B., Nafolin, F. & Short, R. V. *J. Endocr.* **50**, 457-466 (1971).
³¹ Dziuk, P. L. & Cook, B. *Endocrinology* **78**, 208-211 (1966).
³² Kincl, F. A., Banagiano, G. & Angee, I. *Steroids* **11**, 673-680 (1968).
³³ Davidson, J. M., Damassa, D. A., Smith, E. R. & Chung, C. in *Regulatory Mechanisms of Male Reproductive Physiology* (eds Spilman, C. H., Lobl, T. J. & Kirton, K. T.) 151-164 (Excerpta Medica, Amsterdam, 1976).
³⁴ Briggs, M. & Briggs, M. *Nature* **252**, 585-586 (1974).

Anti-Müllerian hormone is a functional marker of foetal Sertoli cells

REGRESSION of Müllerian ducts in male foetuses is mediated by a testicular factor distinct from testosterone¹. This anti-Müllerian hormone (AMH), a protein which has been partially purified², is synthesised by foetal Sertoli cells³. In mammals the first stage of testicular differentiation involves the arrangement of sustentacular or Sertoli cells around the gonocytes and their encasement in testicular cords. Leydig cells appear later in the interstitium and their differentiation is correlated with the onset of steroidogenesis. We thought that there might also be a correlation between the initiation of testicular anti-Müllerian activity and the differentiation of Sertoli cells. Little work has been done on anti-Müllerian activity of the early foetal testis. During the initial stages of testicular organogenesis in the rat⁴ and the guinea pig⁵, AMH production is low, but the rapid pace of sex differentiation in most rodents hampers sequential study. Using sows (*Sus scrofa*), we have avoided this problem, and confirmed that the correlation exists.

Gestational age is known with great accuracy for sows of the large-white breed, bred at the INRA Zootechnical Centre. Foetuses were tested for testicular anti-Müllerian activity at between 27 and 34 d of gestation. Sex was determined by cytogenetic examination of hepatic cells⁶

Fig. 1 Chronological development of anti-Müllerian activity of the foetal pig testis. See text for details. Inc., incomplete.

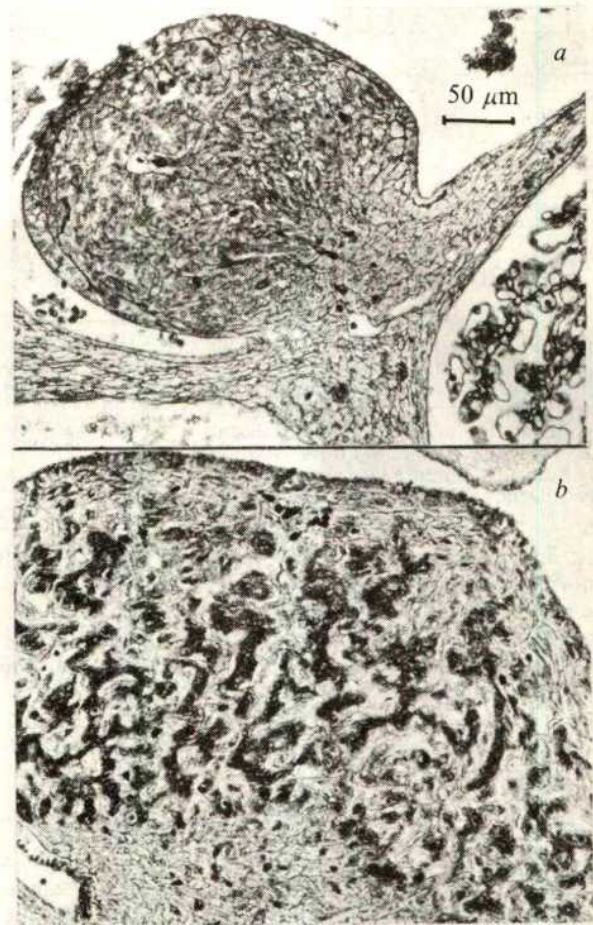
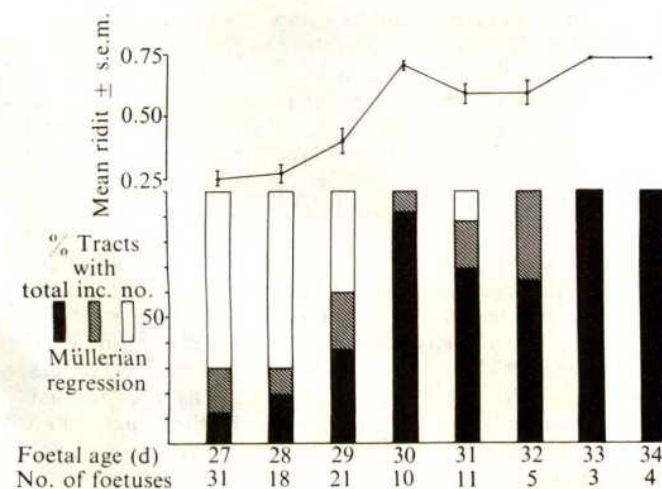


Fig. 2 Histological aspect of the pig foetal testis studied by the silver impregnation method of Gomori¹³. a, 27 d: undifferentiated gonad; testicular cords are not clearly outlined. The mesonephros is on the right. b, 29 d: testicular organogenesis is complete; both the tunica albuginea and testicular cords are well differentiated.

from foetuses up to 30 d and by inspection of the genital tract in older ones. Testicular tissue was explanted on an agar-coated grid placed in a Petri dish filled with Ham's F12 medium, and the anti-Müllerian activity of between two and five explants was tested promptly by association with 14.5-d-old gonadless rat reproductive tracts⁷. In some 27 and 29-d-old pig foetuses one testis was cultured alone for 3 d before testing. The test was terminated after 3 d by fixation and the degree of regression of the Müllerian ducts of individual rat reproductive tracts exposed to testicular explants was scored as before⁸.

Figure 1 shows that anti-Müllerian activity increased steadily from negligible levels at 27 d to maximal values at 33 d. The ridit method^{9,10} of transformation of a non-parametric variable revealed a highly significant correlation ($r = 0.59$, $P < 0.01$) between anti-Müllerian activity and foetal age. We also found that anti-Müllerian activity could be acquired *in vitro*, though at a slower rate. Testes from 27-d-old pig foetuses after culture for 3 d had a significantly higher mean anti-Müllerian activity ($P < 10^{-1}$ by Student's paired *t* test) than the contralateral testes but lower activity ($P < 10^{-7}$ by Student's *t* test) than testes explanted at 30 d of gestation. The same tests performed on day 29 did not yield significant results (Table 1), probably because the basal level of anti-Müllerian activity was already high.

Gonadal sex is recognisable by electron microscopy as early as day 26 in the pig foetus¹¹. Using a light microscope, however, we were unable reliably to identify testicular organogenesis before day 29. Even then, primitive testicular cords, two to three cells wide, as described by

Table 1 *In vitro* development of anti-Müllerian activity of foetal pig testis

Age (d)	No. of pig foetuses	No. of tracts associated	Mean ridit \pm s.e.m.	Comparison with preceding group
27	25	25	0.246 \pm 0.030	
27 + 3*	25	25	0.440 \pm 0.048	$P < 10^{-3} \dagger$
30	10	35	0.697 \pm 0.017	$P < 10^{-7} (\ddagger)$
29	13	13	0.406 \pm 0.048	
29 + 3*	13	13	0.626 \pm 0.055	NS†
32	5	14	0.588 \pm 0.049	NS‡

The mean ridit^{8,10} expresses the probability that a rat reproductive tract selected from those exposed to pig testicular tissue from a given age group will have a greater degree of Müllerian regression than one selected at random from the reference population (here, all 246 tracts used in the study). NS, Not significant.

*Days in culture before test for anti-Müllerian activity.

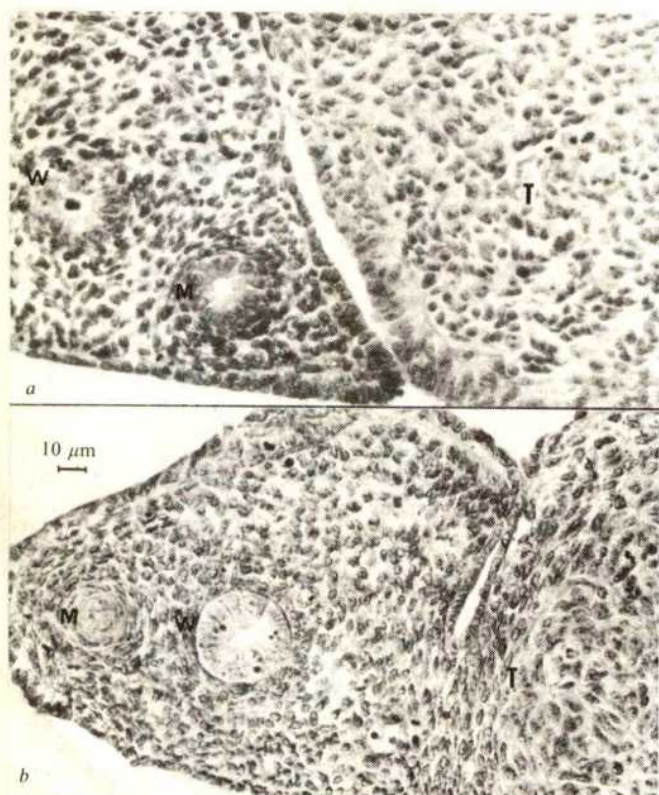
†By Student's paired bilateral *t* test.

‡By Student's bilateral *t* test.

Moon and Hardy¹², were visible only after treatment with Gomori's silver impregnation¹³ (Fig. 2). At day 29, also, testicular anti-Müllerian activity becomes clearly demonstrable (Fig. 3). It seems therefore that cellular rearrangements characteristic of seminiferous tubule organisation must be completed before the activity of early Sertoli cells can be demonstrated physiologically. Similarly, Leydig cells differentiate in the interstitium at approximately day 31 (ref. 12), but peak testosterone concentrations are reached only by day 37 in the testis¹⁴ and day 55 in the plasma¹⁵.

Testicular anti-Müllerian activity wanes and eventually

Fig. 3 Anti-Müllerian activity of foetal pig testis, cocultured for 3 d with 14.5-d-old gonadless rat reproductive tract. *a*, 27 d at explantation: slight inhibition of the Müllerian duct is shown by the formation of a peri-Müllerian fibroblastic ring. *b*, 29 d at explantation: the Müllerian epithelium has almost completely disappeared, replaced by a fibrous whorl. M, Müllerian duct; W, Wolffian duct; T, pig foetal testis. Periodic acid Schiff stain was used.



vanishes after birth (see ref. 2 for review). This led Josso¹⁶ to conclude that AMH is a foetoprotein, that is no longer synthesised after the peri-natal period. In the light of the subsequent demonstration of the Sertoli-cell origin of AMH³, however, an alternative possibility should be considered: formation of the blood-testis barrier could prevent detection of AMH by inhibiting its release from testicular explants. After the formation of occluding junctions between adjacent Sertoli cells, the products of these cells such as inhibin or androgen-binding protein, accumulate in seminal fluid and epididymis^{17,18}, where a search for AMH might be rewarding for the understanding of the relationship between the function of the post-natal Sertoli cells and anti-Müllerian activity.

DIEN TRAN

Unité de Recherches de
Génétique Médicale (INSERM),

NICOLE MEUSY-DESSOLLE

Laboratoire de Physiologie de la Reproduction,
Université Paris VI, France and
Institut National de la Recherche Agronomique,
Centre National de Recherches Zootechniques,
78350 Jouy-en-Josas, France

NATHALIE JOSSO

Unité de Recherches de
Génétique Médicale (INSERM),
Hôpital des Enfants Malades,
75730 Paris, Cédex 15, France

Received 23 May; accepted 27 July 1977.

- Jost, A. *Arch. anat. morph. exp.* **36**, 271-315 (1947).
- Josso, N., Picard, J. Y. & Tran, D. *Rec. Prog. Hormone Res.* **33**, 117-160 (1977).
- Blanchard, M. G. & Josso, N. *Pediat. res.* **8**, 968-971 (1974).
- Picon, R., C. r. heb. Séanc. Acad. Sci., Paris **271**, 2370-2372 (1970).
- Price, D. *Am. Zool.* **15**, Suppl. 1, 173-195 (1975).
- Pelliniemi, L. J. & Saloniemi, A. L. *Acta anat.* **95**, 558-564 (1976).
- Picon, R. *Arch. anat. morph. exp.* **58**, 1-19 (1969).
- Josso, N., Forest, M. G. & Picard, J. Y. *Biol. reprod.* **13**, 163-167 (1975).
- Bross, I. D. *Biometrics* **14**, 18-38 (1958).
- Ben-David, M., Heston, W. E. & Rodbard, D. *J. natn. Cancer Inst.* **42**, 207-218 (1969).
- Pelliniemi, L. J. *Am. J. Anat.* **144**, 89-112 (1975).
- Moon, Y. S. & Hardy, M. H. *Am. J. Anat.* **138**, 253-268 (1973).
- Martoja, R. & Martoja-Pierson, M. *Initiation aux Techniques de l'Histologie Animale* 116 (Masson, Paris, 1967).
- Raesside, J. I. & Sigman, D. M. *Biol. reprod.* **13**, 318-321 (1975).
- Meusy-Dessolle, N. C. r. heb. Séanc. Acad. Sci., Paris **278**, 1257-1260 (1974).
- Josso, N. *Biol. Neonate* **20**, 368-379 (1972).
- Franchimont, P., Chari, S., Hagelstein, M. T. & Duraiswami, S. *Nature* **257**, 402-404 (1975).
- Tindall, D. J., Vitale, R. & Means, A. R. *Endocrinology* **97**, 636-648 (1975).

***In vitro* duplication and 'cure' of haemopoietic defects in genetically anaemic mice**

SEVERAL types of congenital, genetically determined macrocytic anaemias have been described in mice¹⁻⁴. These models offer a means of analysing the effects of mutations at defined loci on the ability of haemopoietic tissue to support stem cell proliferation and differentiation and allow studies on intrinsic defects in differentiation capacity of stem cells. But, analysis of these mutations and their implications in relation to pluripotential stem cell control have been hampered by lack of techniques, particularly of *in vitro* systems, for further defining regulatory interactions between stem cells and the haemopoietic inductive microenvironment. Here we report that the haemopoietic defects seen in W and Steel (Sl) mice have been successfully reproduced *in vitro*.

The pleiotropic effects of mutations at the W and Steel (Sl) loci include macrocytic anaemia, extreme radiosensitivity, sterility and defective pigmentation¹. The lesions in W mice are presumed to be due to intrinsic defects in haemopoietic stem cells, primordial germ cells and melanoblast precursors¹⁻⁴, whereas in Sl mice the defect resides within the environment into which these cells migrate, that is, the haemopoietic microenvironment, the gonads and skin^{1,2,4,5}. Support for this view is provided by the observation that pluripotential stem cells detected by the spleen

Table 1 Production of CFU-c and differentiating haemopoietic cells in continuous cultures of W/W^v , Sl/Sl^d and control bone marrow combinations

Genotype of adherent cells	Genotype of marrow cells added	Total non-adherent cells per culture ($\times 10^{-5}$)						Total CFU-c per culture					
		Weeks cultured*						Weeks cultured					
		1	2	3	4	5	6	1	2	3	4	5	6
$Sl(+/+)$	$Sl(+/+)$	25.0	24.0	10.0	70.0	8.6	14.0	16,000	10,600	10,100	41,700	2,300	12,200
Sl/Sl^d	Sl/Sl^d	18.0	14.0	2.4	1.6	6.0	9.0	21,900	8,300	2,300	540	1,300	90
$W(+/+)$	$W(+/+)$	52.0	24.0	30.0	28.0	ND	ND	32,000	15,200	22,800	23,000	ND	ND
W/W^v	W/W^v	20.0	9.4	4.0	3.2	ND	ND	2,500	430	190	260	ND	ND
Sl/Sl^d	W/W^v	5.6	9.0	6.8	1.2	4.0	ND	150	0	0	0	0	ND
W/W^v	Sl/Sl^d	40.0	7.0	20.0	58.0	30.0	ND	46,200	4,500	11,900	13,800	7,500	ND

ND, Not determined.

*Weeks from time of addition of the second marrow.

colony assay (colony-forming units, CFUs) are greatly reduced or absent in W anaemic mice but are present in normal numbers in Sl mice¹. Furthermore, W mice, both irradiated or unirradiated, support spleen colony formation after inoculation of bone marrow from non-anaemic litter mates or from Sl mice, whereas spleen colony formation does not occur in irradiated Sl mice after normal marrow inoculation^{1,2}. The haematological defects of W mice can, therefore, be cured by an inoculum of marrow cells from Sl mice and the macrocytic anaemia of Sl mice can be reversed after grafting of neonatal spleen or whole bone from W anaemic mice as a source of non-defective haemopoietic microenvironment. A culture system has been developed in which the proliferation of murine pluripotent stem cells (CFU-s), committed granulocyte-macrophage stem cells (CFU-c) and differentiating granulocytic cells can be maintained *in vitro* for several months^{3,4}. In this system mouse bone marrow-derived adherent cell cultures established for 3 weeks are re-inoculated with freshly isolated samples of syngeneic, allogeneic or semi-allogeneic bone marrow cells. The latter subsequently undergo proliferation for several months. This proliferation, differentiation and maturation of haemopoietic cells is dependent on the formation of a bone marrow-derived adherent population comprised of phagocytic mononuclear cells, 'endothelial' cells and giant fat-containing cells which apparently provide an *in vitro* microenvironment necessary for pluripotent stem cell renewal and differentiation⁷. Within this adherent layer extensive cellular interactions occur reminiscent of the intimacy of inductive cell interactions seen in embryogenesis. We have used this system to further explore the haemopoietic defects in W and Sl mice. To establish the cultures, the contents of a single femur from 10–14-week-old $WBB6F_1-W/W^v$ (W/W^v), $WCB6F_1-Sl/Sl^d$ (Sl/Sl^d) or their normal litter mates $W(+/+)$, $Sl(+/+)$, (Jackson Laboratories) were flushed into glass culture bottles containing 10 ml of Fisher's medium (Gibco), supplemented with antibiotics and 25% horse serum (Flow Labs), as described previously⁶⁻⁸. The cultures were incubated at 33 °C (a temperature shown to facilitate the development of the adherent layer of cells and subsequent growth of stem cells) in an atmosphere of 5% CO_2 in air. At weekly intervals the cultures were 'fed' by removal of half the growth medium and addition of an equal volume of fresh medium. Over a period of 2–3 weeks an adherent layer of cells became established and at 3 weeks all the growth medium and suspension cells were removed and the adherent layer re-fed with 10 ml of growth medium containing 10^7 freshly isolated bone marrow cells. The cultures were incubated at 33 °C and at weekly intervals were fed by removal of half the growth medium suspension cells and addition of fresh medium. The suspension cells removed at each weekly depopulation were assayed for granulocyte-macrophage progenitor cell (CFU-c) growth in soft agar using conditioned medium from a murine myelomonocytic leukaemic cell line (WEHI-3) as a source of colony stimulating factor.

Pooled cells from at least six cultures were used for each time point.

As shown in Table 1, extensive cell production was observed over a 6-week period in control cultures of non-anaemic $WBB6F_1$ ($W(+/+)$) or $WCB6F_1$ ($Sl(+/+)$) bone marrow. Production of CFU-c was likewise maintained at a high level throughout the period of culture. In contrast, cultures of Sl/Sl^d marrow or of W/W^v marrow showed considerably reduced production of both haemopoietic cells and CFU-c to as little as 1–3% of litter mate control cultures by 4 weeks. The morphology of the cultured cells showed a preponderance of differentiating granulocytic elements (40–75%) in the first 4–5 weeks of culture with an increasing proportion of phagocytic mononuclear cells at later stages. Transition to a macrophage morphology was observed earlier in W/W^v and Sl/Sl^d cultures than in the control cultures.

Allogeneic cultures were also initiated using Sl/Sl^d marrow to establish an adherent layer which was subsequently fed with W/W^v bone marrow. The additive effects of the Sl environmental defect and the W stem cell defect were apparent in this combination since cell production in culture was again markedly reduced relative to the control cultures and CFU-c were undetectable by the second week of culture. In addition, granulopoiesis in these cultures was no longer evident after the first week and thereafter cell proliferation was exclusively macrophage. The reverse combination using W/W^v to initiate the adherent population and subsequent feeding with Sl/Sl^d marrow resulted in a sustained high level of production of both differentiating haemopoietic cells and CFU-c throughout the period of observation.

While we have not as yet systematically monitored CFU-s in these cultures, the striking defect in generation of CFU-c and of differentiating haemopoietic cells seen in the W/W^v , Sl/Sl^d and $Sl/Sl^d-W/W^v$ cultures clearly points to an underlying defect at the level of interaction of the adherent marrow cells with the pluripotent stem cell.

But, no qualitative or quantitative defect was seen in the capacity of Sl/Sl^d marrow to generate an adherent layer and the defect may lie at the level of cellular interactions. Ultrastructural analysis of the *in vitro* microenvironment is being performed. The duplication of the W and Sl defect *in vitro* and our ability to 'cure' the defect by appropriate combinations of adherent cells and stem cells should permit further insight into genetically determined haemopoietic disorders and inductive cell interactions associated with pluripotent stem cell self renewal and differentiation.

T. M. DEXTER

Paterson Laboratories,
Christie Hospital and Holt Radium Institute,
Withington, Manchester M20, UK

M. A. S. MOORE

Sloan-Kettering Institute for Cancer Research,
410 East 68th Street, New York,
New York 10021

Received 23 May; accepted 1 August 1977.

- ¹ Russel, E. S. & Bernstein, S. E. in *Biology of the Laboratory Mouse*, 2nd ed (ed. Green, E. L.) 351 (1966).
- ² Bernstein, S. E., Russell, E. S. & Keighley, G. *Ann. N.Y. Acad. Sci.* **149**, 475 (1968).
- ³ Bennett, M., Cudkovic, G., Foster, R. S. & Metcalf, D. *J. cell. Physiol.* **71**, 211 (1968).
- ⁴ Mayer, T. C. & Green, M. C. *Devl Biol.* **18**, 62 (1968).
- ⁵ Sutherland, D. J. A., Till, J. E. & McCulloch, E. A. *J. cell. Physiol.* **75**, 267 (1970).
- ⁶ Dexter, T. M., Allen, T. D. & Lajtha, L. G. *J. cell. Physiol.* **91**, 355 (1977).
- ⁷ Allen, T. D. & Dexter, T. M. *Differentiation* **6**, 191 (1976).
- ⁸ Dexter, T. M., Moore, M. A. S. & Sheridan, A. P. C. *J. exp. Med.* **145**, 1612 (1977).

HLA 'help' for human B2-microglobulin across species barriers

HLA antigens comprise products of what is commonly considered to be one of the most polymorphic systems presently known, occurring on chromosome 6. The HLA epitope is present only once¹ on a glycoprotein of molecular weight 45,000. In its naturally occurring form it is tightly, and probably obligatorily, but non-covalently bound to B2-microglobulin, (B2M), a protein of molecular weight 11,600 (ref. 2), determined by chromosome 15. B2M has substantial sequence homology with the C_H3 domain of IgG (ref. 3), and seems to be non-polymorphic. Precisely analogous associations occur between several other MHC antigens in rodents and fowl, and the equivalents of B2M in those species. This leads to the question as to why products of probably the most polymorphic systems should be so strongly associated with a non-polymorphic protein. In this paper we confirm that human B2M is immunogenic in phylogenetically distant species when the molecule is presented alone. When the species barrier is close, immunogenicity declines drastically as, for example, between man and other primate. In contradistinction, however, when B2M is presented bound to the HLA alloantigen-bearing chain, as occurs naturally, the complex is exquisitely immunogenic with respect to both B2M and the alloantigenic determinant if the species are closely related, and may decline as the species barrier widens. This is interpreted in terms of a modified 'intramolecular' help effect across a phylogenetically closely related barrier.

Free human B2M is clearly immunogenic in several

species at doses of 25–100 µg. As might be expected, antibody titres in chickens are higher than in mammals, reflecting a wider species barrier. Hyperimmune rabbit antisera to the unseparated proteins in human serum have also been examined (last line, Table 1). Although B2M was detected in the immunising inoculum at levels of 1–2 µg ml⁻¹, no anti-B2M antibodies were found in the resultant antisera, confirming that, at this dose, B2M is essentially non-immunogenic.

Much smaller doses (0.1–0.2 µg) of B2M bound to HLA antigen are highly immunogenic in rabbits and anti-B2M antibodies rise to levels essentially similar to those found with much higher doses of B2M used alone. As expected, the sera are cytotoxic for peripheral human lymphocytes. Furthermore, the immunogen used did not inhibit the lysis of B cells by a rabbit anti-human B cell-specific antiserum⁴, thereby suggesting the absence of human Ia antigens in the preparations. (Contamination with Ia antigens is, in any case probably ruled out by the anion exchange and gel filtration purification procedures used.)

Absorption of the rabbit anti-HLA sera with human erythrocytes (which are lacking in B2M and HLA antigens), did not reduce the lytic titre by much. Absorption with platelets or crude human spleen membranes negative for the immunising HLA specificity were, however, much more effective in removing lympholytic antibodies. Nevertheless, drastic absorption (>95%) was necessary before moderate HLA specificity (SR>5) was revealed. Antibodies to B2M contributed substantially to the original cytotoxicity. This is most clearly shown by passing antiserum through a B2M affinity column, when 90–95% of cytotoxicity was removed; neither was there a notable increase in HLA allospecificity following such treatment.

In spite of this low level of allospecific globulin, such lytic rabbit sera are substantially inhibited by HLA antigens, thereby confirming earlier work⁵ in which nonspecific cytotoxic xenoantisera prepared against H2 or HLA antigens in soluble or cellular form were inhibited only by those areas of a gel filtration chromatogram (of crude soluble membrane proteins) containing mouse H-2D and H-2K alloantigens or human HLA-A, B, and C alloantigens respectively. Rabbits are known to raise antibodies to at least one common

Table 1 Sensitisation of non-primate species to human B2M

Species*	Injected protein per dose (µg)	B2M form	Adjuvant†	Anti-B2M‡ titre	Human lymphocytotoxic 50% Cytotoxic titre§	SR¶
Chicken	50	Free	C,A	5,000	500 (chicken complement)	1.0
Rat	25	Free	C,A	648 ± 233	—	—
Guinea pig	50	Free	C,A	358 ± 275	—	—
Rabbit	50–100	Free	C,A	1,107 ± 407	60 ± 16	1.0
Rabbit	0.5–1.0**	Papain solubilised HLA antigen††	I,A	838 ± 435	889 ± 265	1.2 ± 0.2
Rabbit	Human serum proteins	Free	C,A	0	—	—

*Groups of four or five animals were usually used. When free B2M was used as antigen, progressively lower doses gave correspondingly lower anti-B2M responses. No detectable response was produced in guinea pigs, chickens, or rabbits with repeated injections of less than 5.0 µg B2M in adjuvant.

†C, Complete Freund's adjuvant; A = alum precipitate; I = incomplete Freund's adjuvant.

‡5 µl of ¹²⁵I-B2M solution was added to 8 µl volumes of fivefold dilutions of antiserum in diluent. After incubating at room temperature for 1 h, 0.180 ml 19% Na₂SO₄ solution was added followed by further incubation at room temperature for 1 h. After centrifugation at 1,000g for 5 min, 50% of the supernatant was sampled. Results were plotted as the percentage radionuclide remaining in solution against antiserum dilution in the reaction volume. The titre of an antiserum is the dilution at which 50% of the nuclide is precipitated. Control samples contained diluent in place of antiserum and results were corrected according to the titration value given by a reference chicken anti-human B2M serum.

§Estimated in microtitre plates using 25-µl volumes of lymphocytes, antiserum dilutions, and rabbit complement, and a Trypan blue indication of cell death.

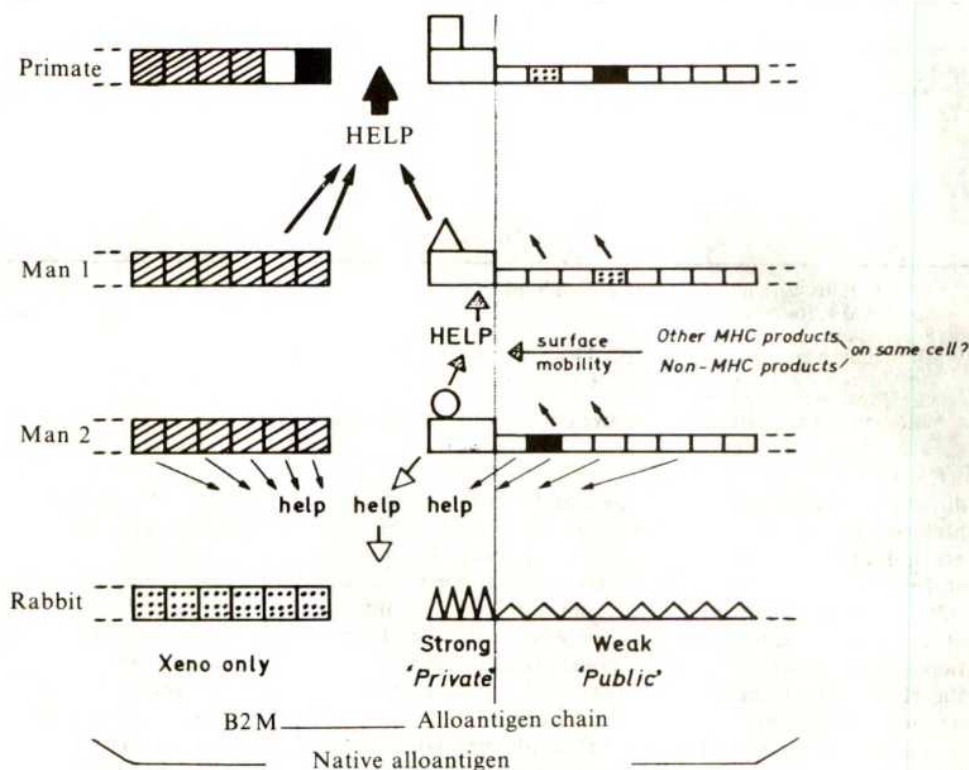
¶SR, Specificity ratio; the ratio of titre with cells positive or negative for an HLA specificity, based on analysis of a population of 20 cell types covering the relevant specificities.

||Values obtained after at least four booster injections in the case of free B2M, but after only two boosts of HLA antigens.

**The protein content of HLA antigen is based on an assumed final purity of 10%, validated by radioimmunoassay of B2M content.

††Specifically purified and separated HLA-A1, A2, B8, and B12 papain-solubilised antigens were used in a total of six rabbits.

Fig. 1 Associative recognition in alloantigens. 'Help' or associative recognition within a species (man 1-man 2) for antibody production to B2M-associated alloantigens occurs in a primary response only when other MHC region products are present at the same time. It seems likely that these products are required to be on the same cell. If spatial relationships are vital in the presentation to recognition units on T cells, then surface mobility, that is, a living membrane will be an equally vital feature of the allogeneic stimulus. These demands disappear when epitopic differences are revealed in the B2M component of alloantigens as in xenogenic but phylogenetically close sensitisations (man-primate). Inherent in this argument is the assumption that the remainder of the alloantigenic glycoprotein is poorly immunogenic for whatever reason, and will remain essentially as weak between related species as it does within species. When the barrier is wide, however (man-rabbit), even this restriction disappears, and enough differences occur between the respective species alloantigens that multiple dual recognitions can arise at various places on the sensitising molecule.



species determinant on the alloantigen-bearing chain of all HLA antigens⁶.

Thus antisera prepared in rabbits, even to purified HLA antigens, are not likely to produce reagents of great value in revealing HLA allospecificity on human lymphocyte targets using lytic assays. The rabbit might be expected to differ from man at several places other than the HLA epitope on the HLA chain, as well as at several places on B2M, so allospecific globulins would comprise only a minor portion of the total antibody formed against the whole alloantigen chain with its attendant B2M. The cytotoxins directed against non-allospecific determinants, both on the HLA chain and on human B2M, can be removed by appropriate absorption with specific immunogen-negative tissue. Such an absorbed antiserum, albeit of low cytotoxic titre, has already been extensively examined (χ^2 with respect to HLA-A1=26.9, cited in ref. 7). What is interesting is that substantially augmented immunogenicity to human B2M is observed when this protein is associated with HLA alloantigen and presented across a species barrier.

The results obtained by immunisation of *Macaca irus* monkeys were quite different. After extensive immunisation with free B2M at doses similar to those which readily produced antibodies in several other non-primate species, much lower humoral immunity was observed with this primate species. Not only were antibody titres lower, but a response was only obtained after six courses of immunisation, compared with two in non-primate species, and the titre has not increased on further immunisation.

Purified HLA substances have previously been shown to be highly immunogenic across this close barrier, producing very high titres of lymphocytotoxic antibodies with a few micrograms of material^{8,9}. The absorbed sera (SR 13.8 \pm 5.6) showed excellent accord with well defined single HLA specificities in population studies. Both the specificity of the unabsorbed sera (SR 4.8 \pm 1.1), and the yield following absorption indicated that anti-HLA globulins formed the majority of the total lympholytic antibody, in contrast to the results obtained with rabbits and shown earlier.

The results of Table 2 indicate that the limited non-

specificity of the primate antisera is largely attributable to their anti B2M content. First, very high titres of anti B2M antibodies were found in the radioimmunoassay. These levels are often substantially higher than those found in non-primate antisera produced by immunisation with much larger quantities of free B2M. (It should be stressed that the amount of B2M administered to primates in HLA-complexed form is 500–1,000-fold less antigen than the dose of free B2M used.) Second, when an animal previously sensitised with HLA-A2 antigen was boosted after 3 yr with free B2M, high levels of anti-B2M were immediately produced, indicative of a pre-existing immunity. Third, all nonspecific cytotoxic activity can easily be removed by a B2M affinity column. In a typical experiment, the anti-HLA activity was recovered in good yield (>90%) following such treatment and was of excellent specificity (SR > 100). These results illustrate that most of the total cytotoxic activity is attributable to specific anti-HLA antibodies, and the remainder is due to nonspecific anti-B2M antibodies. Furthermore, immunosorbents prepared from B2M-absorbed primate monospecific antisera of high titre represent powerful yet simple tools for the purification of HLA antigens from crude starting material, without recourse to prolonged chromatographic separations.

Apart from the mutual augmentation effect of HLA on B2M across a close species barrier, a further interesting feature of all the primate antisera is that they uniformly fail to precipitate B2M either directly or in gel diffusion plates. This is in contrast to the rat, guinea pig, chicken and rabbit antisera, which all precipitate B2M from solution. Preliminary results based on gel filtration of antigen-antibody complexes between primate antisera and B2M indicate that the anti-B2M antibodies are directed against only two sites on the B2M molecule, regardless of whether they are produced in response to free or HLA-linked B2M. This would preclude any lattice formation necessary to produce precipitation with antigen, whereas the radioimmunoassay used here¹⁰ for quantitation of anti-B2M antibodies is not dependent on direct precipitating properties of the antiserum. Rabbit antisera seem to contain antibodies

Table 2 Sensitisation of *Macaca irus* primates to human B2M

Group	Injected protein per dose (μ g)	B2M form*	Anti-B2M titre†	Human lymphocytotoxins 50% Cytotoxic titre‡	SR
1	50–100	Free	93 \pm 13§	8 \pm 2	1.0
2	0.5–1.0¶	Papain-solubilised HLA antigens	2,406 \pm 1,295**	3,250 \pm 1,673	4.8 \pm 1.1

*All injections were in incomplete Freund's adjuvant.

†See Table 1, footnote†.

‡See Table 1, footnote§.

§Detectable responses were only produced after six booster injections.

¶See Table 1, footnote**, the same preparations were used.

||See Table 1, footnote††.

**Responses were detectable after two boosts, titres shown are after three boosts.

directed against three or four sites, and chicken sera detect at least six sites on B2M which would accord with their precipitating properties. The similarity between primate antisera raised either to free B2M or to the HLA-linked B2M in terms of precipitating properties and the number of epitopes recognised, renders it unlikely that conformational changes in B2M are imposed as a consequence of the HLA association and that this results in the augmented immunogenicity noted here.

It may be concluded from these experiments that although free B2M is quite immunogenic across wide species barriers, the immunogenicity declines when the barrier is phylogenetically close. The situation is quite different when B2M bound to HLA antigens is considered. B2M is able to derive immunogenic 'help' by associative recognition, because of its peculiar association with these MHC antigens. Although B2M is not covalently linked to HLA, the bonding is nevertheless strong, in that low pH or very strongly ionic detergents are required to separate the protein chains. The help provided by the alloantigen across a species barrier may thus be intermediate between the two types defined^{11,12}. 'Intramolecular help' refers to a situation where helper and principal determinant are on the same molecule. 'Inter-molecular, intrastructural' which may be somewhat less effective (N. A. Mitchison, personal communication), refers to helper and principal determinants being on different molecules. Because B2M and its attendant alloantigen mobilise jointly on cell surfaces ('capping'), however, and remain tightly bound when solubilised from membrane by enzyme, non-ionic, or weakly ionic detergents, they may for most purposes be considered as being on the same molecule and therefore presented as an immunogenic unit in circumstances such as tissue grafting or the experiments described here with HLA antigen.

Recent evidence (in rats¹³) suggests that within a species, both Ia and AgB antigens must be presented on the same living cell in order to provoke anti-AgB antibodies. Soluble alloantigens, or cellular membranes which do not express Ia antigens, are essentially non-immunogenic in a primary response. Primed animals may be different, however, and it is worth noting that once sensitisation has occurred, help can be provided for particular H2 components by other MHC gene products and/or minor non-MHC antigens¹⁴.

Immunisations across species barriers with purified HLA antigens present quite a distinct contrast. Provided there is some difference at B2M between the species, then even when donor and recipient are phylogenetically close and their MHC antigens might therefore be expected to be quite similar, the high degree of MHC polymorphism is almost certain to ensure a second (HLA) epitopic difference on the two-chain antigen molecule, thereby providing an opportunity for associative recognition and help. It should also be borne in mind that a large proportion of T lymphocytes

is able to recognise MHC antigens within a species^{15,16}, and therefore probably to recognise analogous MHC antigens across a close phylogenetic barrier. It would be expected then that the help provided would be very substantial because of this large existing clone from which T helpers could be derived. This is proposed as the explanation for the high titres of anti-B2M antibodies found here in primates, and it occurs essentially because of the B2M–HLA association. Furthermore, the high degree of allospecificity, especially when anti-B2M antibodies are removed, suggests that the non-alloantigenic portion of the glycoprotein chain is only poorly immunogenic between related species. This conclusion was reached earlier in other primate experiments¹⁷. In dosage terms, the very small amount of soluble alloantigen is as effective outside the species as the same quantity of alloantigen presented in cellular form on viable lymphoid tissue within the species of origin. These inter-relationships are summarised diagrammatically in Fig. 1, where allogeneic, and close or distant xenogeneic immunogenicity is depicted.

In the xenogeneic (mouse–man) primed lymphocyte typing assay it has been found¹⁸ that both the stimulating and target antigens are probably identical to those involved in the allogeneic mouse mixed lymphocyte reaction. The role of B2M was not examined, but when taken in conjunction with the findings reported here, it suggests that the part played by this molecule in xenograft rejection and therefore in species discrimination and interfertility will warrant further investigation.

It is also possible to imply that HLA antigens or their equivalent in other species may serve as 'super carriers' for artificially attached haptens and epitopes when the MHC barrier is crossed within a species or in a closely related species, and this implication is presently under investigation. The examination of what may thus be considered as modified 'non-self' has obvious relevance to the present interest in altered 'self' as a possible mechanism whereby animals combat viruses¹⁹.

ARNOLD R. SANDERSON

McIndoe Memorial Research Unit,
Blond Laboratories,
Queen Victoria Hospital;
East Grinstead,
Sussex, UK

Received 25 March, accepted 2 August 1977.

- ¹ Sanderson, A. R. & Welsh, K. I. *Transplantation* 18, 197–205 (1974).
- ² Peterson, P. A., Rask, L. & Lindblom, J. B. *Proc. natn. Acad. Sci. U.S.A.* 71, 35–39 (1974).
- ³ Cunningham, B. A., Wang, J. L., Berggard, I., & Peterson, P. A. *Biochemistry* 12, 4811–4821 (1973).
- ⁴ Welsh, K. I. & Turner, M. J. *Tiss. Antigens* 8, 197–205 (1976).
- ⁵ Staines, N. A., O'Neill, G. J., Guy, K. & Davies, D. A. *L. Tiss. Antigens* 3, 1–21 (1973).

- ⁶ Tanigaki, N., Nakamura, K., Natori, T., Kreiter, V. P. & Pressman, D. *Transplantation* 18, 74-78 (1974).
⁷ Sanderson, A. R. & Welsh, K. I. *Transplant Proc* 5, 471-475 (1973).
⁸ Sanderson, A. R. & Welsh, K. I. *Transplantation* 16, 304-312 (1973).
⁹ Metzgar, R. S. & Miller, J. L. *Transplantation* 13, 467-471 (1972).
¹⁰ Farr, R. S. *J. infect. Dis.* 103, 239-251 (1958).
¹¹ Rajewsky, K. & Rottlander, E. *Cold Spring Harb. Symp. quant. Biol.* 32, 547-555 (1967).
¹² Mitchison, N. A. *Cold Spring Harb. Symp. quant. Biol.* 32, 431-440 (1967).
¹³ Welsh, K. I., Burgos, H. & Batchelor, J. R. *Ein J. Immun.* (in the press).
¹⁴ Lake, P. & Mitchison, N. A. *Cold Spring Harb. Symp. quant. Biol.* (in the press).
¹⁵ Nisbet, N. W., Simonsen, M. & Zaleski, M. J. *exp. Med.* 129, 459-467 (1969).
¹⁶ Wilson, D. B., Blyth, J. L. & Nowell, P. C. *J. exp. Med.* 128, 1157-1181 (1968).
¹⁷ Sanderson, A. R. & Welsh, K. I. *Transplant Proc* 6, 163-167 (1974).
¹⁸ Peck, A. B., Alter, B. J. & Lindahl, K. F. *Transplant Rev* 29, 189-221 (1976).
¹⁹ Zinkernagel, R. M. & Doherty, P. C. *Nature* 248, 701-702 (1974).

In a radiation chimaera, host H-2 antigens determine immune responsiveness of donor cytotoxic cells

CELL membrane structures controlled by genes in the major histocompatibility complex (H-2 in mice) are involved in most immune interactions between T lymphocytes and other cells¹. Cytotoxic T lymphocytes (CTL) immunised against viruses², haptens³, minor histocompatibility antigens⁴ or tumour antigens⁵, are specific for self H-2 antigens as well as for the foreign antigen. But CTL are not restricted to recognising antigens in combination with only self H-2. H-2^d homozygous CTL which have matured in an irradiated H-2^d/H-2^k host can respond to antigen plus H-2^k in addition to antigen plus H-2^d (refs 6-8). It is not known whether the H-2 environment in which T cells mature influences their range of specificity, that is, whether CTL from a normal mouse can respond quantitatively as well to antigen plus foreign H-2 as they do to antigen plus self H-2. These experiments were designed to test this influence. The results suggest that host H-2 antigens do exert an effect on the specificity of T-cell responses.

A single suspension of bone marrow cells from F₁(BALB/c × BALB B) (F₁(C × C B), H-2^d/H-2^b) mice was used to reconstitute groups of lethally irradiated parental mice, C(H-2^d) ([F₁ → C] chimaeras) and C.B(H-2^b) ([F₁ → C.B] chimaeras). Eight weeks later these chimaeric mice and normal F₁(C × C.B) mice were primed against minor H antigens by injecting 8 × 10⁶ F₁(B10 × B10 D2) (H-2^b/H-2^d) spleen cells. The B10 background offers more than 30 minor histocompatibility antigenic differences that can be recognised by BALB mice^{9,10}. Some weeks later the primed spleen cells were boosted in culture with irradiated F₁(B10 × B10 D2) stimulator cells and assayed for cytotoxicity 5 d later.

Following this immunisation procedure, cells from normal F₁(C × C.B) mice lysed B10 targets and B10 D2 targets almost equally (Table 1). (The two activities are mediated by separate

pools of CTL²⁻⁴) The chimaeras responded differently. In the same conditions of immunisation with F₁(B10 × B10 D2) cells, they responded preferentially to the minor antigens in association with the H-2 antigens of the host CTL from the [F₁ → C] chimaeras killed B10.D2 targets better than B10 targets, whereas CTL from [F₁ → C.B] chimaeras lysed B10 targets better than B10.D2 targets (Table 1).

Spleen cells from five chimaeras were assayed for their content of host and donor cells at time of killing. Complement-mediated lysis with H-2^b anti-H-2^d serum and with H-2^d anti-H-2^b serum indicated that in all cases at least 85% of the cells were of F₁ (donor) origin. The cytotoxic effector cells were also lysed with anti-H-2 serum and complement just before the ⁵¹Cr-release assay (Table 2). Here, the [F₁ → C] chimaera cells lysed B10.D2 targets ninefold more efficiently than they lysed B10 targets (data not shown). The killer cells were treated with antiserum plus complement, washed, and assayed for lysis of labelled B10.D2. Table 2 shows, most importantly, that BALB/c anti-C57BL/6 (anti-H-2^b) serum reduced the cytotoxic activity 86% compared with controls. This antiserum does not lyse BALB/c effector cells. Therefore, at least 86% of the CTL were of F₁ bone marrow origin.

These experiments show that H-2^d/H-2^b cytotoxic cells which mature in an irradiated H-2^d host respond preferentially to antigens plus H-2^d, whereas H-2^d/H-2^b cells which mature in an irradiated H-2^b host respond preferentially to the same antigens in conjunction with H-2^b gene products. The experiments were designed to test the 1971 Jerne hypothesis¹⁴, or a modified version of it^{4,15}. The hypothesis accepts that a somatic theory of generation of receptor diversity is correct and proposes that self-H-2 antigens drive the diversity. Immature T cells first express an anti-self-H-2 receptor, leading to proliferation and to accumulation of V gene mutations until there is no significant reaction with self-H-2. According to this hypothesis, the receptor repertoire of A strain T cells which had matured in an A environment would be quite different from that of A strain T cells which had matured in a B environment. The results presented here are compatible with this hypothesis and with another theory of 'adaptive differentiation'¹.

There is an alternative explanation of the results. It may be that the host haplotype preference seen at the level of effector CTL does not reflect a bias in specificity at the level of precursor CTL. The H-2^d/H-2^b precursor CTL in the H-2^d host may have exactly the same range of reactivity as those in the H-2^b host. The haplotype preference of the effector CTL would then be due to the way antigen is presented to CTL precursors. Even though the immunogen (B10 minor antigens) was introduced on H-2 heterozygous cells, the antigen which was responsible for priming CTL may have been processed antigen presented on radiation resistant host cells⁴. In the [F₁ → C] chimaera such radiation-resistant antigen-present-

Table 1 Specificity of H-2^d/H-2^b cytotoxic cells from normal and chimaeric mice

Responder*	Immunised with†	B10 H-2 ^b	% Specific lysis of targets‡		F ₁ (C × C B) H-2 ^d /H-2 ^b	Ratio of lytic activity on§ B10/B10 D2
			B10 D2 H-2 ^d	B10 BR H-2 ^k		
Experiment 1						
Normal F ₁ (C × C B)(H-2 ^d /H-2 ^b)	F ₁ (B10 × B10 D2)(H-2 ^b /H-2 ^d)	56.2	62.2	1.5	ND	0.7
Chimaera [F ₁ → C]	F ₁ (B10 × B10 D2)(H-2 ^b /H-2 ^d)	19.3	72.7	2.9	ND	0.02
Chimaera [F ₁ → C B]	F ₁ (B10 × B10 D2)(H-2 ^b /H-2 ^d)	51.8	29.1	0.1	ND	5.2
Normal F ₁ (C × C B)	C3H(H-2 ^k)	4.5	3.8	71.5	ND	—
Experiment 2						
Normal F ₁ (C × C B)	F ₁ (B10 × B10 D2)	54.8	68.4	ND	0.9	0.5
Chimaera [F ₁ → C]	F ₁ (B10 × B10 D2)	4.8	80.5	ND	1.5	<0.02
Chimaera [F ₁ → C B]	F ₁ (B10 × B10 D2)	61.3	8.8	ND	0.1	>43.0

*Chimaeras were prepared as follows: BALB/c(C(H-2^d)) and BALB.B(C B(H-2^b)) mice were irradiated with 850 R and reconstituted on the same day with 13.4 × 10⁶ anti-Thy-1 plus complement (C)-treated F₁(C × C B) bone marrow cells. Eight weeks later they were immunised.

†Primed against minor H antigens by injecting 8 × 10⁶ viable F₁(B10 × B10 D2) spleen cells intraperitoneally. Spleen cell suspensions were prepared 4 weeks later (experiment 1) or 6 weeks later (experiment 2), and boosted for 5 d in culture with an equal number of 1,000 R irradiated F₁(B10 × B10 D2) or C3H/He/J spleen cells as described previously¹¹.

‡2-d con A (conavalin A) blasts were labelled with ⁵¹Cr-sodium chromate and used as targets as described previously¹¹. Serial dilutions of the killers were assayed against a constant number of targets, and the figures for specific lysis presented here are for a killer:target ratio of 100:1. Spontaneous release of ⁵¹Cr varied from 18.1-22% in experiment 1 and from 9.6-13.7% in experiment 2.

§Calculated from the titrations of killers:targets^{12,13}. For example, in experiment 1, the [F₁ → C] cells lysed B10 D2 targets 50 times better than they lysed B10 since a 100:1 ratio caused 19.3% specific release from ⁵¹Cr-B10, whereas the same amount of specific lysis of ⁵¹Cr-B10 D2 was obtained at a ratio of 2:1. ND, Not determined.

Table 2 Sensitivity of chimaera cytotoxic cells to anti-H-2 serum plus C'

Cytotoxic cells*	Treatment of effector cells†	% Specific lysis of ⁵¹ Cr-B10 D2‡	% Reduction in lytic activity§
[F ₁ →C] anti-F ₁ (B10×B10 D2)	Medium	34.0	—
	Normal B10 serum + C'	34.3	0
	Anti-H-2 ^b + C'	9.8	86
	Anti-H-2 ^d + C'	5.1	94

*[F₁→C] chimaeras were primed *in vivo* 8 weeks after reconstitution, their spleen cells boosted in culture 20 weeks later and assayed for cytotoxicity on day 5 of culture.

†BALB/c anti-C57BL/6 and C57BL/6 anti-BALB/c sera were prepared by hyperimmunisation with spleen cells. Effector cells were incubated with mouse sera 1:2, washed, and incubated in guinea pig serum 1:9 as a source of complement (C'). Cells were re-suspended to the same volume and assayed.

‡Con A blasts from B10 D2 mice were labelled with ⁵¹Cr and used as target. Data presented are for original number of responder spleen cells: target cells of 7:1. Spontaneous release of ⁵¹Cr was 20.6%.

§Calculated from the titrations of killer: targets as in Table 1.

ing cells would be homozygous H-2^d and would naturally stimulate only anti-B10 D2(H-2^d) CTL, not anti-B10(H-2^b) CTL. Experiments to decide between these interpretations are in progress.

This work was supported by USPHS (grants AI14269 and CA14051).

MICHAEL J BEVAN

Center for Cancer Research and Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received 6 June; accepted 22 July 1977

¹ Katz D H. *Lymphocyte Differentiation, Recognition and Function* (Academic, New York, 1977).

² Doherty P C, Blanden R V & Zinkernagel R M. *Transplant Rev.* 29, 89–124 (1976).

³ Shearer G M, Rehn T G & Schmitt-Verhulst A M. *Transplant Rev.* 29, 222–248 (1976).

⁴ Bevan M J. *Cold Spring Harbor Symp. Quant. Biol.* 41, 519–527 (1976).

⁵ Blank K J, Freedman H A & Lilly F. *Nature* 260, 250–252 (1976).

⁶ Pfizenmaier K *et al.* *J. exp. Med.* 143, 999–1004 (1976).

⁷ Zinkernagel R M. *Nature* 261, 139–141 (1976).

⁸ von Boehmer H & Haas W. *Nature* 261, 141–142 (1976).

⁹ Bailey D W. *Immunogenetics* 2, 249–256 (1975).

¹⁰ Staats J. *Cancer Res.* 36, 4333–4377 (1976).

¹¹ Bevan M J. *J. exp. Med.* 142, 1349–1365 (1975).

¹² MacDonald, H R, Phillips R A & Miller R G. *J. Immunol.* 111, 565–574 (1973).

¹³ Bevan M J. *J. Immunol.* 114, 316–319 (1975).

¹⁴ Jerne N K. *Lin. J. Immunol.* 1, 1–9 (1973).

¹⁵ Nabholz M & Miggiiano V. In *B and T cells in Immune Recognition* (eds Loo F & Roelants G E) (Wiley, Chichester, UK, 1977).

Cytotoxic T lymphocytes induced in mice by inactivated influenza virus vaccine

THERE is abundant evidence that T cell-mediated lysis of virus-infected target cells generally requires H-2K or H-2D region histocompatibility between cytotoxic T lymphocytes (CTL) and target cells^{1–4}. The biological basis of this requirement is still uncertain. Studies using chimaeric mice suggest that T lymphocytes are selectively sensitised by the viral and H-2 antigens expressed on the infected cell and that killing only occurs when the target cell shares H-2K or H-2D identity with the immunising virus-infected cell⁵. It is important to determine whether virus infection of the host tissue is, in fact, necessary for specific CTL immunity to develop. If this were so, the use of inactivated virus or purified viral antigens would not be suitable as vaccines against diseases in which CTL were required to afford optimal protection. We report here that inactivated influenza virus vaccines are, in fact, quite capable of evoking haemagglutinin specific CTL.

Epidemiological and experimental evidence indicate that protective immunity against influenza virus is principally directed against the virus haemagglutinin^{6,7}. We have recently described the development of haemagglutinin-specific CTL in influenza-infected mice⁸. This finding is consistent with these cells playing an important *in vivo* function in influenza immunity. Our finding contrasts with

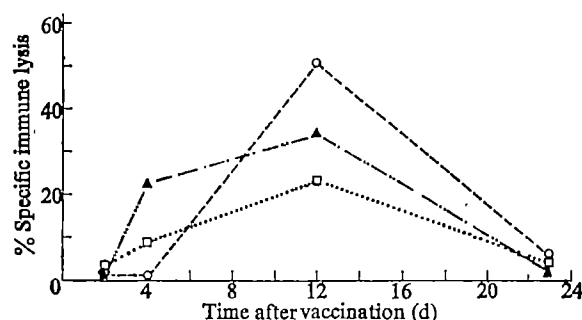


Fig. 1 Specific immune lysis by spleen cells of BALB/c mice immunised intraperitoneally with 5,000 chick cell haemagglutinating units (HAU), (○), 500 HAU (Δ) and 50 HAU (□) of A/Port Chalmers formalin-inactivated whole virus influenza vaccine. The spleen cells were tested at a 100:1 ratio on A/Port Chalmers-infected syngeneic kidney-derived cells in an 18-h ⁵¹Cr-release cytotoxicity assay as described previously¹⁰. The cytotoxic cells generated in vaccine-immunised mice were shown to be T cells by the removal of cytotoxic activity using anti-θ antiserum plus complement. Thus, whereas untreated spleen cells and spleen cells treated with complement alone achieved 42.3 and 37.0% specific lysis respectively, spleen cells treated with anti-θ antiserum plus complement achieved 7.9% specific lysis, which was not significant (*P* > 0.5).

recent studies which failed to demonstrate haemagglutinin-specific CTL in influenza-infected mice^{11–13}. These studies differed from our study, however, in the use as target cells of relatively non-permissive influenza-infected transformed cell lines rather than productively-infected normal tissue-derived target cells¹⁴. In the present study we have similarly tested mice inoculated with influenza vaccine, for haemagglutinin-specific CTL reactive with productively-infected target cells. The virus vaccines used contained an early H3N2 virus strain⁹, A/Aichi/68; a later virus of the same H3N2 subtype, but with a serologically distinguishable haemagglutinin, A/Port Chalmers/73; and an unrelated influenza B virus, B/Hong Kong/72. The target cells were syngeneic kidney-derived infected cells¹⁰. The experiment was carried out in both BALB/c mice (H-2^d) and C3H mice (H-2^k).

Figure 1 depicts the CTL response we observed in BALB/c mice inoculated intraperitoneally with various doses of A/Port Chalmers vaccine (virus vaccine supplied by Merrell-National Laboratories). Significant cytotoxic T-cell response was observed with each of the doses tested. To determine whether the response was restricted to target cells histocompatible with the immunised mice, we tested spleen cells from BALB/c and C3H immunised mice on syngeneic and allogeneic virus-infected target cells. As indicated in Table 1, the cytotoxic response was detectable only on the syngeneic virus-infected target cells. Specificity of the CTL for the haemagglutinin antigen of the immunising vaccine virus was shown in the experiments recorded in Table 2. Thus, CTL-distinguished target cells infected with the immunising strain H3N2 influenza virus, from target cells infected with a serologically different but related

Table 1 Virus specificity of cell-mediated lysis by CTL in influenza vaccine immunised mice

Vaccine virus used as immunogen	Specific immune lysis of target cells*		
	A/Port Chalmers-infected	A/Aichi-infected	B/Hong Kong-infected
A/Port Chalmers/73	96.6	6.2	8.8
A/Aichi/68	5.2	67.0	2.7
B/Hong Kong/72	—6.9	3.2	63.4

*Spleenic lymphocytes were tested 8 d after intraperitoneal immunisation.

Table 2 Allogeneic restriction of cell-mediated lysis by CTL in mice immunised with influenza vaccine

Source of immune lymphocytes*	Specific immune lysis	
	C3H target cells	BALB/c target cells
BALB/c	1.7	21.3
C3H	33.4	1.9

*Splenic lymphocytes were tested 5 d after intraperitoneal immunisation.

H3N2 strain Increasing knowledge is becoming available concerning the structure of the influenza virus haemagglutinin antigen. It should be possible, therefore, to extend the studies reported in this paper to define the precise cellular-antigen interactions involved in the generation of CTL and to determine the basis of the H-2 compatibility requirement between CTL and virus-infected target cells.

The finding that inactivated influenza virus vaccine can induce a haemagglutinin-specific CTL response is in agreement with the recent finding of Schrader and Edelman¹⁵ that inactivated Sendai virus can elicit CTL in primary cultures of mouse spleen cells¹¹, and the observation of Wiktor *et al* that CTL develop in mice inoculated with inactivated rabies virus¹⁶. Together, these findings provide considerable support to the potential development of purified protein vaccines for use against chronic viral diseases and malignancy where T cell-mediated, rather than humoral, immunity seems to be deficient.

We thank Mr S. Daniel for assistance.

FRANCIS A. ENNIS
W. JOHN MARTIN
MARTHA W. VERBONITZ

Division of Virology, Bureau of Biologics,
Food and Drug Administration,
Department of Health, Education
and Welfare,
8800 Rockville Pike,
Bethesda, Maryland 20014

Received 22 June, accepted 8 August 1977

¹ Zinkernagel, R. M. & Doherty, P. C. *J. exp. Med.* 141, 1427 (1975)

² Kosinowski, U. & Ertl, H. *Nature* 225, 552 (1975)

³ Blanden, R. V., Doherty, P. C., Dunlop, M. B. C., Gardner, I. D. & Zinkernagel, R. M. *Nature* 254, 269 (1975)

⁴ Zinkernagel, R. M. *J. exp. Med.* 143, 437 (1976)

⁵ Blank, K. J., Freedman, H. A. & Lilly, F. *Nature* 260, 250 (1976)

⁶ Gomard, E., Duprez, V., Henin, Y. & Levy, J. P. *Nature* 260, 707 (1976)

⁷ Zinkernagel, R. M. *J. exp. Med.* 144, 933 (1976)

⁸ Kilbourne, E. D. in *The Influenza Viruses and Influenza* (ed. Kilbourne, E. D.) 483 (Academic, New York, 1975)

⁹ Schulze, I. T. in *The Influenza Viruses and Influenza* (ed. Kilbourne, E. D.) 53 (Academic, New York, 1975)

¹⁰ Ennis, F. A., Martin, W. J., Verbonitz, M. W. & Butchko, G. M. *Proc. natn. Acad. Sci. U.S.A.* 74, 3006 (1977)

¹¹ Effros, R. B., Doherty, P. C., Gerhard, W. E. & Bennink, J. *J. exp. Med.* 145, 557 (1977)

¹² Doherty, P. C., Effros, R. B. & Bennink, J. *Proc. natn. Acad. Sci. U.S.A.* 74, 1209 (1977)

¹³ Zweerink, H. J., Courtneidge, S. A., Skehel, J. J., Crumpton, M. J. & Askonas, B. *J. Nature* 267, 354 (1977)

¹⁴ Ennis, F. A., Martin, W. J. & Verbonitz, M. W. *J. exp. Med.* (in the press)

¹⁵ Schrader, J. W. & Edelman, G. M. *J. exp. Med.* 145, 523 (1977)

¹⁶ Wiktor, T. J., Doherty, P. C. & Koprowski, H. *Proc. natn. Acad. Sci. U.S.A.* 74, 334 (1977)

Induction of plaque-forming cells in cultured human lymphocytes by combined action of antigen and EB virus

PRIMARY *in vitro* immune responses of human peripheral blood lymphocytes (PBL), desirable for both basic and clinical studies, have been rather difficult to achieve. Positive results were obtained when other stimulatory factors, besides antigen, were given to the cells^{1,2}, and other reports have suggested a critical role of antigen presentation and antigen concentration^{3,4}. In view of the difficulty encountered in stimulating an antibody response with antigen alone,

we have attempted to use Epstein-Barr virus (EBV) together with sheep red blood cells (SRBC) to induce a specific response. Human B lymphocytes possess membrane receptors for EBV^{5,6} and the virus stimulates DNA synthesis in human PBL⁷, so EBV provides a 'nonspecific' stimulus in addition to the specific one given by the antigen. We report here that use of EBV and SRBC in this way consistently induces an anti-SRBC response from human PBL.

Human PBL, from different donors, were cultured in the conditions previously described¹. The source of the virus was the EBV-secreting marmoset lymphoid cell line B 95-8 (ref. 8). Table 1 shows that the appearance of plaque-forming cells (PFC) was a rare and inconsistent event when human PBL were cultured in the presence of SRBC only. Addition of EBV alone led, with some donors, to a measurable but transient appearance of background PFC. But, when both antigen and virus were present in the culture from the very beginning a significant immune response was regularly observed with the majority of donors. Ultraviolet inactivation of the virus did not abrogate this effect. There was no stimulation if the addition of the virus was delayed by 48 h, or if culture supernatant freed of virus was added.

To determine whether the PFC responses were antigen specific, either SRBC or horse red blood cells (HRBC) were added to cultures in the presence of EBV. Each culture was screened separately for PFC using SRBC and HRBC as indicator cells. The results in Table 2 show that the responses obtained were specific for the antigen added. The occasional appearance of nonspecific PFC with some donors could be due either to an antigen-independent response induced by EBV or to a cross-reactivity between the two antigens.

Though the basic phenomenon described above is remarkably reproducible, the kinetics and height of the response vary considerably. PFC levels were very low before day 4 and usually reached a maximum between days 7 and 9. While in some cases PFC were present over 2 or 3 days only, cells of other donors yielded PFC in high numbers over 1 week or more. In some experiments a second burst of PFC (occasionally amounting up to a small percentage of

Table 1 Dependence of anti-SRBC response on antigen and virus

Donor	EBV	SRBC	PFC per well	
			Day 7	Day 9
LG	+	+	292	106
	—	+	4	0
	+	—	90	0
JH	+	+	361	167
	—	+	1	3
	+	—	39	6
AW	+	+	250	1953
	—	+	0	0
	+	—	0	0
JB	+	+	853	973
	—	+	0	4
	+	—	426	60

Human lymphocytes were prepared from heparinised blood by centrifugation on a Ficoll-Urovison gradient. Adherent cells were removed and the non-adherent cells were suspended at a density of 9×10^6 per ml in tissue culture medium according to previously described methods² with a few modifications. Foetal calf serum was substituted with human AB serum, heat inactivated and absorbed three times in the cold with SRBC. The concentration of AB serum was 8% in the medium and 10% in the nutritional mixture. Antigen was added (0.05 ml of 1% SRBC per ml of culture) and the cell suspension was distributed in aliquots of 0.1 ml in the wells of Microtest plates. 50 μ l of 10-fold concentrated virus-stock⁸ were added to each well. The cultures were incubated at 37 °C in the presence of 5% CO₂, and 24 h after initiation 50 μ l of nutritional mixture were pooled in each well. At the time of assay the content of five wells was pooled, washed and plaqued with a modification of the haemolysis in gel method². Figures represent PFC per well.

Table 2 Antigen specificity of the EBV-assisted response

Donor	Antigen in culture	Day 7		Day 8		Day 10	
		SRBC	HRBC	SRBC	HRBC	SRBC	HRBC
BM	SRBC	238	9	580	334	13	2
	HRBC	33	298	25	858	28	244
JR	SRBC	1,125	6	1,410	0	574	0
	HRBC	4	117	18	50	7	5
LG	SRBC	1,422	0	910	0	2,200	0
	HRBC	122	318	71	166	0	89
CP	SRBC	1,134	7	6,400	18	7,500	2
	HRBC	250	741	79	167	33	8
PC	SRBC	59	0	41	0	122	0
	HRBC	5	12	0	55	0	2,400

Procedure as in the legend to Table 1. The cultures stimulated with HRBC received 0.05 ml of 1% HRBC per ml of culture. The AB serum used for these cultures was absorbed three times in the cold with HRBC. Figures represent PFC per well.

the recovered viable cells) arose beyond day 10, suggesting a biphasic event (data not shown). Moreover, comparing different culture wells of the same preparation, large variability was observed in some experiments but not in others. There were also significant differences between donors: among the 35 tested, some gave consistently high responses in up to six experiments over a period of 8 months, while others responded poorly and a few not at all. Whether this is due to technical reasons or reflects differences in the immune status of the donor is under investigation. There is apparently no correlation between the capacity to respond in the present system and previous exposure to EBV, as judged by the presence of anti-viral antibodies in the serum.

The mechanism of action of EBV is a matter for speculation, but any model would have to consider either direct action of the virus on B cells or induction of helper T cells.

In the presence of antigen the binding of EBV to its receptor could provide a sufficient signal for B-cell triggering, and bypass the requirement for T-cell help. In its extreme form this concept would attribute to the EBV receptor a role in the binding of physiological T-cell factor(s)⁸. But, what we observed could just as well be an instance of a much more general phenomenon: the threshold for B-cell induction is lowered by the binding of some agent to its appropriate receptor on the cell surface. It has been shown¹⁰ that binding of an antibody directed against a surface component on mature murine B lymphocytes markedly enhances the appearance of PFC in conditions of suboptimal antigen doses and can substitute for T-helper activity. Furthermore, EBV can act as a polyclonal activator¹¹. Its mode of action may therefore be analogous to the stimulatory effect of lipopolysaccharide (LPS) on the immune response to various antigens in mice¹².

The alternative model implies that EBV indirectly activates T cells which would then provide, in the presence of antigen, sufficient help for B-cell induction. Support for this interpretation comes from the clinical syndrome of infectious mononucleosis. During the acute phase of this EBV-induced disease mostly thymus-derived lymphocytes proliferate and appear in the peripheral blood and in the T-dependent areas of lymph nodes¹³. It seems that the stimulus for the T-cell response comes from virus-determined structures on B cells⁹. A similar mechanism in our *in vitro* system could provide sufficient T-cell help to promote the specific antigen-dependent response. Experiments are in progress to determine the mode of action of EBV by culturing separately purified populations of T and B lymphocytes.

We thank Ms Lotte Kuhn for technical assistance, Drs J. Zeuthen and G. Klein for providing the B95-8 cell line,

and Drs H. Cosenza, S. Fazekas de St Groth and S. de Petris for useful discussions.

A. L. LUZZATI
H. HENGARTNER
M. H. SCHREIER

Basel Institute for Immunology,
487 Grenzacherstrasse,
Postfach 4005 Basel 5, Switzerland

Received 23 May; accepted 15 August 1977.

1. Mann, P. L. & Falk, R. E. *Proc. Leucocyte Cult. Conf.* 7, 39-48 (1973).
2. Luzzati, A. L., Taussig, M. J., Meo, T. & Pernis, B. J. *Expl Med.* 144, 573-585 (1976).
3. Delfraissy, J. F., Galanaud, P., Dormont, J. & Wallon, C. J. *Immun.* 118, 630-635 (1977).
4. Dosch, H. M. & Gelfand, E. W. J. *Immun.* 118, 302-308 (1977).
5. Jondal, M. & Klein, G. J. *exp. Med.* 138, 1365-1378 (1973).
6. Greaves, M. F., Brown, G. & Rickinson, A. B. *Clin. Immun. Immunopath.* 3, 514-524 (1975).
7. Gerber, P. & Hoyer, B. H. *Nature* 231, 46-47 (1971).
8. Miller, G. & Lipman, M. *Proc. natn. Acad. Sci. U.S.A.* 70, 190-194 (1973).
9. Greaves, M. F. *Scand. J. Immun.* 5, Suppl. 5, 113-123 (1976).
10. Huber, B., Gershon, R. K. & Cantor, H. J. *exp. Med.* 145, 10-20 (1977).
11. Rosén, A., Gergely, P., Jondal, M., Klein, G. & Britton, S. *Nature* 267, 52-54 (1977).
12. Watson, J., Trenkner, E. & Cohn, M. J. *exp. Med.* 138, 699-714 (1973).
13. Carter, R. L. *Lancet* i, 846-849 (1975).

EB virus-induced B lymphocyte cell lines producing specific antibody

EPSTEIN-BARR virus (EBV) is a lymphotropic herpesvirus that converts normal human B lymphocytes into established lines. This 'immortalisation' preserves the characteristics of the original B cell, including EBV receptors, complement receptors, surface immunoglobulin and secretory immunoglobulin. Surface immunoglobulin is most frequently IgM. EBV-immortalised lines carry multiple copies of the viral genome, detected by nucleic acid hybridisation, and regularly express an EBV-specific nuclear antigen, EBNA. It has been suggested¹ that all B-cells carry EBV receptors. If so, and if all B cells can be transformed into lines by EBV, it should be feasible to establish permanent lines from B lymphocytes capable of producing specific antibodies against appropriate antigens. Clearly, in view of the polyclonal transformation obtained after infection of B lymphocytes with a transforming virus strain, for example, B95-8, it will be necessary to pre-select lymphocytes with the appropriate antigen combining site. Rosetting with antigen-coupled erythrocytes is one of the conceivable methods for such pre-selection. While this will select lymphocytes with the appropriate surface immunoglobulin receptors, it was recently shown that EBV-transformation activates normal lymphocytes and induces the release of secretory immunoglobulin². If the procedure is successful, the established lines might therefore be expected to carry the appropriate surface receptor and also to secrete the corresponding antibody. This was actually found in this study, opening the way to the establishment of cell lines producing specific antibodies of choice.

We have selected as lymphocyte donors three members of the laboratory staff in Helsinki who were shown to have relatively high natural antibody titres against the synthetic hapten NNP (4-hydroxy-3,5-dinitrophenacetic acid) and whose peripheral blood lymphocytes showed specific NNP rosetting.

Lymphocytes were isolated on Ficoll³ and rosetted with autologous-NNP coupled erythrocytes⁴. NNP-azide, prepared according to Brownstone *et al.*⁵ was coupled to erythrocytes using a hapten concentration of 2 mg ml⁻¹ in the final conjugation mixture with the erythrocytes⁶. The percentage of rosettes ranged between 0.08 and 0.6% of the total lymphocytes. The rosettes were spun down on Ficoll-Isopaque (density 1.077) for 20 min at 400g to remove the non-rosetting cells, and then infected with B95-8-derived EBV. As the virus source, we used supernatants of

Table 1 Anti-NNP rosette and plaque formation by EBV-transformed lines derived from NNP rosetting and unfractionated cells, respectively

Cell lines	Source of EBV-transformed explant	% RFC*	% PFC†	% Ig positive cells‡	EBNA staining§
EP	NNP selected	15	11	100	+
LP	NNP selected	13	ND	100	+
SP	NNP selected	18	ND	100	+
S (Unf)	Unfractionated	0	0	100	+
L (Sup)	NNP rosette deprived	0	0	100	+
E (Unf)	Unfractionated	0	0	100	+
EP re-selected	NNP re-selected	86	69	100	+

*Rosette-forming cells (RFC): 5×10^6 cells in 1 ml were washed and mixed with 0.01 ml human NNP-coupled erythrocytes, spun down for 15 min by low velocity centrifugation at 4°C, and incubated further for 30 min at 4°C. The % of rosettes was assessed after resuspension of the pelleted cells. No rosettes were formed with uncoupled erythrocytes.

†Plaque-forming cells (PFC): equal volumes of 1/5 guinea pig serum, 10% NNP-coupled erythrocytes and different numbers of cells were mixed and inserted between two slides⁸. Plaques were counted 90 min later. The plaques were inhibited if 10^{-4} M NaN₃ was added to the mixture. No plaques were detected with uncoupled erythrocytes. ND, not determined.

‡Cells were washed and stained with FITC-labelled rabbit anti-human Ig.

§EBV-determined nuclear antigen, stained according to Reedman and Klein¹². Staining was 90–100% in all positive cultures.

||EP, LP, and SP are NNP-receptor positive lines established from three donors (E, L, and S) as described in the text. S(Unf) and E(Unf) are lines established from EBV infected unfractionated lymphocytes of donor S and E. L (Sup) is a line established from EBV infected lymphocytes, deprived of NNP-receptor positive cells. 'EP re-selected' is a line re-selected by NNP-erythrocyte rosetting of the EP line.

mycoplasma-free B95-8 cultures: 1 ml of the supernatant induced 20% EBNA positive cells in 10^6 cells of the BJAB line⁷ after 48 h incubation. As controls we infected either unfractionated lymphocytes from the same donor or the NNP-rosette-deprived fraction. The infected cells were incubated in RPMI-20% foetal calf serum, at 37°C, 5% CO₂. Permanent lines were established from all three donors after approximately 4 weeks. All viable cells of all lines were brilliantly EBNA positive and grew with a doubling time of approximately 24 h.

The established lines were tested for rosette formation and for plaque formation with NNP-coupled human erythrocytes⁸. The supernatants were assayed for specific NNP agglutination titre and for inhibition of plaque formation with 4-hydroxy-5-iodo-3-nitrophenacetic acid coupled T4 phage (NIP-cap-T4) (refs 9, 10). NIP show >90% cross

reactivity with the NNP-hapten¹¹. The immunoglobulin class of the product was assayed by sucrose gradient sedimentation and by incubating NNP-coupled erythrocytes in the supernatants from the different lines, followed by staining with FITC-labelled anti-human immunoglobulin sera.

As shown in Table 1, the cell lines derived from the EBV transformation of the NNP-rosetting fraction contained 13–18% NNP rosetting cells, whereas the lines derived from unfractionated cells or cells remaining in the supernatant after NNP rosetting gave no specific rosettes. EP line was re-selected by renewed rosetting. In this re-selected line, the frequency of the rosette forming cells rose to 86%. The same line also formed 69% NNP-specific plaques. Plaque formation was inhibited by sodium azide, confirming that it must have been due to active secretion. Since the frequency of plaque-forming cells nearly equalled the

Table 2 Summary of anti-NNP antibody tests with culture supernatants

Supernatant*	Source of EBV-transformed explant	NNP-Agglutination titre†	Haptenated phage inactivation titre	Haptenated phage inactivation titre after 2ME treatment§	NNP-coupled erythrocytes exposed to culture supernatants, stained for				
					Ig	IgM	IgG	lambda	kappa
EP	NNP selected	256	ND	ND	+	+	—	—	+
LP	NNP selected	64	86	<3	+	+	—	—	+
SP	NNP selected	64	981	<3	+	+	—	—	+
E (Unf)	Unfractionated	0	ND	ND	—	—	—	—	—
L (Sup)	NNP deprived	0	ND	ND	—	—	—	—	—
S (Unf)	Unfractionated	0	ND	ND	—	—	—	—	—
EP re-selected	NNP re-selected	1,024	4,070	<3	+	+	—	—	+
RPMI-10% FCS		0	8	<3	—	—	—	—	—

*As in Table 1. ND, not determined.

†Aliquots of 0.1 ml of twofold dilutions of 0.22-µm filtered supernatants in BSS were mixed with 0.02 ml of 2% NNP-coupled erythrocytes. Agglutination was read after 60 min at 37°C. No agglutination was detected with uncoupled erythrocytes.

‡Aliquots of 0.3 ml of supernatant dilutions (filtered through 0.22-µm filter) were mixed with 0.3 ml of NIP-cap-T₄ suspension containing 200 PFU (plaque-forming units) and incubated for 4 h at 37°C. 5 ml 0.7% fluid agar containing about 10^8 *Escherichia coli* was then added, poured on to a solid 2% agar plate, and incubated overnight at 37°C. The titres shown are the reciprocals of the dilution causing 50% inactivation of haptenated phages.

§Supernatants were treated with 0.3 M 2-mercaptoethanol for 60 min at 37°C before mixing with the NIP-cap-T₄ phage.

||NNP-coupled erythrocytes were incubated with the culture supernatants for 60 min, at 37°C, washed and stained with FITC conjugated antisera. Uncoupled erythrocytes showed no staining after the same treatment. The following FITC conjugates were used: (1) rabbit anti-human IgG, IgA, IgM, kappa, lambda, (2) anti-IgM, specific for μ chains, (3) anti-IgG specific for Fc fragment; (4) anti-kappa, light chains (Bence-Jones); (5) anti-lambda, light chains (Bence-Jones). They were all products of Dakopatts, Denmark. The following cell lines were included as positive and negative controls in each test; BJAB¹⁴: IgM-kappa positive; Ramos¹⁴: IgM-lambda positive; Rael¹⁵: IgG-lambda positive.

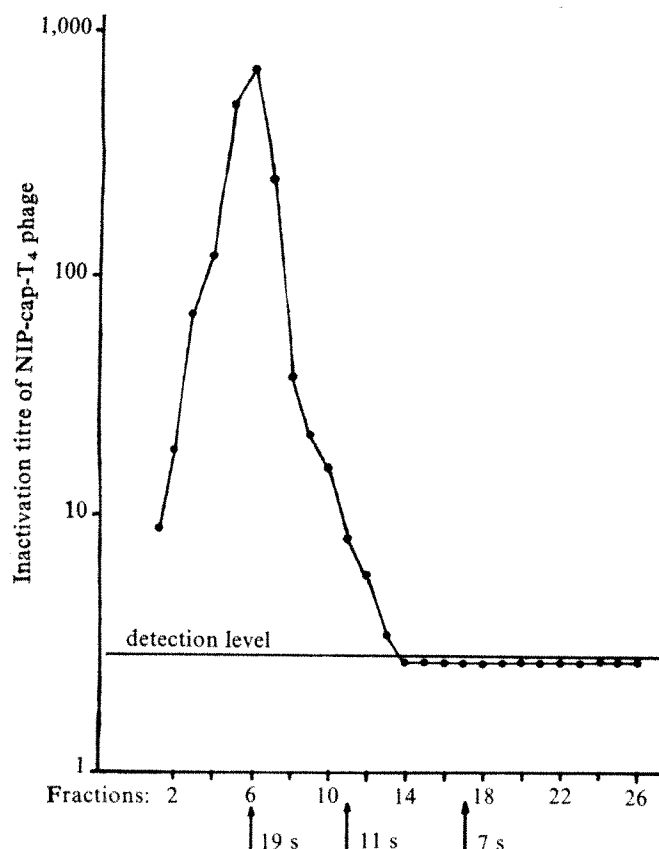


Fig. 1 EP culture supernatant was run on 5–20% sucrose gradient, Beckman SV41 39,000 r.p.m. for 13 h. The fractions were tested for inhibition of NIP-cap-T₄ phage plaque formation on *E. coli* as described in Table 2. NIP shows >90% cross reactivity with the NNP hapten.

frequency of rosette-forming cells, the PFC and RFC forming populations were either identical or largely overlapping.

The agglutination titre of the supernatants ranged between 64 and 1,024. The 50% plaque inhibition titre with the NIP-cap-T₄ phage ranged between 86 and 4,070. All inhibitory activity was destroyed after treating the supernatant with 2-mercaptoethanol, indicating that the antibody was IgM, or at least not IgG.

Table 2 shows the results of all immunological tests, including staining of NNP-coupled erythrocytes with the culture supernatants, followed by FITC-conjugated anti-immunoglobulin reagents, as indicated. All three NNP-selected, transformed lines released anti-IgM-kappa antibody, according to the erythrocyte staining test.

Figure 1 shows a sucrose gradient pattern of the EP supernatant. It is clear that the specific plaque inhibition peaked at 19S. This is in line with the NNP-coupled erythrocyte staining test.

Since EBV-transformed lymphoblastoid cell lines are polyclonal for a long time after their establishment¹², the lines will have to be cloned before it is possible to say whether 100% antibody producing cells can be obtained in this way.

These results show that specific antibody-forming human cell lines can be established by a combination of B-lymphocyte selection, based on specific antigen affinity, and subsequent EBV immortalisation. It should be possible to manufacture lines, selected for the production of particularly interesting antibodies. Monospecific anti-HLA activity would be an obvious example.

This work was supported by contract NO1-CP-33316 of the virus cancer program of the USNCI, by the Swedish Cancer Society and the King Gustaf V Jubilee Fund.

Michael Steinitz is the recipient of a fellowship from the US Cancer Research Institute.

MICHAEL STEINITZ
GEORGE KLEIN

Department of Tumor Biology,
Karolinska Institutet,
S-104 01 Stockholm 60,
Sweden

SAJJI KOSKIMIES
OLLI MAKEL

University of Helsinki,
Department of Serology and Bacteriology,
Helsinki 29, Finland

Received 17 June; accepted 10 August 1977.

- Greaves, F. M., Brown, G. & Rickinson, A. B. *Clin. Immun. Immunopath.* **3**, 514–524 (1975).
- Rosén, A., Gergely, P., Jondal, M., Klein, G. & Britton, S. *Nature* **267**, 52–54 (1977).
- Böyum, A. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 77–89 (1968).
- Osoba, D. *J. exp. Med.* **132**, 368–383 (1970).
- Brownstone, A., Mitchison, N. A. & Pitt-Rivers, R. *Immunology* **10**, 465–479 (1966).
- Koskimies, S., Sarvas, H. & Kaartinen, M. *Immun. Commun.* **2**, 1–14 (1973).
- Klein, G., Sugden, B., Leibold, W. & Menezes, J. *Intervirology* **3**, 232–234 (1974).
- Cunningham, A. J. & Szenberg, A. *Immunology* **14**, 599–600 (1968).
- Mäkelä, O. *Immunology* **10**, 81–86 (1966).
- Becker, M. & Mäkelä, O. *Immunochimistry* **12**, 329–331 (1975).
- Karjalainen, K. & Mäkelä, O. *Eur. J. Immun.* **6**, 88–93 (1976).
- Béchet, J. M., Fialkow, P. J., Nilsson, K. & Klein, G. *Expl Cell Res.* **89**, 275–282 (1974).
- Reedman, B. M. & Klein, G. *Int. J. Cancer* **11**, 499–520 (1973).
- Yefenof, E. & Klein, G., *Expl Cell Res.* **99**, 175–178 (1976).
- Klein, G., Dombos, L. & Gothoskar, B. *Int. J. Cancer* **10**, 44–51 (1972).

Interferon-induced transfer of viral resistance between animal cells

MANY animal cell types exhibit the ability to communicate between themselves, both *in vivo* and *in vitro*¹. This phenomenon is thought to occur through gap junctions which allow cells to share their metabolites and small control molecules^{2–6}. The overall effect of this cellular communication seems to be a coordinated control of growth and function of different cells and cell types. Interferon is believed to act at the cell membrane in a fashion similar to polypeptide hormones^{7–9}. This membrane interaction in turn leads to de-repression and production of the antiviral protein^{10,11}. If, as is the case with polypeptide hormones¹², the induction of the antiviral protein is mediated through secondary molecules, these might influence adjacent cells. The presence of such intermediary molecules during interferon induction of the antiviral protein has been inferred from kinetic studies^{13,14}. The many instances of the species specificity of interferon action¹⁵ make this a testable hypothesis. We show here that cells made resistant to virus infection by treatment with their homologous interferon can transfer viral resistance to cells of a heterologous species insensitive to that interferon and also to cells of the homologous species.

The experimental plan was to determine whether interferon treated mouse L cells could transfer their antiviral resistance to co-cultivated heterologous cells. Mouse L cells were cultured in various ratios with either human (WISH) or baby hamster kidney (BHK) cells in the presence or absence of mouse C243 cell interferon. Controls consisted of an equivalent total number of either cell species alone in the presence or absence of mouse interferon.

Table 1 shows that whereas WISH or BHK cells alone are not sensitive to the action of mouse interferon, co-cultivation of these cells in the presence of mouse interferon and sensitive mouse L cells resulted in a marked inhibition of the expected yield of virus from the interferon-insensitive cells. Control cell mixtures, in the absence of interferon (Table 1, experiments 1 and 3), yielded at least the expected amount of virus as compared with virus yield from either cell type alone. These data indicate

Table 1 Interferon-induced transfer of viral resistance between heterologous animal cells

Cell ratio (2.25×10^5 cells per well)	Mouse interferon (7,500 PDD ₅₀ U ml ⁻¹)*	Experiment 1			Experiment 2			Experiment 3		
		log ₁₀ virus yield Observed	log ₁₀ virus yield Expected	log ₁₀ inhibition†	log ₁₀ virus yield Observed	log ₁₀ virus yield Expected	log ₁₀ inhibition†	log ₁₀ virus yield Observed	log ₁₀ virus yield Expected	log ₁₀ inhibition†
L cell: WISH (2 : 1)	+	ND			4.2	5.28	1.08	ND		
	—	ND			ND			ND		
L cell: WISH (1 : 1)	+	3.80	5.75	1.95	4.5	5.46	0.96	4.88	6.63	1.75
	—	6.05			ND			6.93		
L cell: WISH (1 : 2)	+	ND			4.98	5.58	0.60	ND		
	—	ND			ND			ND		
L cell: BHK (2 : 1)	+	3.00	5.28	2.28	ND			ND		
	—	5.76			ND			ND		
L cell: BHK (1 : 1)	+	3.70	5.41	1.71	ND			4.41	6.55	2.14
	—	5.71			ND			6.85		
L cell	+	< 3.00		> 2.69	< 4.5		> 1.47	< 3.00		> 3.95
	—	5.69			5.97			6.95		
WISH	+	5.16		0.19	5.76		0.07	6.00		0.11
	—	5.35			5.83			6.11		
BHK	+	5.37		-0.02	ND			6.47		0.08
	—	5.35			ND			6.59		

Mouse L cells and human WISH cells or baby hamster kidney (BHK) cells in Eagle's medium (supplemented with 10% foetal calf serum) were cultured in various ratios in Micro Test II tissue-culture plates (Falcon Plastics, Oxnard, California). The total number of cells in each well (about 28 mm²) was 2.25×10^5 . Controls consisted of an equivalent total number of either cell species alone. Interferon (final concentration 7,500 PDD₅₀ U ml⁻¹) or an equal volume of medium was added; cultures were incubated overnight at 37 °C in a 4% CO₂ atmosphere. Supernatant fluids were decanted and each well infected with 5×10^4 p.f.u. of VSV. After 1.5 h at 37 °C, inoculum was decanted, cell sheets were washed once and replenished with fresh medium. Virus yields from pooled triplicate cultures were determined approximately 24 h later by a slightly modified microplaque assay in which methylcellulose (0.5%) was substituted for carboxymethylcellulose¹⁹. Since interferon-treated L cells produce a negligible amount of virus, the expected yield is calculated from the percentage of the WISH cells in a cell mix without interferon (experiments 1 and 3) or a percentage of the heterologous cell yield with interferon (experiment 2).

*The 50% plaque-depressing dose (PDD₅₀) is defined as the amount of an interferon preparation that inhibited 50% of the plaques from developing as compared with controls. In our assay system, i U of National Institutes of Health (NIH) mouse reference interferon preparation had a titre of 2 PDD₅₀ U.

†Any difference greater than 0.5 log₁₀ is significant at $P < 0.05$.

that the presence of interferon with its homologous cell can induce antiviral activity in heterologous cells. The controls show that inhibition of virus yield does not result from co-cultivation of different cell species but results from the presence of the mouse interferon preparation with the co-cultivated cells. In addition, the degree of inhibition of virus yield in mixed cultures in the presence of mouse interferon seems to be directly related to the ratio of L cells to WISH or BHK cells (Table 1, experiments 1 and 2); that is, the higher the proportion of L cells the more inhibition of virus yield from the heterologous cells.

Initially, it was important to determine that the observed transfer of viral resistance from L cells to heterologous cell species was initiated by mouse interferon and also that the transferred resistance had the characteristics of interferon action.

Table 2 shows that when partially purified mouse L-cell interferon, 100 PDD₅₀ U ml⁻¹ (10^7 U per mg protein), were incubated with a 1:1 mix of L and WISH cells there was 90% inhibition of the expected virus yield. Thus a partially purified interferon preparation caused transfer of resistance. Further, interferon produced by mouse L cells was as effective as that produced in C243 cells.

One hallmark of interferon action is its sensitivity to inhibition by actinomycin D. L cells were treated for 1 h at room temperature with actinomycin D $5 \mu\text{g ml}^{-1}$, washed to remove the unbound antibiotic and mixed with an equal number of untreated WISH cells in the presence or absence of mouse interferon. Control experiments had shown that actinomycin D $2 \mu\text{g ml}^{-1}$ caused 97% inhibition of RNA synthesis in 15 min. As seen in Table 2, actinomycin D blocked both the action of mouse interferon on L cells and the ability of L cells to transfer viral resistance to WISH cells.

Another criterion of interferon action is its inhibitory effect on a wide range of viruses. At the low multiplicity of infection (MOI) usually used, vaccinia virus was inhibited by mouse interferon in L cells alone (98.5%) as

well as in the 1:1 L cell: WISH cell mixture (93.3%) (Table 2). In higher MOI conditions (5×10^4 p.f.u. per well), vaccinia virus was not sensitive to mouse interferon in L cells alone, and transfer of resistance to WISH cells was not observed (data not shown). In our experience, therefore, the susceptibility of vaccinia virus to mouse interferon in L cells is highly dependent on the input MOI. Likewise, the ability of L cells to transfer viral resistance to WISH cells was similarly dependent on the input MOI. These data also show that interferon induced transfer of viral resistance can be demonstrated for both a DNA (vaccinia virus) and a RNA (vesicular stomatitis virus, VSV) virus in parallel with their sensitivity to interferon.

Taken together, Table 2 results strongly suggest that initiation of the transfer process was due to mouse interferon, since it occurred with crude as well as highly purified interferon preparations. Its action was on the cell rather than the virus, since resistance was manifested in the absence of interferon but in the presence of cells which had been acted on by interferon; and the transfer process was initiated by mouse interferon produced in two different cell lines (L and C243). These two interferon preparations have been shown to have all the classic characteristics of interferon (that is, species specificity, broad virus specificity, requirement of RNA and protein synthesis for action, inhibition by anti-mouse interferon antisera, non-dialysability, trypsin sensitivity, pH 2 resistance and so on)¹³. The results also indicate that transferred resistance has the characteristics of the interferon system in that it was blocked by actinomycin D, was effective against both a RNA (VSV) and a DNA (vaccinia virus) virus, and that the level of transferred resistance showed the same MOI dependency as resistance in the homologous cell.

If transfer of resistance is dependent on cell proximity, then the degree of transfer should be controlled by both the donor (L) to receptor (WISH or BHK) cell ratio (shown in Table 1) as well as the absolute cell density

Table 2 Characteristics of the transfer of viral resistance

Cells (2.25×10^5 cells/well)	% Inhibition of expected virus yield (P^*) after treatment with:			
	Purified L cell interferon ($100 \text{ PDD}_{50} \text{ U ml}^{-1}$) + VSV	Actinomycin D ($5 \mu\text{g ml}^{-1}$) + VSV	VSV	Vaccinia virus (1×10^4 p.f.u. per well)
L cell: WISH cell (1 : 1)	90 (< 0.025)	0 (> 0.5)	78 (< 0.05)	93.3 (< 0.01)
L cells	> 99.98 (< 0.025)	56 (> 0.2)	99.8 (< 0.025)	98.5 (< 0.025)
WISH cells	0 (> 0.5)	0 (> 0.5)	0 (> 0.5)	8 (> 0.5)

For experimental conditions see Table 1.

*Statistical analysis was carried out using Students *t* test.

at a given ratio. To test the variable of cell concentration the effect of variation of cell density of a constant ratio of L and WISH cells was studied. Specifically, L and WISH cells each at a concentration of 1.5×10^6 cells ml^{-1} were serially diluted in twofold steps. L cells at each dilution were mixed 1 : 1 with WISH cells of the same dilution and the cell mixtures were plated in the presence or absence of mouse interferon ($7,500 \text{ PDD}_{50} \text{ U ml}^{-1}$). It was found that at an L cell-WISH cell ratio of 1 : 1, the demonstration of transfer of viral resistance was dependent on the absolute cell density (Fig. 1a). Interferon-induced transfer of viral resistance was not observed until the majority of cells were in close contact with neighbouring cells.

Since our initial experiments were carried out using very high levels of interferon ($100 \text{ PDD}_{50} \text{ U ml}^{-1}$), it was important to determine if transfer of viral resistance could be demonstrated at more physiological concentrations. Mouse L cells were cultured in a 1 : 1 ratio with WISH cells (cell number per well = 2.25×10^5) in the presence or absence (control) of different concentrations of mouse interferon or in its absence. Other controls consisted of an equivalent number of either cell type alone in the presence or absence of mouse interferon. Fig. 1b shows that transfer of viral resistance requires levels of interferon above $5 \text{ PDD}_{50} \text{ U ml}^{-1}$. Almost maximum transfer of attainable resistance can be demonstrated with $15 \text{ PDD}_{50} \text{ U ml}^{-1}$ of mouse interferon indicating that in the experimental conditions there is a limit to the degree of resistance which can be transferred. These data show that whereas transfer of viral resistance to heterologous cells requires more interferon than development of resistance in homologous cells the process is, nonetheless, fairly efficient in terms of the concentrations of interferon required. The concentrations of interferon necessary for transfer are well within physiological limits.

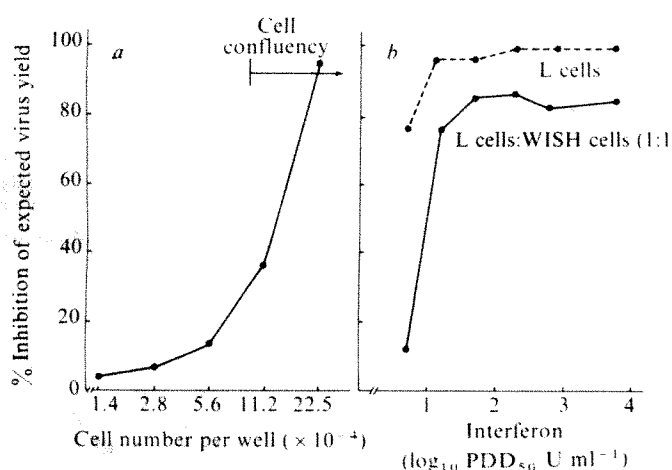
Although the exact mediator of the transfer of viral

resistance is unknown at present, it seems clear that it does not result from production of interferon by the heterologous cells in the mixture. The conclusion is based on two observations. First, interferon is thought to be externalised from a cell before acting¹⁶; however, in a mixture of 1 L : 1 WISH cell with mouse interferon, transfer of viral resistance occurred in the presence of antisera to human fibroblast interferon (enough to neutralise $300 \text{ PDD}_{50} \text{ U ml}^{-1}$ of any interferon which might be produced, data not shown). Second, transfer of viral resistance can occur from L cells to a line of African Green Monkey kidney cells (Vero) which have lost the ability to synthesise interferon (data not shown)¹⁷.

Another possibility is that human WISH cells are made sensitive to mouse interferon by co-cultivation with mouse L cells. Preliminary experiments indicate that this is probably not the case, since L cells treated with mouse interferon and washed extensively to remove the interferon can transfer resistance to WISH cells that are added after interferon have been removed (data not shown).

There are several implications of interferon-induced transfer of viral resistance between animal cells. First, some part(s) of the interferon system are apparently not 'species' specific because interferon-treated mouse L cells can transfer viral resistance to human WISH and baby hamster kidney cells. Second, the natural mechanism of interferon protection may include action on cells near the interferon-responding cell by means of this transfer mechanism. Such an idea has been proposed¹⁸ to explain the shallowness of the slopes of interferon dose-response curves¹⁸. This type of secondary action would amplify the efficiency of the interferon system. Third, since cell lines differ in the rate at which they develop resistance in response to interferon, one might expect in conditions of transfer of resistance in mixed populations of cells as occurs *in vivo*, more slowly responding cells might be influenced by cells which respond more rapidly to interferon. In other words, the rate of development of interferon-induced resistance in a mixed population of cells would be determined by the first responding cell type. In fact, in carefully controlled kinetic studies human WISH cells treated with $30 \text{ PDD}_{50} \text{ U ml}^{-1}$ human interferon developed significant resistance (50% inhibition of virus yield) to virus infection after 30 min treatment whereas human amnion U cells required 2.5 h to develop an equivalent degree (58% inhibition of virus yield) of resistance. When these two cell lines were mixed in a 1 : 1 ratio, the mixed population of cells developed significant viral resistance (63% inhibition of virus yield) after 30 min. That is, the mixed population assumed the kinetics of development of viral resistance of the more rapidly responding of the two cell types rather than an average (data not shown). This phenomenon would also tend to amplify the efficiency of the interferon system. Furthermore, this finding suggests that interferon-induced transfer of viral resistance occurs between cells of the same species and supports the notion of a function *in vivo* for transfer of viral resistance. A second example of homologous transfer of resistance is provided by our finding that interferon-treated L cells, which have been washed and treated with excess antiserum

Fig. 1 Effect of cell density (a) and interferon concentration (b) on transfer of viral resistance between L cells and WISH cells. Since interferon-treated L cells produce a negligible amount of virus, the percentage inhibition of expected virus yield is calculated by dividing the yield from an interferon-treated L cell-WISH cell mix (1 : 1) by half of the yield from a similar but untreated L cell-WISH cell mix, times 100.



to mouse interferon, can transfer viral resistance to untreated L cells (data not shown).

A fourth implication is that interferon-induced transfer of viral resistance may represent a system for studying transfer of biological activity between cells. Finally, the interaction of interferon at the cell surface may cause the formation of a chemical intermediate which is responsible for induction of the antiviral protein. Such a chemical mediator might be passed from cell to cell with a resultant transfer of viral resistance. Preliminary experiments have indicated that cell-free supernatant fluids from interferon-treated L cells can transfer viral resistance to human WISH cells. The elucidation of the nature of the mediator of transfer of viral resistance would greatly enhance our understanding of the induction process for the antiviral state. Should this mediator prove to be a simple molecule, the potential for control of viral infections is obvious.

In summary, we have shown that interferon-treated mouse L cells can transfer their antiviral resistance to co-cultured heterologous (human WISH or hamster BHK) cells which are insensitive to mouse interferon. Transfer of viral resistance seems to be initiated by interferon itself. Once transferred, the viral resistance has the characteristics of the interferon-induced antiviral state. The transferred resistance occurs between several cell species. The transfer of resistance depends on the ratio of cells homologous (to interferon) to cells which are heterologous as well as to the absolute cell density at a given ratio. The transfer process is efficient in that it requires relatively small amounts of interferon. Finally, we propose that this phenomenon is a natural process for amplification of the interferon system, and preliminary evidence indicates that it occurs by cell to cell transfer of an interferon-induced molecule.

This study was supported by the James W. McLaughlin Fellowship Fund.

J. E. BLALOCK
S. BARON

Department of Microbiology,
University of Texas Medical Branch,
Galveston, Texas 77550

Received 7 June; accepted 18 July 1977.

- ¹ Lowenstein, W. R. in *Cell Membranes: Biochemistry, Cell Biology and Pathology* (eds Weissmann, G. & Claiborne, R.) 105-114 (HP Publishing, New York, 1975).
- ² Pitts, J. D. in *Ciba Foundation Symposium on Growth Control in Cell Cultures* (eds Wolstenholme, G. E. W. & Knight, J.) 89-105 (Churchill-Livingstone, London, 1971).
- ³ Sheridan, J. D., Finbow, M. E. & Pitts, J. D. *J. Cell Biol.* 67, 396a (1975).
- ⁴ Pitts, J. D. & Finbow, M. E. in *Intercellular Communication* (ed. De Mello, W. C.) 61-86 (Plenum, New York, 1976).
- ⁵ Fentiman, I., Taylor-Papadimitriou, J. & Stoker, M. *Nature* 264, 760-762 (1976).
- ⁶ Pitts, J. D. & Burk, R. R. *Nature* 264, 762-764 (1976).
- ⁷ Baron, S. in *Interferons* (ed. Finter, N. B.) 291-292 (North-Holland, Amsterdam, 1966).
- ⁸ Ankel, H., Chany, C., Galliot, B., Chevalier, M. J. & Robert, M. *Proc. natn. Acad. Sci. U.S.A.* 70, 2360-2363 (1973).
- ⁹ Chany, C., Ankel, H., Galliot, B., Chevalier, M. J. & Gregoire, A. *Proc. Soc. exp. Biol. Med.* 147, 293-299 (1974).
- ¹⁰ Taylor, J. *Biochem. biophys. Res. Commun.* 14, 447-451 (1964).
- ¹¹ Taylor, J. *Virology* 25, 340-349 (1965).
- ¹² Cuatrecasas, P. in *Cell Membranes: Biochemistry, Cell Biology and Pathology* (eds Weissmann, G. & Claiborne, R.) 177-184 (HP Publishing, New York, 1975).
- ¹³ Dianzani, F. & Baron, S. *Nature* 257, 682-684 (1975).
- ¹⁴ Dianzani, F., Levy, H. B., Berg, S. & Baron, S. *Proc. Soc. exp. Biol. Med.* 152, 593-597 (1976).
- ¹⁵ Lockhart, R. Z. in *Interferon and Interferon Inducers* (ed. Finter, N. B.) 11-27 (North-Holland/American Elsevier, Amsterdam and New York, 1973).
- ¹⁶ Pitha, P. M., Vengris, V. E. & Reynolds, F. H., Jr *J. supramolec. Struct.* 4, 467-473 (1976).
- ¹⁷ Desmyter, J., Melnick, J. L. & Rawls, W. E. *J. Virol.* 2, 955-961 (1968).
- ¹⁸ Gifford, G. E. & Koch, A. L. *J. theor. Biol.* 22, 271-283 (1969).
- ¹⁹ Campbell, J. B., Grunberger, J., Kochman, M. A. & White, S. L. *Can. J. Microbiol.* 21, 1247-1253 (1975).

Hyperacute autoimmune encephalomyelitis induced by a synthetic autoantigen

EXPERIMENTAL autoimmune encephalomyelitis (EAE) is an organ-specific autoimmune disease of the central nervous system (CNS) induced by immunisation with CNS tissue in

complete Freund's adjuvant (CFA). It is generally regarded as an animal model of acute disseminated encephalomyelitis. In Lewis rats a hyperacute variant of EAE can be induced using appropriate CNS immunogen with *Bordetella pertussis* vaccine as adjuvant with or without CFA¹⁻³. Hyperacute EAE (HEAE) differs from ordinary EAE clinically in its early onset and in its rapid and severe course, with high incidence of cerebral involvement and mortality. Histological lesions of HEAE are characterised by the presence in the CNS parenchyma of polymorphonuclear leukocytes, and necrosis, haemorrhage, oedema and fibrin^{1,2}. Because HEAE and ordinary EAE overlap histopathologically and immunologically³ they provide experimental evidence supporting the concept that certain human demyelinating diseases represent a spectrum of autoimmunity ranging from chronic multiple sclerosis to acute haemorrhagic necrotising leukoencephalopathy, for which HEAE is a model^{1,2,4}. We report here that the induction of HEAE is favoured by the absence of a methyl group in a sequence of 14 amino acids of the primary structure of the immunogen.

The autoantigen in CNS tissue which induces and is the target of the immune response in EAE is the basic protein of myelin. Few differences are found in the sequences of the approximately 170 amino acids of mammalian basic proteins which have been characterised (reviewed in ref. 5). Numerous encephalitogenic determinants have been identified in the basic protein molecule (reviewed in ref. 6). In contrast to the similarities in amino acid composition of their basic proteins, different species vary markedly in their susceptibility to EAE induction by defined peptide regions of the basic protein. For example, the major encephalitogenic determinant for guinea pigs is the 'tryptophan' peptide (residues 113-121) (ref. 7). That peptide also induces EAE in rabbits^{8,9}, but is inactive in rats⁹. Peptide 66-75 is encephalitogenic for rabbits¹⁰, but is inactive in rats¹¹, and a peptide comprising residues 70-88 of guinea pig basic protein is known to be encephalitogenic for rats¹²⁻¹⁴. With *B. pertussis* as adjuvant, we found that guinea pig basic protein was unique in its capacity to induce HEAE in rats when compared with basic proteins from seven other species⁵. We further established that the determinant of the guinea pig basic protein responsible for HEAE induction resided in the region 70-88 (ref. 12). We have now confirmed by synthesis that the unique antigenicity of the guinea pig basic protein leading to HEAE induction is determined by the primary amino acid sequence of a 14 amino acid peptide. The corresponding region of the rat basic protein, which differs by only a single amino acid substitution at residue 79 (threonine/serine), induces ordinary EAE but not HEAE.

Guinea pig and rat basic proteins were prepared from brain tissues⁵ and the corresponding midpeptides (residues 45-88) were isolated after 20 min of peptic digestion⁸. The midpeptides were further digested by rapid proteolysis with α -chymotrypsin (20-30 s at 37 °C). Preparative electrophoresis in pyridine-acetic acid-butanol-H₂O (1:1:2:36) yielded two fragments of 19 and 25 amino acids. Amino acid analyses identified the fragments as residues 70-88 and 45-69 respectively (Table 1). Peptide 73-86 (guinea pig sequence) was synthesised by the Merrifield solid phase technique.

Graded doses of basic proteins and peptides were prepared in saline, emulsified with CFA and injected intradermally into 10-week-old female Lewis rats as described previously³. *B. pertussis* vaccine (2×10^{10} organisms) was injected subcutaneously. Clinical signs of ordinary EAE and HEAE were scored daily. Ordinary EAE generally appeared around day 12 or later and consisted of flaccid weakness or paralysis of limbs and/or tail, and incontinence, and was usually not fatal. HEAE was readily distinguished from ordinary EAE clinically by the additional combination

Table 1 Encephalitogenicity of graded doses of guinea pig and rat myelin basic proteins and peptides of their midregions

Immunogen	Dose (nmol)	Mean day of onset	Clinical EAE Total incidence of signs	Incidence of 'cerebral' signs	Mortality	Mean day of death
Guinea pig basic protein	0.53	10	5/5	4/5	5/5	12
	0.17	10	5/5	4/5	5/5	15
	0.053	12	4/5	0/5	0/5	—
Rat basic protein	0.21	16	4/5	0/5	0/5	—
	0.067	—	0/5	0/5	0/5	—
Guinea pig residues 45–88	1.0 –3.1	9	24/24	20/24	22/24	12
	0.62	11	10/10	6/10	4/10	17
	0.062–0.21	12	8/10	0/10	1/10	12
	0.021	—	0/5	0/5	0/5	—
Rat residues 45–86	0.64 –3.2	12	22/22	5/22	2/22	(12,21)
	0.21	14	2/4	0/4	0/4	—
	0.021–0.064	—	0/7	0/7	0/7	—
Guinea pig residues 45–69*	0.55 –5.5	—	0/9	—	0/9	—
Guinea pig residues 70–88†‡	0.16 –2.3	9	33/33	22/33	27/33	12
	0.0046–0.037	13	13/14	4/14	7/14	15
	0.0028	—	0/4	—	0/4	—
Rat residues 68–86†	0.60 –2.3	11	25/25	15/25	3/25	13
	0.16	13	4/5	0/5	0/15	—
	0.018 –0.037	—	0/5	—	0/5	—
Guinea pig residues 73–86§ (synthetic)	1.2	10	12/12	11/12	8/12	13
	0.15	13	8/8	3/8	3/8	18

HEAE was distinguished from ordinary EAE clinically by a combination of early onset (usually 9–10 d), rapid and severe course and high incidence of mortality and cerebral signs (irritability, seizures, coma and spasticity). Amino acid analyses were determined using a Beckman 121 analyser after hydrolysis for 24 h in 6 M HCl.

*Amino acid analysis: Phe (1.0), Gly (3.0), Ser (3.0) Asp (2.0) Arg (3.0), Ala (4.0), Pro (1.0), Lys (2.0), His (3.0), Thr (2.0), Tyr (1.0).

†The rat sequence (68–86) is identical to the guinea pig sequence (70–88) except for a threonine for serine substitution.

‡70 Gly-Ser-Leu-Pro-Gln-Lys-Ser-Gln-Arg-[Ser]-Gln-Asp-Glu-Asn-Pro-Val-Val-His-Phe
(1.0)(3.0)(1.0)(2.0)(4.0)(1.0) (1.0) (2.0) (2.0) (1.0)(1.0)

§73 Pro-Gln-Lys-Ser-Gln-Arg-[Ser]-Gln-Asp-Glu-Asn-Pro-Val-Val
(2.0)(4.2)(1.0)(1.8) (1.0) (2.0) (1.8)

of early onset of signs (9–10 d), severe motor impairment with a high incidence of cerebral involvement (irritability, seizures, coma and spasticity) and a uniformly rapid and fatal course. Brains and spinal cords were examined histologically³.

Table 1 compares the encephalitogenicity of the whole guinea pig basic protein, its midpeptide (residues 45–88) and smaller fragments of that peptide. Doses as low as 0.17 nmol of guinea pig basic protein, 1.0 nmol of peptide 45–88 and 0.16 nmol of the chymotryptic fragment 70–88 induced HEAE. Ordinary EAE was induced by doses of peptide 70–88 as low as 0.0046 nmol. The larger chymo-

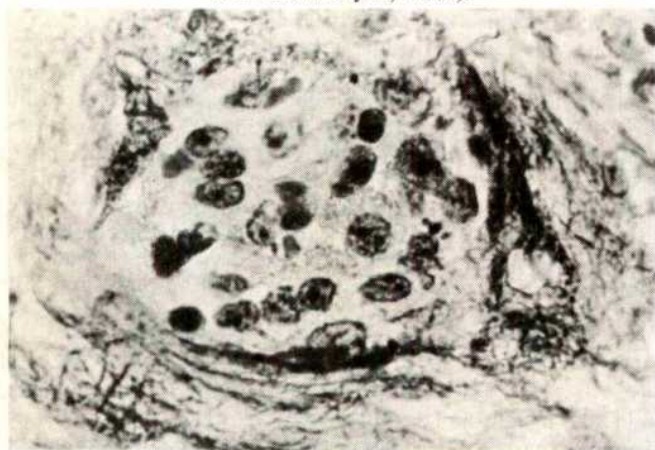
tryptic fragment 45–69 induced neither clinical nor histological signs of EAE at 5.5-nmol doses. The synthetic peptide comprising residues 73–86 induced HEAE (both clinical and histological signs (Fig. 1)) at a dose of 1.2 nmol.

Peptides corresponding to guinea pig residues 45–88 and 70–88, but derived from syngeneic rat basic protein, induced ordinary EAE, but not HEAE, when tested over a broad range of doses with identical adjuvants. Doses of 2.3 and 3.2 nmol induced very severe signs of ordinary EAE but cerebral signs were uncommon and most rats recovered. Thus the unique capacity of the guinea pig basic protein to induce HEAE (ref. 3) is not simply a dose effect but is determined by the amino acid sequence of the region 73–86. The only sequence difference in this region of the basic proteins of guinea pig (which induces HEAE) and rat (which induces only ordinary EAE even at high doses) is a serine/threonine substitution at residues 79. Thus the antigenic requirements for induction of two clinically distinct autoimmune syndromes are determined by one methyl group.

The fact that HEAE was induced by the synthetic peptide 73–86 rules out the possibility that the enzymatically derived guinea pig peptide might have been contaminated with another encephalitogenic fragment. Synthesis of additional analogues of the region comprised in residues 73–86 should allow definition of (1) the minimal antigenic requirements for induction of HEAE and (2) the essential difference between determinants inducing HEAE and ordinary EAE. The availability of defined synthetic autoantigens presents a unique opportunity to analyse the different immunopathogenic mechanisms involved in ordinary and hyperacute EAE.

We thank C. Sommers, J. Clark, R. Rubin and E. Ward for technical assistance and Eli Lilly and Co. for a gift of *B. pertussis*. This work was supported by grants NS 12391

Fig. 1 A small blood vessel with intraluminal inflammatory cells in the spinal cord parenchyma of a rat inoculated 12 d earlier with synthetic peptide 73–86 (Pro-Gln-Lys-Ser-Gln-Arg-[Ser]-Gln-Asp-Glu-Asn-Pro-Val-Val). The prominent extravasation of fibrin is characteristic of HEAE (phosphotungstic acid-haematoxylin, $\times 779$).



and NS 11719 from the US NIH and by the Kroc Foundation, Santa Ynez, California.

FRED C. WESTALL
MILLIE THOMPSON
VANDA A. LENNON

The Salk Institute for Biological Studies,
Post Office Box 1809,
San Diego, California 92112

Received 8 June; accepted 29 July 1977.

- ¹ Levine, S. & Wenk, E. J. *Science* **146**, 1681-1682 (1964).
- ² Levine, S. *Acta Neuropath.* **28**, 174-189 (1974).
- ³ Lennon, V. A., Westall, F. C., Thompson, M. & Ward, E. *Eur. J. Immun.* **6**, 805-810 (1976).
- ⁴ Alvord, E. C. in *Handbook of Clinical Neurology* **9**, (eds Vinken, P. J. & Bruyn, G. W.) 500-571 (North-Holland, Amsterdam, 1970).
- ⁵ Dunkley, P. R. & Carnegie, P. R. *Biochem. J.* **141**, 243-255 (1974).
- ⁶ Bergstrand, H. in *Immunocytochemistry of Proteins I*, (ed. Atassi, M. Z.) 315-370 (Plenum, New York, 1977).
- ⁷ Westall, F. C., Robinson, A. B., Caccam, J., Jackson, J. & Eylar, E. H. *Nature* **229**, 22-24 (1971).
- ⁸ Eylar, E. H., Westall, F. C. & Brostoff, S. J. *biol. Chem.* **246**, 3418-3424 (1971).
- ⁹ Lennon, V. A. thesis, Univ. Melbourne (1972).
- ¹⁰ Shapira, R., Chou, F. C.-H., McKneally, S., Urban, E. & Kibler, R. F. *Science* **173**, 736-738 (1971).
- ¹¹ McFarlin, D. E., Blank, S. E. & Kibler, R. F. *Science* **179**, 478-480 (1973).
- ¹² Westall, F. C., Thompson, M. & Lennon, V. A. *Fedn Proc.* **35**, 436 (1976).
- ¹³ Chou, C.-H. J., Chou, F. C.-H., Kowalski, T. J., Shapira, R. & Kibler, R. F. *J. Neurochem.* **28**, 115-119 (1977).
- ¹⁴ Martenson, R. E., Nomura, K., Levine, S. & Sowinski, R. J. *Immun.* **118**, 1280-1285 (1977).

Increased endoneurial fluid pressure in experimental lead neuropathy

THE endoneurial compartment of peripheral nerve is relatively inaccessible because of its small size and is maintained in a specialised environment by means of a perineurial barrier¹, a blood-nerve barrier² and a cerebrospinal fluid barrier. We have gained access to the endoneurial compartment of mammalian peripheral nerve by means of small polyethylene matrix (PEM) capsules and have recorded endoneurial fluid pressure (EFP) using an active servo null system³. The solid PEM capsules have pores of approximately 60 μ m which facilitate entry of fluid into the interstices and connecting polyethylene tubing, but unlike hollow capsules⁴, are not invaded by connective tissue. We have used PEM capsules to make serial measurements of EFP in control and lead-fed rats and watched the pathological changes. We did this because of the prominence of endoneurial oedema in, for example, inflammatory polyradiculoneuropathy⁵, diabetic neuropathy⁶, acromegalic neuropathy⁷ and experimental lead⁸ and galactose^{9,10} neuropathies, and because external pressure causes segmental demyelination and even axonal degeneration of peripheral nerve fibres¹¹. We found that EFP increased progressively in lead-fed rats and preceded the onset of fibre pathology. The time course of the increase and of fibre pathology suggests that increased EFP is a cause of segmental demyelination in lead neuropathy.

PEM capsules (external diameter 0.5 mm) tightly wedged in a PE-10 tubing were implanted into the left sciatic nerve of 18 Sprague-Dawley rats and EFP recordings were made 1 month later. Pressures from the capsules were measured using a modification of an active servo-null device originally developed by Wiederhielm¹². A micropipette containing hyperosmolar saline forms one arm of a balanced Wheatstone bridge adjusted for null. When the micropipette is wedged into a tubing connected to the capsule the EFP causes a shift in the fluid interface at the tip of the pipette, causing an error voltage. This voltage is amplified and applied to a bellows system which generates a counter-pressure within the pipette, restoring the fluid interface to its original position. The hydraulic pressure is measured with a transducer and recorded³.

After the initial recording nine rats were placed on a

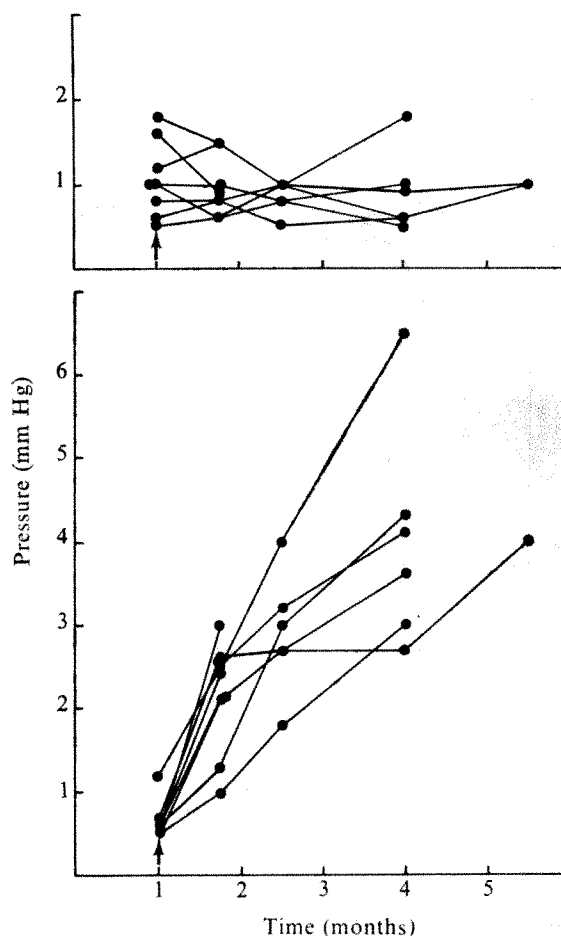
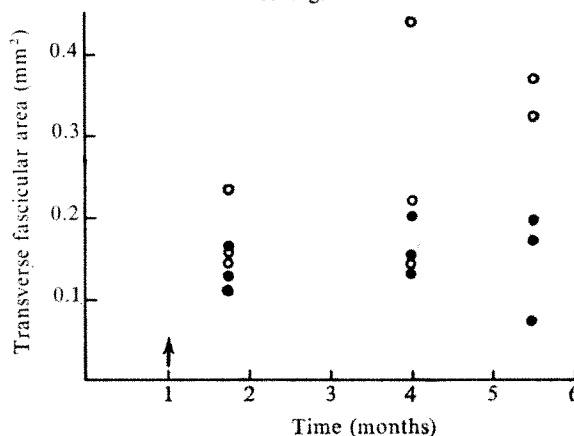


Fig. 1 EFP does not change significantly with time in control rats (upper) but increases progressively in lead rats (lower). Arrow indicates onset of test feeding.

conventional diet to which 4% lead carbonate had been added (lead rats). The remaining nine animals were pair-fed the same diet but without the added lead (control rats). EFP recordings were repeated 3 weeks, 6 weeks, 3 months and 4.5 months after commencement of test feeding (Fig. 1). EFP recordings were successful in eight of nine control rats 1 month after implantation of the capsule and ranged from 0.5 to 1.8 mm Hg. They did not alter significantly in subsequent serial recordings. By contrast, the EFP was increased in seven of nine lead rats by 3 weeks after start of lead feeding and progressively increased in serial recordings (Fig. 1).

Three animals from each group were killed 3 weeks

Fig. 2 Transverse fascicular area of control (closed circles) and lead rats (open circles) at different times after start (arrow) of test feeding.



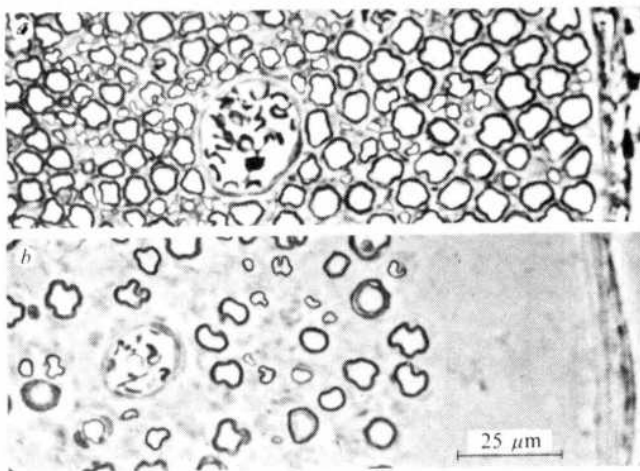


Fig. 3 Transverse section of peroneal nerve from control (upper) and lead rat (lower) after 3 months of lead feeding. Note the marked pericapillary and subperineurial oedema in the nerve of the lead rat.

and 3 months after start of test feeding and the remaining three controls and two lead rats (one meanwhile had died) were killed at 4.5 months. All morphological studies were done on the right peroneal nerve.

The transverse fascicular area (TFA) of right peroneal nerve was digitised on photographic enlargements ($\times 230$) of transverse sections of nerves embedded in Epon. The TFA was increased at 4½ months after test feeding and was variably increased even earlier in lead rats (Fig. 2). The number of measurements were small but the trend paralleled that of EFP recordings.

On pathological evaluation, endoneurial oedema was absent or mild at 3 weeks and prominent by 3 months. Oedema tended to be most pronounced around small blood vessels and in the subperineurial area (Fig. 3). Fibre pathology, as assessed by phase contrast microscopy of sections and by evaluation of teased fibres (100 fibres per nerve) consisted chiefly of segmental demyelination and remyelination. Axonal degeneration was uncommon. In Table 1 fibres showing segmental demyelination with or without regeneration are grouped under percentage demyelination. Segmental demyelination was not found in nerves of control rats or in nerves of rats fed lead for 3 weeks. At 3 months, however, segmental demyelination was seen in all lead rats and the changes were marked by 4½ months (Table 1).

Our study provides the first evidence that endoneurial pressure is increased in a neuropathy. The rise in EFP in experimental lead neuropathy is small in absolute terms and relative to the externally applied pressures which have been used to produce segmental demyelination^{11,13}; however, the relative increase above baseline values is considerable. The

mean EFP after 6 weeks of lead feeding is approximately four times and at 3 months six times baseline values. Moreover, the mode of action of experimental compression is mechanical¹⁴ and acute, compression being applied for minutes to an hour or two, whereas the pressure rise in experimental lead neuropathy as reported here is maintained over months. The magnitude of the increase in pressure is not sufficient to collapse endoneurial and perineurial capillaries¹⁵ so that ischaemia from capillary collapse, as was postulated by Sunderland¹⁶ for median nerve entrapment in man, should not be the mechanism of segmental demyelination in this model.

This investigation was supported in part by grants from NIH and by the Upton, Herrick, Gallagher and Schmitter Funds. P.A.L. was supported in part by grants from the Royal Australian College of Physicians and the postgraduate medical foundation, University of Sydney, NSW, Australia.

PHILLIP A. LOW
PETER JAMES DYCK

Mayo Clinic and Mayo Foundation,
Rochester, Minnesota 55901

Received 13 May; accepted 25 July 1977.

- Shanthaveerappa, T. R. & Bourne, G. H. *J. Anat.* **96**, 527–537 (1962).
- Olsson, Y. *Peripheral Neuropathy*, 190–212 (Saunders, Philadelphia, 1975).
- Low, P., Marchard, P., Knox, F. & Dyck, P. J. *Brain Res.* **122**, 373–377 (1977).
- Guyton, A. C., Granger, H. J. & Taylor, A. E. *Physiol. Rev.* **51**, 527–563 (1971).
- Haymaker, W. & Kernohan, J. W. *Medicine* **28**, 59–141 (1949).
- Thomas, P. K. & Lascelles, R. G. *Q. J. Med.* **35**, 489–509 (1966).
- Woltman, H. W. *Arch. Neurol. Psychiat.* **45**, 680–682 (1941).
- Ohnishi, A. *et al.* *J. Neuropath. exp. Neurol.* (in the press).
- Gabbay, K. H. & Snider, J. J. *Diabetes* **21**, 295–300 (1972).
- Sharma, A. K., Thomas, P. K. & Baker, R. W. *R. J. Neurol. Neurosurg. Psychiat.* **39**, 794–802 (1976).
- Dyck, P. J. *Arch. Neurol.* **21**, 73–95 (1969).
- Wiederhielm, C. A., Woodbury, J. W., Kirk, S. & Rushmer, R. F. *Am. J. Physiol.* **207**, 173–176 (1964).
- Fowler, T. J., Danta, G. & Gilliat, R. W. *J. Neurol. Neurosurg. Psychiat.* **35**, 638–647 (1972).
- Ochoa, J., Fowler, T. J. & Gilliat, R. W. *J. Anat.* **113**, 433–455 (1972).
- Zweifach, B. W. *Circulat. Res.* **34**, 858–866 (1974).
- Sunderland, S. J. *J. Neurol. Neurosurg. Psychiat.* **39**, 615–626 (1976).

Development-related changes of triiodothyronine binding to brain cytosol receptors

SEVERAL lines of evidence have established that thyroid hormones have a major role in the functional differentiation of the mammalian central nervous system^{1–4}. Clinical or experimental hypothyroidism in early life results in behavioural disturbances and mental dysfunction which are apparently the outward manifestation of well-defined biochemical and structural lesions observed in the cerebellum and cerebral cortex of experimental animals. Significantly, the defects arising from the hypothyroid state are amenable to hormone therapy only during an early critical age period. Furthermore, unlike the liver and most other tissues, the mature brain is not responsive to the manipulation of the thyroid hormone state at least in terms of certain biochemical parameters. The brain is thus uniquely dependent on thyroid hormones for the full expression of functional development during a limited age period which is analogous to the situation in amphibian metamorphosis⁵. I reasoned that the acquisition and disappearance of brain sensitivity to thyroid hormones may be related to their binding properties with cellular receptors. I show here that the affinity of rat brain (cerebellum) cytosol for triiodothyronine (T_3) is high during an early age period when cytodifferentiation is most intense and declines dramatically with age with no apparent change in the number of binding sites. In sharp contrast, the liver shows a marked increase in the number of binding sites with age with little change in affinity.

The rat cerebellum was chosen for study since both the structural and functional differentiation of this region at an early age and its responsiveness to the thyroid hormones

Table 1 Percentage of teased fibres (of 100) showing segmental demyelination with or without remyelination

Duration of study (weeks)	Nerve no.	Control	Lead
3	1	0	0
	2	0	0
	3	0	0
12	1	0	96
	2	0	—*
	3	0	—*
18	1	0	94
	2	0	80
	3	0	—

*Improperly prepared for adequate teased assessment—most fibres of both nerves showed segmental demyelination with or without remyelination.

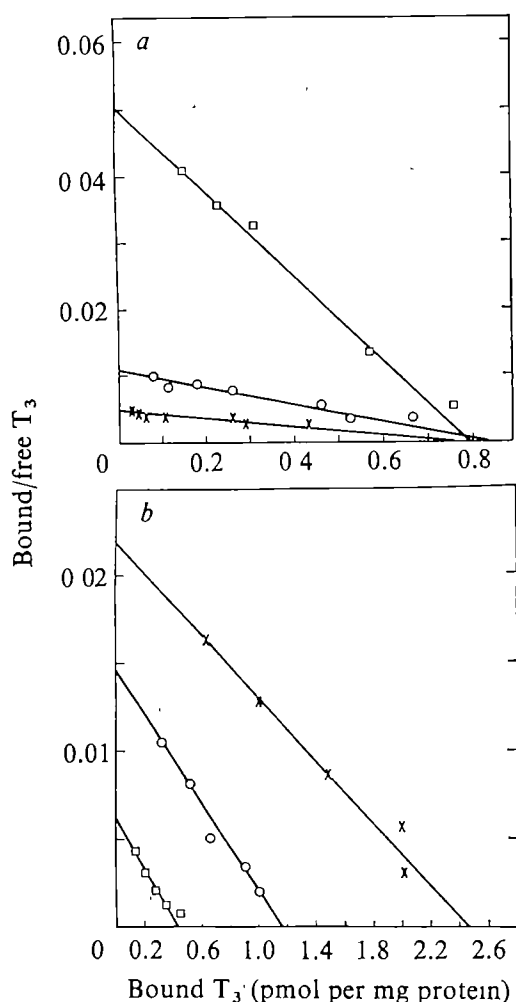


Fig. 1 Scatchard analysis of binding of $^{125}\text{I}-T_3$ to cytosol prepared from rat (a) cerebellum and (b) liver at 10 (\square), 20 (\circ) and 50 (\times) days of age. Cytosol (100–150 μg protein) was incubated with increasing concentrations of $^{125}\text{I}-T_3$ (4×10^{-10} to 1×10^{-8} M, final concentration) for 2 h at 0°C . Bound hormone was separated from free hormone by the addition of 0.2 ml of a dextran-coated charcoal suspension (5% charcoal: 1% dextran of molecular weight 60,000–90,000 in 20 mM Tris-HCl, pH 7.8). The tubes were vortexed for 6 s, kept on ice for 10 min and centrifuged at 4000g for 10 min. A portion of the clear supernatant containing the bound $^{125}\text{I}-T_3$ was rapidly removed and counted in a Packard Auto-gamma spectrometer. Sephadex G25 chromatography²¹ of the bound fraction confirmed that 94% of the radioactivity was in the form of T_3 . The magnitude of the high affinity, saturable binding was estimated by subtraction of the radioactivity bound in the presence of a 1,000-fold molar excess of native T_3 . The free hormone concentration was calculated by subtraction of the total $^{125}\text{I}-T_3$ bound from the total $^{125}\text{I}-T_3$ added per assay and expressed as nmol per l of cytosol. The dextran-coated charcoal procedure removed more than 98% of the $^{125}\text{I}-T_3$ at all concentrations in the absence of cytosol. The removal of the bound hormone by Sephadex G25 gel filtration gave comparable results with the charcoal procedure. The values are means of two determinations which did not differ by more than 3% from the mean. The values are not corrected for the dissociation of the bound hormone in the presence of charcoal.

during this period have been extensively characterised. Cytosol was prepared from the cerebellum and liver (perfused through the portal vein with 25 mM KH_2PO_4 , 0.1 M NaCl, pH 7.4) of 10-, 20- and 50-d old Sprague-Dawley rats. The tissues were homogenised with a motorised Teflon pestle in 5 volumes of 0.32 M sucrose, 20 mM Tris, 1.0 mM MgCl_2 (pH 7.6). After removal of the mitochondrial fraction by centrifugation in the cold at 12,000g for 20 min, the supernatant was centrifuged at 130,000g for 90 min at 4°C . The supernatant, which represented the cytosol, was aspirated (excluding the surface lipid in the case of the

liver) and stored in small portions at -20°C for no longer than 2 weeks. Cytosol, equivalent to 100–150 μg protein was incubated in the presence of $^{125}\text{I}-T_3$ (specific activity 290 Ci mmol^{-1} , Abbott) in 12 \times 75-mm polypropylene tubes for 2 h at 0°C in a final volume of 0.5 ml. The reaction medium contained 0.25 M sucrose, 20 mM Tris, 1.0 mM MgCl_2 , 1 mM dithiothreitol and 5% glycerol (pH 7.6) and was used for the dilution of cytosol and labelled and unlabelled hormones. The presence of dithiothreitol was found to be necessary for maximal binding and glycerol was included to prevent receptor decay particularly over long incubation intervals. A second identical series of tubes contained, in addition, 1,000-fold molar excess of unlabelled T_3 as a competitor for high affinity (saturable) $^{125}\text{I}-T_3$ binding. $^{125}\text{I}-T_3$ was at least 92% pure as determined by thin-layer chromatography. The level of cytosol protein as used in the assay was within the range where binding was directly proportional to protein for all age groups.

Representative Scatchard plots of the binding of increasing concentrations of $^{125}\text{I}-T_3$ to cerebellar and liver cytosol during development are shown in Fig. 1a and b, respectively. Saturation was achieved with about 1×10^{-8} M T_3 at all ages. The binding parameters, affinity constant and maximum binding capacity, derived from these curves are listed in Table 1. The striking decline in the affinity constant for the cerebellum with age apparently correlates with the limited period of responsiveness of the brain to thyroid hormones during and early critical period of development. In the liver, on the other hand, which does not demonstrate an age-dependent tissue sensitivity to thyroid hormones, the number of binding sites increases markedly with age with little change in the affinity constant.

The development of the cerebellar cortical architecture in the rat is almost exclusively confined to the first 3 weeks of life and the sequence of events have been clearly documented⁸. These maturational processes are dependent on the presence of thyroid hormones; thus, neonatal hypothyroidism retards cellular proliferation and differentiation and impairs the growth of neuronal processes, the formation of synapses and their organisation⁷⁻⁹. Physiological levels of hormone are capable of reversing these aberrant changes if administered at an early age¹⁰.

It is significant that the affinity constant for the cerebellum is high at an age when cellular proliferation and migration are maximal and cytodifferentiation is proceeding rapidly. This relationship implies that the regulation of gene expression may come under hormonal control. Evidence that thyroid hormones bind to nuclear receptors with high affinity in several tissues, including brain¹¹⁻¹³, provides support for this contention.

The significance of recognition sites for T_3 in brain cytosol shown here and earlier in several mammalian non-neuronal tissues^{12,16-22}, is not clear. The T_3 binding characteristics in cytosol seem to be considerably different from nuclear preparations^{17,19}. Moreover, unlike steroid hormones, cytosol binding is apparently not a prerequisite for the interaction of thyroid hormones with nuclear re-

Table 1 Association constants (K_a) and maximal binding capacity (M) of cerebellum and liver during development

Tissue	Age (d)	K_a (l per mol $\times 10^{-7}$)	M (pmol per mg protein)
Cerebellum	10	6.40	0.79
	20	1.25	0.87
	50	0.66	0.75
Liver	10	1.43	0.43
	20	1.25	1.16
	50	0.88	2.45

K_a and M were derived from Fig. 1 where the slope represents $-K_a$ and the intercept of the line in the abscissa equals M .

ceptors^{11,13,14,17}. Cytosol binding sites may serve to provide a readily available supply of hormone for biological action at some additional intracellular site and thus may be regarded as 'acceptors'. Alternatively, cytosol binding sites may indeed be regarded as one of several primary receptors in accordance with the multiple actions of thyroid hormones.

This study was supported by a US NIH grant from the National Institute of Neurological and Communicative Disorders and Stroke.

S. E. GEEL

Department of Neurology,
School of Medicine,
University of California,
Davis, California 95616

Received 30 March; accepted 17 June 1977.

- ¹ Eayrs, J. T. in *Endocrinology and Human Behavior* (ed. Michael, R. P.) 239–255 (Oxford University Press, London, 1968).
- ² Hamburg, M. *Curr. Top. Dev. Biol.* **4**, 109–148 (1969).
- ³ Balazs, R. in *Cellular Aspects of Growth and Differentiation in Nervous Tissues* (ed. Pease, D. C.) 273–311 (University of California, Los Angeles, 1971).
- ⁴ Sokoloff, L. & Kennedy, C. in *Biology of Brain Dysfunction 2* (ed. Gaul, G. E.) 295–332 (Plenum, New York, 1973).
- ⁵ Frieden, E. & Just, J. in *Biochemical Actions of Hormones* (ed. Litwack, G.) 1–52 (Academic, New York, 1970).
- ⁶ Altman, J. *J. comp. Neur.* **145**, 353–514 (1972).
- ⁷ Legrand, J. *Arch. Anat. Micro. Morph. exp.* **56**, 206–244 (1967).
- ⁸ Nicholson, J. L. & Altman, J. *Brain Res.* **44**, 13–38 (1972).
- ⁹ Hajos, F., Patel, A. J. & Balazs, R. *Brain Res.* **50**, 387–401 (1973).
- ¹⁰ Clos, J. *et al. Gen. comp. Endocr.* **23**, 178–192 (1974).
- ¹¹ Samuels, H. H., Tsai, J. S., Casanova, J. & Stanley, F. *J. clin. Invest.* **54**, 853–865 (1974).
- ¹² Thomopoulos, P., Dastugue, B. & Defer, N. *Biochem. biophys. Res. Commun.* **58**, 499–506 (1974).
- ¹³ Tata, J. R. *Nature* **257**, 18–23 (1975).
- ¹⁴ De Groot, L. J. & Torresani, J. *Endocrinology* **96**, 357–369 (1975).
- ¹⁵ Surks, M. I., Koerner, D. H. & Oppenheimer, J. H. *J. clin. Invest.* **55**, 50–60 (1975).
- ¹⁶ Spindler, B. J., Macleod, K. M., Ring, J. & Baxter, J. D. *J. biol. Chem.* **250**, 4113–4119 (1975).
- ¹⁷ Doctor, R., Visser, T. J., Stinis, J. T., van den Hout-Goemaat, N. L. & Hennemann, G. *Acta Endocr.* **81**, 82–95 (1976).
- ¹⁸ Eberhardt, N. L., Valcana, T. & Timiras, P. S. *Psychoneuroendocr.* **95**, 492–498 (1974).
- ¹⁹ Dillman, W., Surks, M. I. & Oppenheimer, J. H. *Endocrinology* **95**, 492–498 (1974).
- ²⁰ Sterling, K., Saldanha, V. F., Brenner, M. A. & Milch, P. O. *Nature* **250**, 661–663 (1974).
- ²¹ Davis, P. J., Handwerker, B. S. & Glaser, F. *J. biol. Chem.* **249**, 6208–6217 (1974).
- ²² Visser, T. J., Bernard, H. F., Doctor, R. & Hennemann, G. *Acta endocr.* **82**, 98–104 (1976).
- ²³ Hamada, S. & Fukase, M. *J. clin. Endocr. Metab.* **42**, 302–308 (1976).
- ²⁴ Green, W. L. *J. Chromat.* **72**, 83–91 (1972).

Axonal accessibility and adaptation to osmotic stress in an extreme osmoconformer

It is generally assumed that neuronal function is critically dependent on a relatively stable chemical environment. In most animals investigated homeostasis of the neuronal microenvironment is achieved by regulation of the chemical composition of the body fluids and, in higher vertebrates^{1–3} and insects⁴, by additional homeostatic control within the nervous system. The axons of an extreme invertebrate osmoconformer⁵ (the worm *Mercierella enigmatica* Fauvel) have been shown, however, to adapt and to continue to produce sodium-dependent action potentials when exposed to massive blood dilution^{6,7}. It has also been suggested that short-term protection is provided by a facultative blood-brain barrier which enables the axons to adapt relatively slowly to hyposmotic stress⁷. We now report that the effects of isosmotic and hyposmotic dilution of the surrounding medium on the giant axons of *Mercierella* are direct, and that axons respond rapidly to changes in ionic concentration and adapt to extreme osmotic stress almost immediately.

Electron microscopy showed that the giant axon is overlaid by narrow glial processes which form an incomplete investment. Where the covering is more complete the intercellular clefts which link the extra-axonal fluid with the

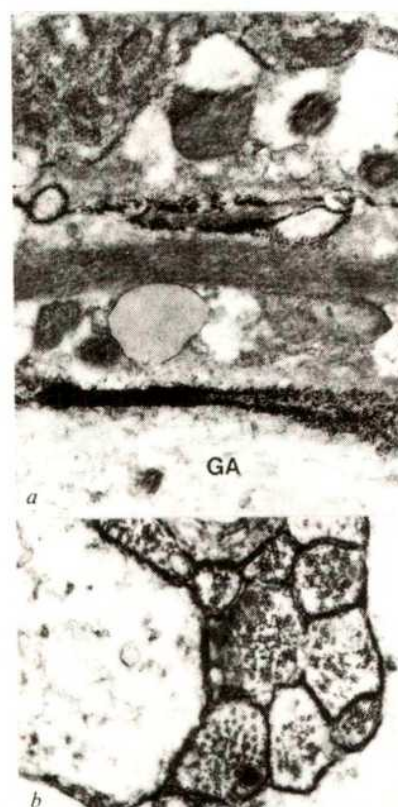


Fig. 1 *a*, Giant axon (GA) and surrounding tissue of an animal adapted to seawater showing penetration of lanthanum through the glial clefts to the axon surface. Animals were incubated for 30 min in normal saline with 10 mM LaCl_3 added before fixation by the method devised by Baskin⁸ ($\times 20,500$). *b*, Small axons from the connective of a seawater-adapted animal exposed briefly to dilute saline, a treatment previously supposed to limit access to the axon surface. Lanthanum is shown here to penetrate to the intercellular spaces between the axons. Animals briefly exposed to dilute saline were incubated in 50 mM LaCl_3 for 2 h before fixation with glutaraldehyde in dilute saline, enriched with phosphate to a final concentration of 0.05 M and with added calcium. The preparation was post-osmicated and stained *en bloc* with uranyl acetate ($\times 31,000$).

blood, or bathing medium, do not seem to be occluded by junctional complexes.

Externally-applied lanthanum was found to penetrate to the axon surfaces in seawater-adapted individuals bathed in normal (Fig. 1*a*) and diluted (10%) physiological saline (Fig. 1*b*). There is therefore no ultrastructural evidence for a significant restriction of intercellular diffusion to the axon surface in hyposmotic conditions.

We also found that the axonal responses to hyposmotic solutions depend on the method of dilution of the fluid in the recording chamber. With continuously flowing saline, as used before⁷, the lack of effect of rapid hyposmotic dilution resulted from fluid stratification which produced an unstirred layer of relatively high salinity in the immediate vicinity of the axon. When hyposmotic dilution was achieved by vigorously syringing the solution directly on to the preparation, relatively rapid axonal responses to ionic dilution could be observed in isosmotic and hyposmotic conditions (Fig. 2).

Our results indicate that the giant axon of *Mercierella* is not structurally protected from the immediate effects of hyposmotic stress. Even if some protection is provided in the intact animal by fluid stratification in the immediate vicinity of the axon surface, the giant axon can adapt to hyposmotic stress in experimental conditions much more rapidly than has been previously supposed⁷.

Intact colonies of *Mercierella* can function normally in distilled water for several weeks. Fig. 3*b* and *c* illustrates the

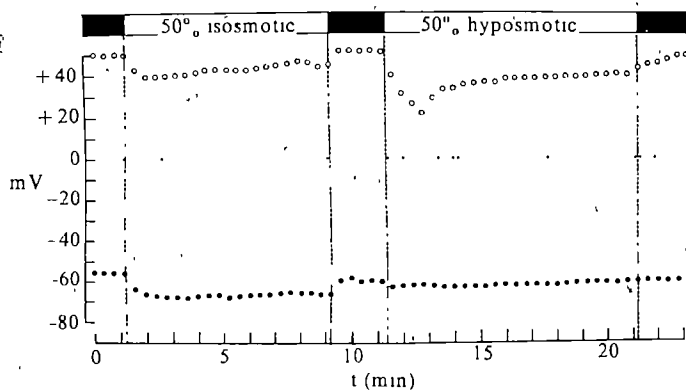
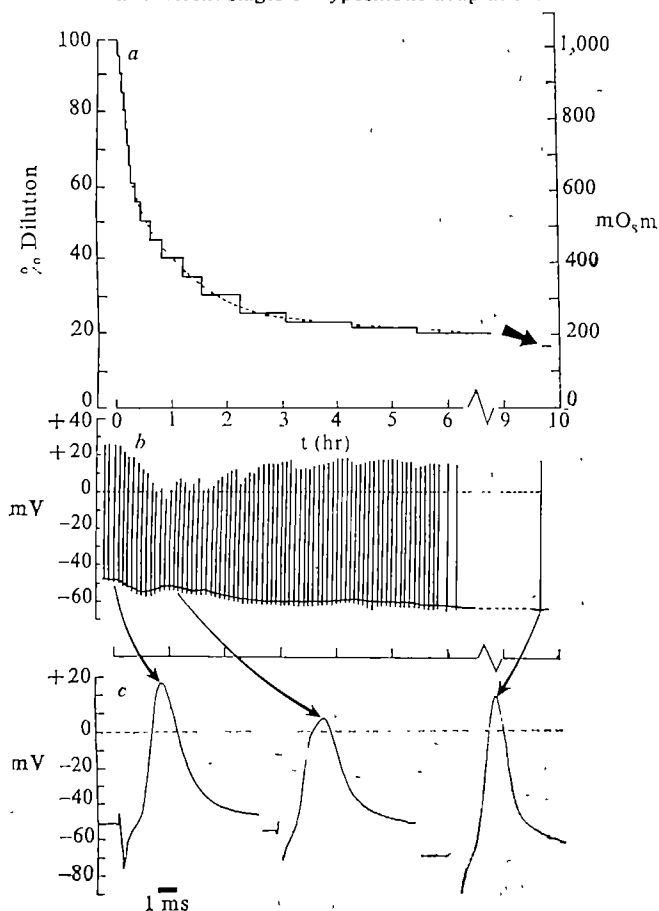


Fig. 2 Effects of 50% dilution of the physiological saline (1,024 mOsm) on the action and resting potentials, recorded with a conventional intracellular microelectrode (40 M Ω), in a giant axon of *Meiclerella*. Hypoosmotic saline was made by diluting standard saline with distilled water. Isosmotic dilution was achieved by addition of mannitol to diluted standard saline. In these experiments the solutions were syringed (at a rate of 0.5 ml s⁻¹) directly on to the preparation. The standard saline was based on the ionic and osmotic concentrations of seawater-adapted animals (mM: Na⁺, 482.3; K⁺, 30; Mg²⁺, 77; Ca²⁺, 31, SO₄²⁻, 26; Cl⁻, 663.8; OH⁻, 12.5 and PIPES, 7.5 mM).

responses of the giant axon to a dilution regime (Fig. 3a) similar to that in the blood of intact animals after transfer of a colony from seawater to distilled water. In spite of extreme osmotic stress, action potentials persisted throughout the experiment, with some diminution in amplitude only during the initial period of dilution. The hypoosmotically-

Fig. 3 Effects of progressive hypoosmotic dilution on the resting and action potentials in a giant axon of *Meiclerella a*. The broken line illustrates the rate of dilution of the blood measured on transfer of colonies of *Meiclerella* from seawater to distilled water⁵. The continuous line indicates the step changes in dilution used in this experiment. **b**, Continuous recording of the resting and action potentials recorded by a transient signal processor and displayed on a chart recorder. **c**, Action potentials at different stages of hypoosmotic adaptation.



adapted action potentials were of larger amplitude than those recorded initially in full strength saline. As shown from axons from individuals which were hypoosmotically adapted in distilled water for several weeks⁷, the *in vitro* adaptation involves a relatively slow hyperpolarisation of the axon membrane. This, together with an apparent reduction in intracellular sodium concentration⁷, facilitates overshooting action potentials of large amplitude in the face of the extreme dilution of the ionic and osmotic concentration of the bathing medium.

These observations indicate that the giant axons in this euryhaline osmoconformer can withstand and adapt to extreme changes in the osmotic and ionic composition of its immediate fluid environment. This establishes the important principle that neuronal homeostasis can be achieved not only by regulation of the extracellular fluid (as in higher vertebrates and insects) but also by relatively rapid adaptations by the nerve cells themselves.

This research was supported in part by a grant from the US European Research Office

J. E. TREHERNE
J. A. BENSON
H. le B. SKAER

Department of Zoology,
University of Cambridge,
Cambridge, UK

Received June 8, accepted 21 July 1977

- 1 Davson, H. in *Physiology of Cerebro-spinal Fluid* (Churchill, London, 1967).
- 2 Rapport, S. I. *Blood-Brain Barrier in Physiology and Medicine* (Raven, New York, 1976).
- 3 Abbott, N. J. & Treherne, J. E. in *Transport of Ions and Water* (ed. Gupta, B. L., Moreton, R. B. & Wall, B. J.) (Academic, New York, in the press).
- 4 Treherne, J. E. in *Insect Neurobiology* (ed. Treherne, J. E.) (North-Holland, Amsterdam, 1974).
- 5 Skaer, H. le B. *J. exp. Biol.* 60, 321-330 (1974).
- 6 Carlson, A. D. & Treherne, J. E. *J. exp. Biol.* 67, 205-215 (1977).
- 7 Treherne, J. E., Carlson, A. D. & Skaer, H. le B. *Nature* 265, 550-553 (1977).
- 8 Baskin, D. G. *Tiss. Cell* 3, 579-588 (1971).

Modification of synaptic input following unilateral labyrinthectomy

UNILATERAL vestibular nerve transection is followed by postural and locomotor disturbances that disappear to a large extent with time after the lesion¹. This compensation requires a central readjustment of the tonic and phasic output from the remaining vestibular apparatus to the motor nuclei involved in the control of limb and head muscles. To study the mechanisms of this type of motor learning at the single neurone level, we have used the frog (*Rana temporaria*), because in this species the time course of the postural compensation following hemilabyrinthectomy has been thoroughly studied². Furthermore, the synaptic circuitry and functional organisation of the peripheral and central vestibular systems are well known for this species³. Since the vestibular nuclei are the first integrative structures in which bilateral vestibular and other sensory inputs converge, and since their output influences motor systems directly or indirectly, we began to look for plastic changes in second order vestibular neurones. Many of these neurones are monosynaptically excited from the ipsilateral⁴ and disynaptically through commissural fibres from the contralateral⁵ labyrinth.

After unilateral labyrinthectomy via a ventral, extracranial approach, acutely (≤ 12 h) and chronically (≥ 60 d) deafferented vestibular neurones were activated by electrical stimulation of the anterior branch of the intact contralateral VIIIth nerve. Dissection, stimulation, and recording procedures were as described previously^{4,5}. Synaptic potentials were averaged with a Nicolet computer and corrected for the extracellularly recorded field potential⁶. Duration of excitatory postsynaptic potentials (e.p.s.ps) were measured at half amplitudes and the times-to-peak from the points of divergence of the intra- and extracellular records to the peak of the e.p.s.ps. To facilitate comparison of amplitudes, threshold values, and shape indices

of e.p.s.ps⁷ amongst different animals, the stimulation intensity was expressed in multiples of the N_1 field potential threshold (T_{N_1}) recorded in the ipsilateral vestibular nucleus⁴ and was restricted to $\leq 5 \times T_{N_1}$.

In acutely operated animals stimulation of the intact VIIIth nerve elicited field potentials in the vestibular nuclei on both sides (Fig. 1A) as described previously⁴. The presence of these field potentials served as a convenient measure of electrode location and the state of the animal.

Intracellular recordings from vestibular neurones located ipsi- and contralaterally to the stimulation side revealed e.p.s.ps with significant differences in their shape indices (Fig. 1B). The e.p.s.ps elicited from primary afferent fibres had a much greater amplitude (2.6 ± 1.0 mV; $n = 45$) and a much shorter time-to-peak (4.2 ± 1.6 ms; Fig. 2) than those elicited through commissural fibres (1.6 ± 1.1 mV; $P < 0.001$ and 12.4 ± 3.2 ms; $P < 0.001$; $n = 75$; Fig. 2). With increasing stimulus intensity, the time-to-peak of the e.p.s.ps did not shorten systematically. The duration of the e.p.s.ps was in general about three times the time-to-peak but was often much longer because of the arrival of late polysynaptic potentials. The e.p.s.ps induced by primary afferent fibres regularly triggered bursts of partial⁴ and full action potentials whereas e.p.s.ps induced by commissural fibres elicited single spikes in only 22.1% and repetitive firing in only 2.6% of the cells. The slower time-to-peak and the lower efficacy of commissural e.p.s.ps to provoke action potentials fully corroborate the suggestion⁵ that commissural fibres form synapses more peripherally on the dendrites of vestibular neurones than primary afferent fibres which are known to synapse on and close to the soma⁸.

In chronic animals the vestibular-evoked field potentials

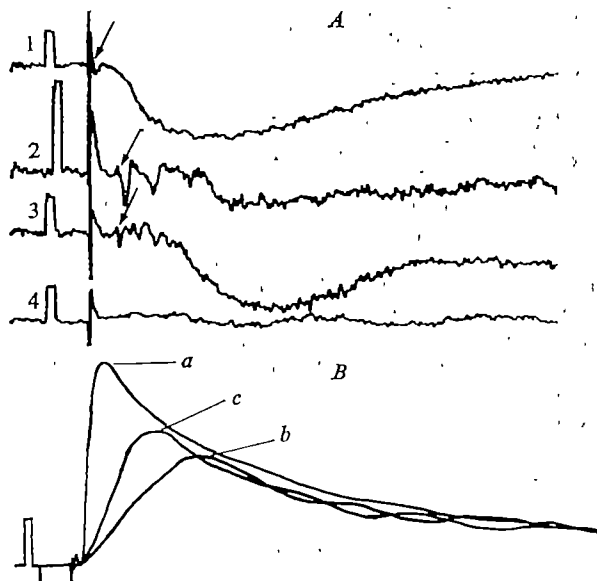


Fig. 1 A, Field potentials in the vestibular nucleus recorded on the ipsilateral sides (1) and contralateral (2, 3, 4) to VIIIth nerve stimulation from an acutely (2) and a chronically (1, 3, 4) operated animal. The presynaptic components (arrows) indicate the arrival of impulses in primary afferent (1) and in commissural fibres (2, 3) respectively. Cutting the vestibular commissure abolished both the prepotentials as well as the later slow negative field potentials (4) recorded previously at the same depth (500 μ m, 3) but left the ipsilateral field potential (1) unchanged. The depth profiles of the slow negative field potentials of acute and chronic animals were similar (maximal response 450–500 μ m), but their amplitudes were different (compare 2 with 3). Calibration pulse: 0.5 mV; 1 ms, positivity upwards. B, E.p.s.ps recorded in vestibular neurones were induced by primary afferent fibres (a) and by commissural fibres (b, c). b, was recorded in an acutely and c in a chronically deafferented neurone. Note the shorter time-to-peak and the larger amplitude of c in comparison with b. Traces are redrawn from the computer-averaged e.p.s.ps (16 sweeps) and were superimposed at the point of their onset. Downward artefacts represent stimulation; calibration pulse: +1 mV; 1 ms.

recorded in the deafferented vestibular nucleus (Fig. 1A) were of larger amplitude (two to three times) than those recorded in acute animals. Usually bursts of evoked spikes were superimposed on the field potential, a finding which was rarely observed in intact⁵ or in acute animals from this series of experiments. In chronic preparations, in contrast to acute animals, many vestibular neurones were spontaneously active with firing rates (1–10 Hz) similar to those recorded on the intact side.

The increased excitability of deafferented vestibular neurones might have been caused by a change in the excitability of vestibular neurones located on the intact side which project to the deafferented nucleus. To investigate this possibility, we recorded extra- and intra-axonally from commissural fibres on the lesioned side of acute and chronic animals as well as in the depth of the brainstem where these fibres are known to cross⁹. A comparison of the data obtained in these two groups of experimental animals showed practically identical values for the thresholds of single spikes ($1.6 \times T_{N_1}$), bursts ($2.3 \times T_{N_1}$) and for the number of spikes per burst (2.9) as well as for the inter-spike interval (2.4 ms). These findings indicate that the excitability and the output pattern of vestibular neurones on the intact side have not changed postoperatively.

Intracellular recordings from vestibular neurones on the chronically deafferented side (Fig. 1B) showed that they receive predominantly excitatory inputs from vestibular commissural fibres as in intact animals⁶. The major characteristics of commissurally evoked e.p.s.ps from acute and chronic animals are compared in Table 1. As can be seen, e.p.s.ps in both groups of animals had similar thresholds and latencies. In chronic frogs, however, the amplitude of the evoked e.p.s.ps was significantly larger, and generated in many more cells partial and full action potentials at a significantly lower stimulus intensity than in acutely operated or intact animals⁶. The rise time of the e.p.s.ps was also significantly shorter in chronic than in acute animals (Fig. 2) and varied only a little (± 1.5 ms) with increasing stimulus intensity. The duration of the e.p.s.ps varied greatly, but many of the e.p.s.ps recorded in chronic animals with a shorter time-to-peak had a longer duration. In addition polysynaptic components of the e.p.s.ps could prolong the falling phase considerably.

The changes in e.p.s.p shape indices and spike thresholds were observed in chronically deafferented neurones in spite of the absence of changes in threshold and bursting pattern of commissural input fibres. This indicates that the higher synaptic efficacy of the crossed excitatory input must be produced on the deafferented side somewhere between commissural terminals and postsynaptic neurones. One possible mechanism that could explain all of the observed changes in e.p.s.p. parameters would be axonal sprouting of commissural terminals resulting in the formation of new functional synapses on the vacated space on soma and proximal dendrites of vestibular neurones. As a result of the cable properties of dendrites⁷, these mixed den-

Table 1 Excitation of deafferented second-order vestibular neurones

Time after lesion	< 12 h	> 60 d
Number of e.p.s.ps	75	78
$T_{c.p.s.p}$	1.8 ± 0.8	1.6 ± 0.5
Latency*	5.5 ± 1.5	5.8 ± 1.6
Time to peak*	12.4 ± 3.2	8.6 ± 3.1 †
Amplitude*	1.6 ± 1.1	2.1 ± 0.9 †
Partial spikes in % of cells	49.3	87.5
$T_{spike < 5 \times T_{N_1}}$ in % of cells	22.1	55.1
$T_{spike < 5 \times T_{N_1}}$	3.4 ± 1.0	2.6 ± 1.1 †
$T_{burst < 5 \times T_{N_1}}$ in % of cells	2.6	46.4

T refers to the threshold for the evoked N_1 -potential on the stimulated side.

*Mean values \pm s.d.

†Significantly different ($P < 0.01$) from < 12 h.

‡Significantly different ($P < 0.001$) from < 12 h.

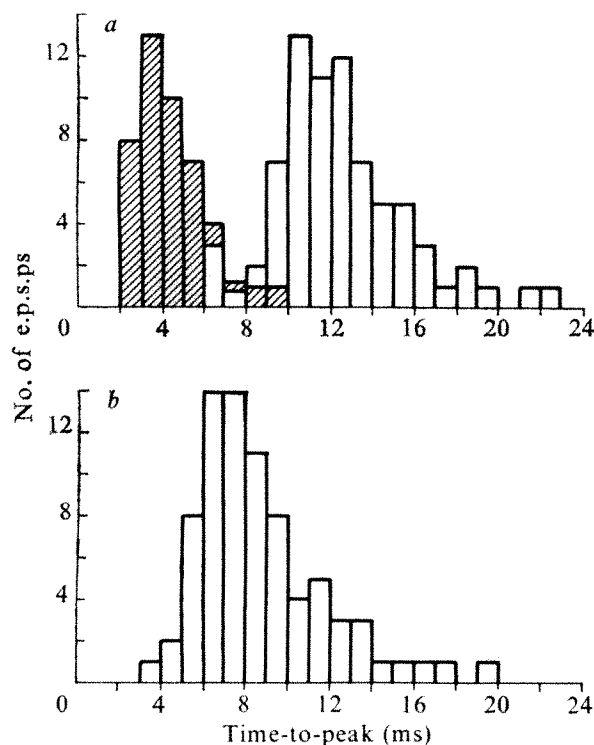


Fig. 2 Distribution of times-to-peak of e.p.s.ps in acutely (a) and chronically (b) deafferented second-order vestibular neurones. The e.p.s.ps elicited from primary afferent fibres (cross-hatched bars) have a much shorter rise time than those elicited from commissural fibres. After chronic deafferentation the same commissural input induces e.p.s.ps with a significantly shorter rise time (b).

dritic-somatic e.p.s.ps would be less attenuated in their rise times and amplitudes and, therefore, more effective in driving the cell. Similar changes in the shape indices of e.p.s.ps have been observed in other partially deafferented nuclei¹⁰ where sprouting was shown to occur¹¹. Another mechanism, for example, increased chemosensitivity of the denervated neurones to a given transmitter, is unlikely since the time-to-peak of the response does not seem to be affected¹². Also, neither the ipsilateral nor the contralateral group of e.p.s.ps showed systematic shortening of the time-to-peak with increasing stimulus intensity. Changes in passive membrane properties, although not excluded, cannot explain all the alterations observed.

The increase in excitatory synaptic efficacy of the commissural system may provide part of the background activation of chronically deafferented vestibular neurones necessary to counterbalance the asymmetry in the output of the two vestibular nuclei at rest. As a result of this newly acquired symmetrical output, the animals will resume their normal pose. Furthermore, the enhanced crossed excitation will also be of use for dynamic postural reflexes requiring a symmetrical output to somatic motoneurones. To obtain functional recovery of all vestibular reflexes, crossed inhibitory mechanisms must also be postulated¹³; these will be subject of a later report.

N. DIERINGER
W. PRECHT

Neurobiologische Abteilung,
Max-Planck-Institut für Hirnforschung,
6 Frankfurt/M.-71 FRG

Received 8 June; accepted 2 August 1977.

- ¹ Schaefer, K. P. & Meyer, D. L. in *Handbook of Sensory Physiology*, VI/2 (ed. Kornhuber, H. H.) 463–490 (Springer, Berlin, Heidelberg & New York, 1974).
- ² Kolb, E. Z. *vergl. Physiol.* 37, 136–160 (1955).

- ³ Precht, W. in *Frog Neurobiology* (eds Llinás, R. & Precht, W.) 481–512 (Springer, Berlin, Heidelberg & New York, 1976).
- ⁴ Precht, W., Richter, A., Ozawa, S. & Shimazu, H. *Expl Brain Res.* 19, 377–393 (1974).
- ⁵ Ozawa, S., Precht, W. & Shimazu, H. *Expl Brain Res.* 19, 394–405 (1974).
- ⁶ Fadiga, E. & Brookhart, J. M. *Am. J. Physiol.* 198, 693–703 (1960).
- ⁷ Rall, W. *Neurophysiology* 30, 1138–1168 (1967).
- ⁸ Hillman, D. E. *Prog. Brain Res.* 37, 329–339 (1972).
- ⁹ Fuller, P. M. *Brain Behav. Evol.* 10, 157–169 (1974).
- ¹⁰ Tsukahara, N., Hultborn, H., Murakami, F. & Fujito, Y. *J. Neurophysiol.* 38, 1359–1372 (1975).
- ¹¹ Nakamura, Y., Mizuno, N., Konishi, A. & Sato, M. *Brain Res.* 82, 298–301 (1974).
- ¹² Kuffler, S. W., Dennis, M. J. & Harris, A. J. *Proc. R. Soc. B177*, 555–563 (1971).
- ¹³ Precht, W., Shimazu, H. & Markham, C. H. *J. Neurophysiol.* 29, 996–1010 (1966).

Enkephalin effects on release of brain acetylcholine

MORPHINE and other narcotic analgesics depress the spontaneous release of acetylcholine (ACh) from the rat cerebral cortex *in vivo*^{1,2}. The effect of these agents on the central release of ACh, like their inhibitory effects on the peripheral release of ACh in the guinea pig ileum, is stereospecific³ and can be fully antagonised by naloxone⁴. It is well recognised that the endogenous opiate peptides, enkephalins^{5,6} and endorphins⁷, depress the output of ACh from the electrically stimulated guinea pig ileum by a specific action on opiate receptors. This suggests that in the central nervous system endogenous opiates may exert a significant influence on the release of ACh. We have examined the effects of two enkephalins, methionine-enkephalin (Met-enkephalin) and leucine-enkephalin (Leu-enkephalin) on the cortical release of ACh *in vivo* following intraventricular injections. Our observations show that natural enkephalins may interact with opiate receptors in the brain to modulate the release of ACh from the cholinergic neurones.

Experiments were carried out on Sprague-Dawley rats (250–300 g), lightly anaesthetised with a pentobarbital-urethane mixture. The spontaneous release of cortical ACh, which occurs in the presence of neostigmine (50 $\mu\text{g ml}^{-1}$), and atropine (0.5 $\mu\text{g ml}^{-1}$), was measured using the cup technique of MacIntosh and Oborin⁸. Experimental details of this procedure in rats, and the estimation of ACh, have been described elsewhere^{2,4}. In this study, the collection periods for cortical ACh release were shortened to 10 min from the 20–30 min used in earlier tests. The samples of cortical fluid (0.2 ml each) were collected bilaterally and both samples were pooled before estimation of ACh. The peptides, in most experiments, were administered by intraventricular (i.v.t.) injection through a fine cannula placed in the lateral ventricle on the left side. In a few tests with Met-enkephalin, the intravenous (i.v.) route of administration was used. Naloxone was always administered by i.v. injection through a femoral catheter.

Met-enkephalin (20 μg) depressed the release of ACh during the first 10-min collection period after a single i.v.t. injection. Recovery of release to pre-injection levels occurred after about 50 min. When administered intravenously Met-enkephalin (2.5 mg per kg) was completely ineffective. In subsequent experiments effects of both Met-enkephalin and Leu-enkephalin on ACh release were investigated, and in these tests naloxone was administered to animals during the peptide-induced response. As shown in Fig. 1a and b, both enkephalins depressed ACh release to the same degree and their effect was fully reversed by naloxone. When administered before enkephalins, naloxone completely blocked the inhibitory effect of enkephalins on ACh release (Fig. 1c and d). In previous work with morphine, both depressant and stimulant effects on central ACh release have been reported^{9,10}. In some cases low doses of opiates depress central ACh release while higher doses enhance this release^{3,10}. To observe if enkephalin has a dual action on release, effects of several doses of Met-enkephalin on cortical ACh release were tested. Met-enkephalin produced only a dose-related inhibition of ACh release (Fig. 2). The inhibition produced by lower doses of

Met-enkephalin was fully reversed by naloxone when it was injected after the peptide, but the inhibition produced by larger doses was only partially reversed by the antagonist (Fig. 2).

Our findings show that both Met-enkephalin and Leu-enkephalin inhibit the spontaneous release of ACh from the cerebral cortex *in vivo*. The fact that their effect could be antagonised by the specific opiate antagonist naloxone suggests that these enkephalins are acting selectively on opiate receptors to influence the release of cortical ACh. In the guinea pig ileum⁸, and the opiate receptor binding assay¹¹, Met-enkephalin is twice as potent as Leu-enkephalin. As only one dose (10 µg) of Leu-enkephalin was tested here, no definite conclusion can be drawn regarding the potency of action of two enkephalins on the central ACh release. At this dose, however, the effects of Met-enkephalin and Leu-enkephalin on ACh release were very similar and their action was completely blocked by naloxone. In previous work with opiate analgesics we have observed that when naloxone is administered after morphine-induced depression of ACh release, it produces a rebound effect on this release⁴. The post-naloxone level of output in such tests greatly exceeds the pre-drug control level^{3,4}. Interestingly, no rebound effect could be observed when naloxone was given after the enkephalin induced depression. Furthermore, we did not observe the enhancement of ACh release by opiates which has been reported in certain circumstances^{3,9,10}. Further investigation is necessary to clarify the basis for differences between the action of opiate analgesics and opiate peptides. In this regard the use of more stable analogues of enkephalins, endorphins or agents which inhibit the degradation of natural enkephalins may prove useful.

Naloxone administered by itself enhances the electrically

Fig. 1 Inhibitory effect of (a), Met-enkephalin and (b), Leu-enkephalin on the release of ACh from the intact rat cerebral cortex. The antagonism of the effect of (c) Met-enkephalin and (d) Leu-enkephalin by pretreatment with naloxone (Nal). Enkephalins were administered by intraventricular injection in a 10 µl volume, and naloxone was given intravenously in all experiments. Control release represents average release of ACh during two collections preceding the first injection. Mean \pm s.e.m. of 3–6 experiments is shown.

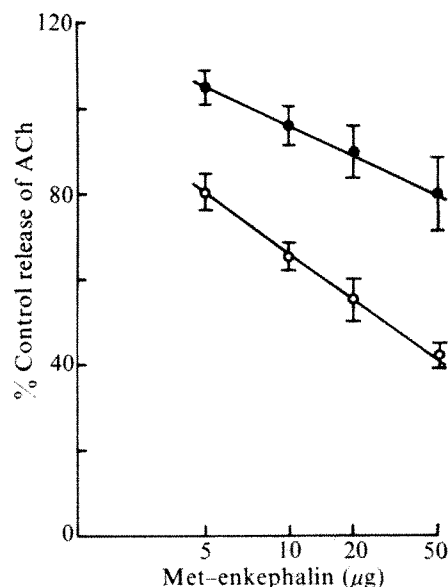
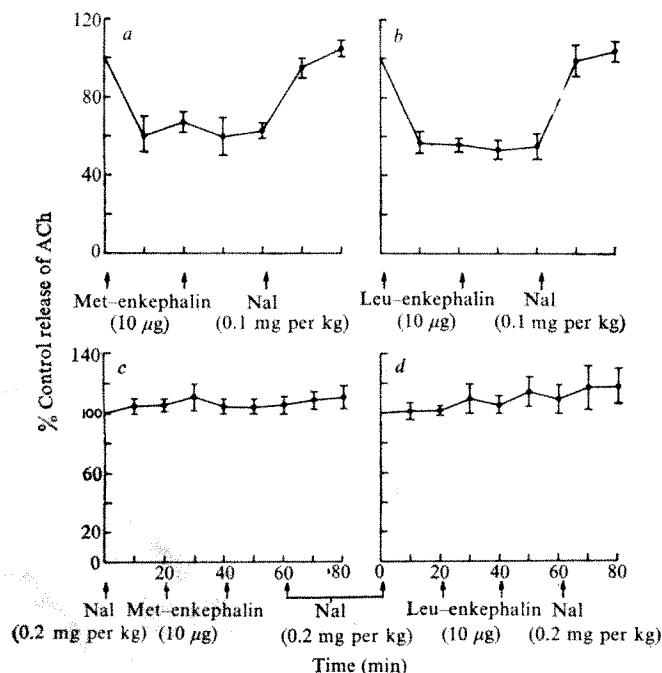


Fig. 2 Inhibition of cortical ACh release by several doses of Met-enkephalin, and the reversal of this inhibition by naloxone. ○, represents the release of ACh 10 min after intraventricular injection of Met-enkephalin; ●, represents release of ACh 10 min after intravenous injection of naloxone in the same animal. A separate group of animals was used for each dose of Met-enkephalin. Mean \pm s.e.m. of 3–4 experiments is shown.

stimulated release of ACh from the rat cortex⁴, and it has been suggested that this facilitatory effect of naloxone may be due to the antagonism of an endogenous opiate released by stimulation. This suggestion is strengthened by this demonstration of an inhibitory action of enkephalins on ACh release. Other investigators have reported an inhibition of noradrenaline¹² and dopamine¹³ release from brain slices by endogenous opioid peptides. In view of these reports, and present findings in an *in vivo* model of ACh release, an attractive suggestion is that the enkephalins or endorphins in the central nervous system may serve to modulate the release of neurotransmitters resulting from activity in the catecholaminergic and cholinergic pathways.

This work was supported by the MRC of Canada. J.S. is the recipient of a research scholarship from the Addiction Research Foundation of Ontario. We thank Endo Laboratories for the gift of naloxone.

K. JHAMANDAS
J. SAWYNOK
M. SUTAK

Department of Pharmacology,
Queen's University,
Kingston, Ontario, Canada

Received 25 May; accepted 18 July 1977.

- Matthews, J. D., Labrecque, G. & Domino, E. F. *Psychopharmacologia* 29, 113–120 (1973).
- Jhamandas, K. & Sutak, M. *Br. J. Pharmac.* 50, 57–62 (1974).
- Jhamandas, K., Hron, V. & Sutak, M. *Can. J. Pharmac. Physiol.* 50, 540–548 (1975).
- Jhamandas, K. & Sutak, M. *Br. J. Pharmac.* 58, 101–107 (1976).
- Hughes, J. *Brain Res.* 88, 295–308 (1975).
- Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. & Morris, H. R. *Nature* 258, 577–579 (1975).
- Goldstein, A. *Science* 193, 1081–1086 (1976).
- MacIntosh, F. C. & Oborin, P. E. *Abstr. XIX Int. Physiol. Cong.* 580–581 (1953).
- Mullin, W. J., Phillips, J. W. & Pinsky, C. *Eur. J. Pharmac.* 22, 117–119 (1973).
- Nistri, A. *Brain Res.* 110, 403–406 (1976).
- Simantov, R. & Snyder, S. H. *Molec. Pharmac.* 12, 987–998 (1976).
- Taube, H. D., Borowski, E., Endo, T. & Starke, K. *Eur. J. Pharmac.* 38, 377–380 (1976).
- Loh, H. H., Brase, D. A., Sumathy, S. K., Jeffrey, B. M., Way, E. L. & Li, C. H. *Nature* 264, 567–568 (1976).

Polymerising ability of C₆ glial cell microtubule protein decays much faster than its colchicine-binding activity

BECAUSE of its high degree of specificity¹, the binding of colchicine to tubulin has become a powerful tool for the quantification and subcellular localisation of the protein. The mechanism of the binding reaction is not yet completely understood, but several of its characteristics are well documented. One of these is the decay of binding capacity with time, which follows the kinetics of a first-order reaction^{2,3} and is accentuated at elevated temperatures⁴. Although correlation between this rate of decay and the loss of other characteristic functions of tubulin, such as its ability to polymerise into microtubules, has not been directly established by experiment, carefully controlled measurements of the decay of colchicine binding seem to be useful for estimating the stability of the tubulin molecules *per se*. In contrast, the decay rate of the polymerisability of tubulin preparations should depend not only on the stability of the tubulin molecules themselves but also on that of other factors that may be essential for polymerisation⁵⁻⁷. As part of our recently initiated^{8,9} attempt to study the mechanisms regulating microtubule assembly in cultured cells, we report here that tubulin preparations from rat glial C₆ cells exhibit a decay rate of colchicine-binding activity that is quite different from the decay rate of polymerisability.

Tubulin preparations purified from crude cell extracts by one cycle of *in vitro* polymerisation (with 4 M glycerol) and depolymerisation^{8,10}, which by SDS-gel electrophoresis scans contained about 70% tubulin, were used in time course studies of both the capacity to bind colchicine and the ability to polymerise. The depolymerisation was carried out by suspending the tubulin pellet in glycerol-free buffer, since the stabilising effect of this reagent on polymerisability¹⁰ and possibly on colchicine-binding activity¹¹, might have obscured the results. The decay rates of colchicine-binding activity were determined by pre-incubation of sample aliquots at 37 °C for various lengths of time before assaying the binding of ³H-colchicine¹. The results of such an experiment, carried out with purified C₆ tubulin, are shown in Fig. 1. Typically, the binding activity decayed in an apparent first-order manner with a half life of 230 ± 37 min. In the same conditions, hog brain tubulin (three polymerisation cycles) had a half life of 293 ± 20 min. Purification of the C₆ tubulin by repeated cycles of polymerisation had no effect, within experimental error, on the kinetics of decay.

In other studies (G.W., L.S.H. and R.D.C., unpublished), it was found that *in vitro* microtubule formation from depolymerised C₆ tubulin is very similar to that of hog brain tubulin in that alike polymeric intermediates in the form of rings and planar and twisted ribbons are observed. The occurrence of such structures during the assembly of microtubules from hog brain tubulin has been studied extensively¹²⁻¹⁴. The decay rate of the polymerisability of depolymerised C₆ tubulin preparations may be determined directly, by measuring from electron micrographs¹² the total amount of polymer, that is, ribbons and tubules, that formed after incubation at 37 °C after various time intervals, following the last cold spin, at 0 °C. Monitoring assembly by quantitative electron microscopy has the advantage of allowing a distinction to be made between these ordered tubulin polymers and amorphous tubulin aggregates which affect measurements made by light scattering or viscometry. Throughout this study the incubation periods at 37 °C extended beyond the time during which ring structures could still be observed. The

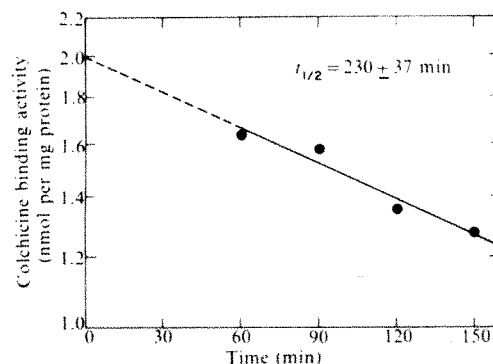


Fig. 1 Decay rate of colchicine-binding activity. $t_{1/2} = 230 \pm 37$ min. Rat glial cells (clone C₆)⁸ were grown suspended in Minimal Essential Medium (Joklik-Modified, No. F-13, Grand Island Biological, Grand Island, New York), supplemented with 5% calf serum, to a final density of approximately 1×10^8 cells ml⁻¹, and collected by centrifugation at 700g for 10 min at room temperature. Tubulin was purified from the packed cells by a modification of the Shelanski method¹⁰ of *in vitro* polymerisation (but only one cycle) as described previously⁸. Pellets of polymerised tubulin were resuspended in 100 mM sodium *N*-morpholinoethanesulphonate (MES), pH 6.4, 1.0 mM EGTA, 0.5 mM MgCl₂, and 1.0 mM GTP, and chilled on ice for 1 h for depolymerisation. The suspensions were then centrifuged at 100,000g for 1 h at 4 °C, and the pellets discarded. Aliquots of the supernatants (purified tubulin) were added to mixtures that finally contained: 8.5 µg protein, 33 mM MES, 0.33 mM EGTA, 3.7 mM MgCl₂, 0.4 mM GTP, and 6.7 mM sodium phosphate, pH 6.8, in a volume of 150 µl; and pre-incubated for various times at 37 °C. At the end of each pre-incubation period, 5 µl of an aqueous 0.186 mM ³H-colchicine (New England Nuclear) solution (2.16 Ci mmol⁻¹) were added to the samples to be tested for binding activity, and an additional incubation was carried out for 1 h at 37 °C. Subsequently the samples were analysed using DEAE-Sephadex column chromatography¹¹. All assays were run in triplicate and the data points shown represent the average values. The curve was constructed by linear least-squares fitted to these values. The total time includes pre-incubation plus the 1 h of incubation with ³H-colchicine. Protein was determined according to the method of Lowry *et al.*¹⁵.

bulk (about 90%) of the polymers formed consisted of tubules; the rest were ribbons.

A plot of the assembly data of one experiment is shown in Fig. 2, in which the decay was measured over a period of 2 h. As with the decay of colchicine-binding activity, the loss of polymerisability followed apparent first-order kinetics. Its rate seemed to be faster, however, than that of the former by an order of magnitude. The half life was calculated to be only 25.0 ± 1.8 min. Addition of supplementary fresh GTP to the solutions did not affect the amount of polymer formed at any time. Since these data suggested that the decay of some factor other than tubulin itself might have been responsible for the rapid decay of polymerisability, we attempted to supplement C₆ tubulin preparations with microtubule-associated protein, τ factor³, which has been shown to be essential for the formation of microtubules from preparations of hog brain tubulin. The addition of such hog brain factor to polymerisation mixtures of C₆ tubulin not only increased the amount of C₆ polymers formed in general, but, most interestingly, it induced the formation of tubules even at times after the polymerisability of non-supplemented tubulin had decreased to zero. Table 1 presents data obtained from such an experiment. Note that even after a pre-incubation of 510 min (about 3 h after the polymerisation of the tubulin preparation alone became undetectably low) the amount of polymer formed in the presence of heterologous τ factor exceeded by 19-fold the quantity formed with comparatively fresh preparations (60 min pre-incubation) in the absence of added factor.

Our results strongly suggest that the polymerisability of C₆ tubulin preparations depends on the integrity of some

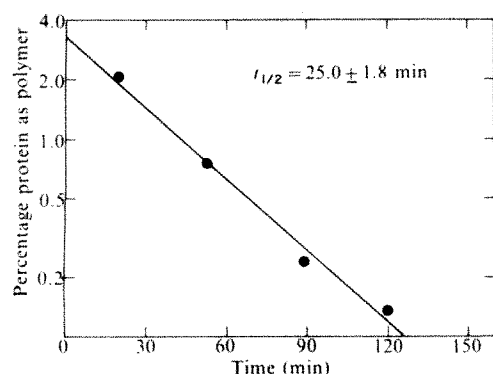


Fig. 2 Decay rate of polymerisability. $t_{1/2} = 25.0 \pm 1.8$ min. Preparations of depolymerised, purified tubulin were obtained as described in Fig. 1, except that the pellets of polymerised tubulin were resuspended in 25 mM MES, pH 6.4, 1.0 mM EGTA, 0.1 mM EDTA, 0.5 mM $MgCl_2$ and 1.0 mM GTP. At the time indicated, 50- μ l aliquots were incubated for 15 min at 37 °C. A standard solution of tomato bushy stunt virus (BSV) was then added and the mixture sprayed with 5% uranyl acetate, using a dual nebuliser, on to a carbon-coated collodion grid¹². The number of BSV was counted and the lengths of the microtubules and ribbons measured in three drop patterns for each data point. The percentage of the protein present as polymer was then calculated from these data, with knowledge of the final concentrations of tubulin (6.80 mg ml⁻¹) and BSV (0.495 mg ml⁻¹)¹².

endogenous factor other than tubulin itself. Alternatively, it is possible that tubulin could exist in multiple states with the polymerising capacity of a tubulin molecule decaying independently from the colchicine-binding ability and that τ factor could reverse the loss of polymerising function. But the simpler explanation, involving only loss of a factor, seems more likely. The decay of this factor must be more rapid than that of the tubulin, since the tubulin could still be polymerised at a time when the activity of this factor was already too low to induce any polymer formation. The data also demonstrate that even initially the concentration of this factor in active form is a limiting element in microtubule assembly from C₆ tubulin.

The experimental conditions used for measuring the decay of colchicine-binding activity and that of polymerisability differed from each other in several aspects, such as the protein concentrations of the assay mixtures, the temperatures of pre-incubations before the tests and the slightly different compositions of the buffer solutions. We believe, however, that the results shown in this report provide a conservative estimate of the divergence between the polymerisability and colchicine-binding decays. For

Table 1 Influence of heterologous τ factor on polymer formation

Pre-incubation time on ice (min)	τ concentration (mg ml ⁻¹)	Percentage protein as polymer
60	0	0.28
330	0	< 0.01
510	0	< 0.01
510	0.61	5.32

A fresh preparation (see Fig. 2) of depolymerised tubulin (7.5 mg ml⁻¹) was incubated at 0–4 °C. At the times indicated, aliquots were taken and incubated for 15 min at 37 °C. The extent of polymer formation (percentage total protein as polymer) was then determined by quantitative electron microscopy as described in Fig. 2. The formation of polymers in the presence of τ factor was determined after mixing tubulin and τ factor at a ratio of 2:1 (v/v) before incubation at 37 °C. The τ factor fraction was isolated by phosphocellulose chromatography⁹ from hog brain microtubule protein that had been purified by three cycles of polymerisation and depolymerisation¹⁰. After elution from the column, the preparation was dialysed against 50 mM ammonium acetate, pH 6.4, and 10 mM 2-mercaptoethanol, concentrated by vacuum dialysis against the same buffer, and then stored frozen. Each data point shown represents the average measurement of three drop patterns.

example, if we had been able to increase the protein concentration of the colchicine-binding assay mixture to the level used for measuring polymerisation, the difference between the two measured decay rates would have been even greater than it was, since the decay of colchicine-binding activity is known to slow down as tubulin concentrations are elevated¹. Similarly, the performance of pre-incubations at 0–4 °C (before colchicine-binding activity assays) instead of at 37 °C, only lengthened the half-life of the colchicine-binding activity (ref. 4 and G.W., unpublished). The modest levels of glycerol remaining in the tubulin preparations actually resulted in underestimation of the difference in decay rates; the concentration of residual glycerol was 100 times less in colchicine binding than during polymerisation.

In conclusion, we have shown that with tubulin purified from cultured C₆ glial cells, the capacity for microtubule assembly decays at a much faster rate than the tubulin molecules themselves. Presumably, the decay rate of polymerisability expresses primarily alteration or loss of endogenous protein factors that co-purify with tubulin through repeated cycles of *in vitro* polymerisation and depolymerisation.

We thank R. C. Williams for advice and the use of his laboratory; and Ms Nancy Steinhaus and Ms Carol Hensley for assistance. This work was supported by the NSF, the USPHS and the National Cancer Institute.

GERHARD WICHE*
LAWRENCE S. HONIG
R. DAVID COLE

Department of Biochemistry,
Department of Molecular Biology,
University of California,
Berkeley, California 94720

Received 27 May; accepted 1 August 1977.

*Present address: Institute of Biochemistry, University of Vienna, 1090 Vienna, Austria.

- Wilson, L., Bamberg, J. R., Mizel, S. B., Grisham, L. M. & Creswell, K. M. *Fedn Proc.* **33**, 158–166 (1974).
- Weisenberg, R. C., Borisy, G. G. & Taylor, E. W. *Biochemistry* **7**, 4466–4479 (1968).
- Wilson, L. *Biochemistry* **9**, 4999–5007 (1970).
- Pfeffer, T. A., Asnes, C. F. & Wilson, L. *J. Cell Biol.* **69**, 599–607 (1976).
- Weingarten, M. D., Lockwood, A. H., Hwo, S.-Y. & Kirschner, M. W. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1858–1862 (1975).
- Murphy, D. B. & Borisy, G. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2696–2700 (1975).
- Keates, R. A. B. & Hall, H. R. *Nature* **257**, 418–421 (1975).
- Wiche, G. & Cole, R. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1227–1231 (1976).
- Wiche, G., Lundblad, V. J. & Cole, R. D. *J. biol. Chem.* **252**, 794–796 (1977).
- Shelanski, M. L., Gaskin, F. & Cantor, C. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 765–768 (1973).
- Frigon, R. P. & Lee, J. C. *Arch. Biochem. Biophys.* **153**, 587–589 (1972).
- Kirschner, M. W., Honig, L. S. & Williams, R. C. *J. molec. Biol.* **99**, 263–276 (1975).
- Erickson, H. P. *J. Cell Biol.* **60**, 153–167 (1974).
- Olmsted, J. B., Marcum, J. M., Johnson, K. A., Allen, C. & Borisy, G. G. *J. supramolec. Struct.* **2**, 429–450 (1974).
- Lowry, O. H., Rosebrough, N. H., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).

Translocation of intracellularly stored calcium during the contraction-relaxation cycle in guinea pig taenia coli

ALTHOUGH it has been shown that, in vertebrate smooth muscles, cation binding sites are localised in some intracellular structures^{1–7}, and that isolated microsomal fraction can accumulate Ca in the presence of ATP (refs 8–10), the role of the Ca-accumulating structures in the contraction-relaxation cycle is not firmly established. Using potassium pyroantimonate, which is known to penetrate intact cell membrane in the presence of osmium to produce an electron-opaque precipitate with intracellular cations^{11,12},

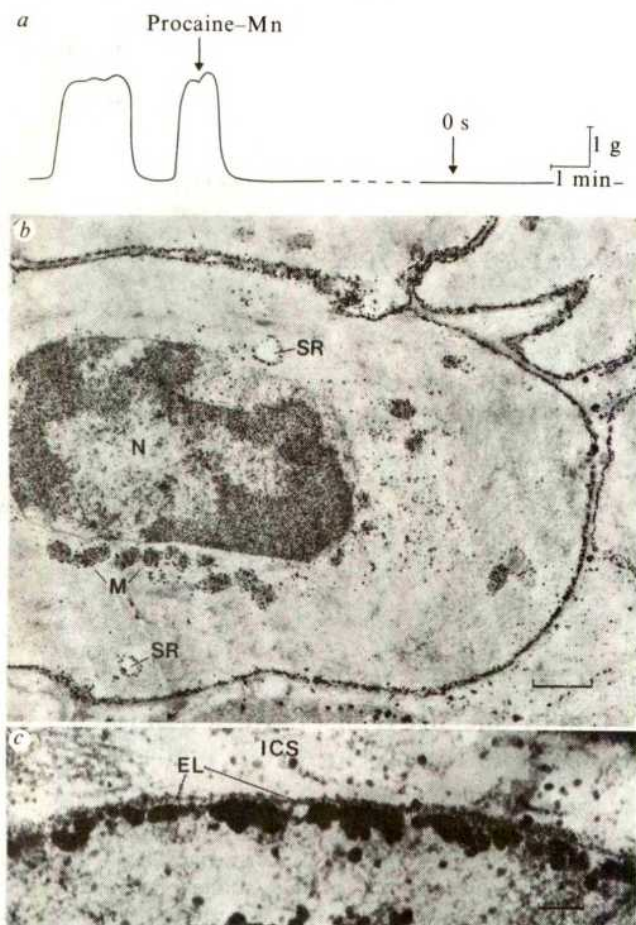


Fig. 1 *a*, Record of isometric tension during the procedure used to determine Ca localisation in the resting fibres. Spontaneous rhythmic contractions were eliminated by the addition of 1 mM procaine and 5 mM Mn ions before the application of the pyroantimonate-osmium solution (Os). *b*, Cross section of the resting fibres showing the localisation of the precipitate around the peripheral part of the fibre. N, nucleus. SR, sarcoplasmic reticulum. Unstained, scale bar 1 μ m. *c*, High-magnification view around the plasma membrane. The precipitate is mostly located inside the external lamina (EL) of the plasma membrane. ICS, intercellular space. Unstained, scale bar 0.1 μ m.

experiments on a molluscan smooth muscle have provided evidence that Ca localised in some intracellular structures is released into the myoplasm during mechanical activity^{13,14}. We have used this method to investigate the role of the intracellular Ca-accumulating structures in the contraction-relaxation cycle of guinea pig taenia coli. When guinea pig taenia coli was fixed at rest the electron-opaque precipitate containing Ca was observed at or near the plasma membrane. If the fixation was made at the peak of mechanical activity, the precipitate was diffusely distributed in the myoplasm.

Pieces of taenia coli (5–10 mm long) were mounted horizontally in an experimental chamber (3 ml) filled with oxygenated Krebs solution at 37 °C; one end of the preparation was clamped and the other end connected to a strain gauge to record isometric tension. After each experiment the preparation was fixed by replacing the experimental solution with 1% OsO₄ solution (adjusted to pH 7.4 with 0.01 M acetic acid) containing 2.5% potassium pyroantimonate (K[Sb(OH)₆]), dehydrated with ethanol, and embedded in Epon 812. Sections with gold colour were examined with a Hitachi HU-12 electron microscope.

The localisation of Ca-accumulating structures in the resting muscle fibres was examined in the following way.

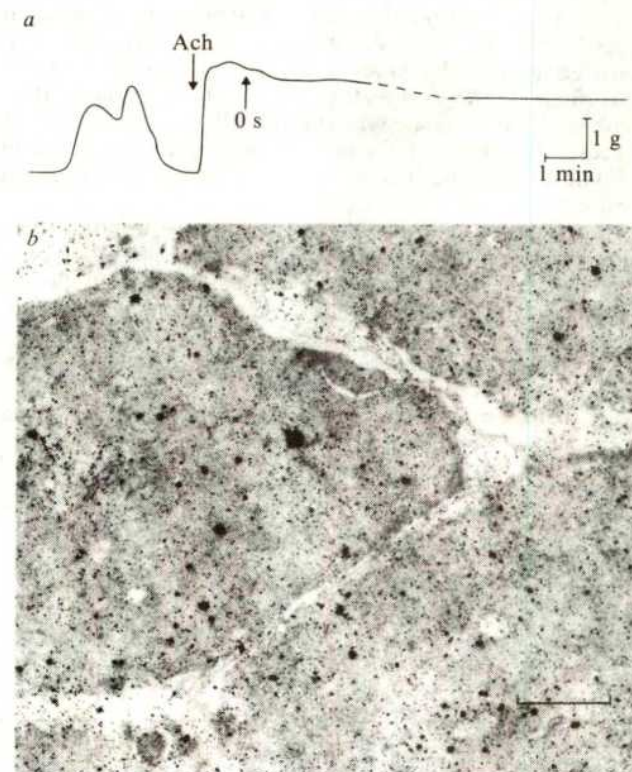
The preparation was first kept in Krebs solution for more than 20 min, and immersed in isotonic KCl solution containing 2.5 mM potassium pyroantimonate for 10 min to introduce the pyroantimonate into the fibres during membrane depolarisation (it did not readily penetrate polarised resting membrane). The preparation was then returned to Krebs solution, and after 5–10 min procaine (2 mM) and Mn ions (5 mM) were added to eliminate spontaneous mechanical activity (Fig. 1*a*); the preparation was fixed with the pyroantimonate-osmium solution with no detectable tension development.

Figure 1*b* shows the cross section of the resting fibres fixed by the above method. It can be seen that particles of electron-opaque precipitate are mostly located around the peripheral part of the fibre. Examination of the plasma membrane with higher magnifications (Fig. 1*c*) indicates that a large amount of precipitate is located inside the external lamina of the plasma membrane (probably at the inner surface of the plasma membrane and at the sarcoplasmic reticulum). The precipitate was also observed at the nucleus. Similar localisation of calcium oxalate deposits has been reported in guinea pig taenia coli fixed after treatment with potassium oxalate⁴.

In other experiments the preparation was made to contract with acetylcholine (10⁻⁵ M) and fixed at the peak of resulting contracture tension (Fig. 2*a*). The fall of tension at the completion of fixation was 20–30%. As shown in Fig. 2*b*, the precipitate was diffusely distributed in the myoplasm in the form of a number of particles, while little precipitate was seen at the periphery of the fibre. The amount of precipitate at the nucleus apparently remained unchanged. Similar results were obtained in the fibres fixed at the peak of spontaneous contractions.

For chemical identification of the precipitate, the sections

Fig. 2 *a*, Record of contracture tension induced by 10⁻⁵ M acetylcholine (ACh), and its decline after 30 min in the pyroantimonate-osmium solution (Os). *b*, Cross section of the fibres fixed at the peak of the contracture tension. The precipitate is diffusely distributed in the myoplasm in the form of a number of particles, while it is hardly observable around the peripheral part of the fibre. Unstained, scale bar 1 μ m.



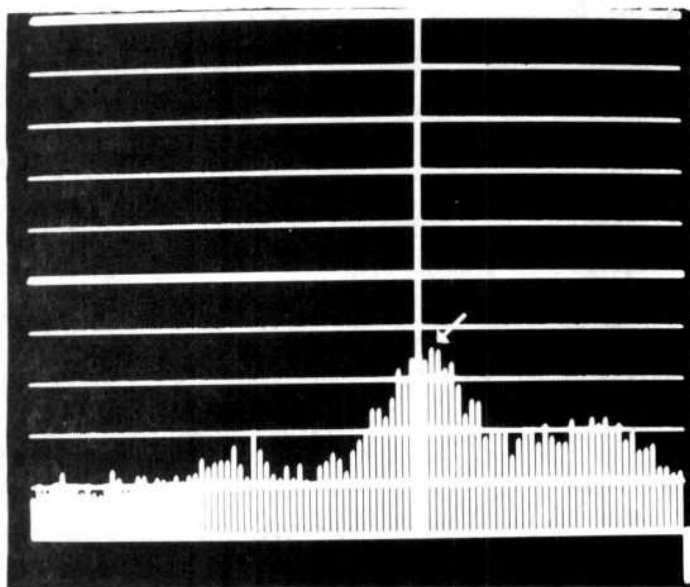


Fig. 3 X-ray spectrograph of the precipitate. The peak at 3,620 eV (arrow) is regarded as a combined spectrum of Sb-L α and Ca-K α lines. Thick vertical line indicates the standard peak of Sb-L α line (3,600 eV).

were analysed with an energy dispersive X-ray microanalyser (EDAX-707A) attached to a Hitachi HHS-2R electron microscope (accelerating voltage 25 kV, counting time 400 s). The X-ray spectrograph of the precipitate in both the resting and the contracted fibres showed a distinct peak at 3,620 eV (Fig. 3). Model tests with Ca and antimonate¹⁵ suggest that this spectrum results from the combination of Sb L-line (3,600 eV) with Ca K-line (3,690 eV). This indicated the presence of calcium pyroantimonate in the precipitate. Quantitative analysis of the precipitate was further made with another energy dispersive X-ray microanalyser (EDAX-707B) attached to a JEOL JEM-100C electron microscope together with a computerised EDIT system (accelerating voltage 40 kV, counting time 400 s). As shown in Table 1, the result again showed the presence of Ca in the precipitate in both the resting and the contracted fibres, though its concentration ratio was smaller than those of K and Na. The presence of Mg was not detected. But, considering that the amount of Ca ions involved in the activation of the contractile system is far smaller than those of K and Na ions in the myoplasm¹⁶, the values of concentration ratio of Ca in the precipitate may be taken to indicate that the precipitate serves as a measure of Ca localisation.

In conclusion, the translocation of the precipitate containing Ca from the peripheral intracellular structures into the myoplasm during mechanical activity seems to be consistent with the view that, in guinea pig taenia coli, the contraction-relaxation cycle is regulated by the release of Ca from, and its uptake by, the intracellular Ca-accumulating structures.

We thank Dr Suechika Suzuki and Miss Yoko Narikawa

for technical assistance, and the staff of the Electron Optics Division, JEOL Ltd. for providing facilities to use the micro-analyser with the EDIT system.

HARUO SUGI
TATEO DAIMON

Departments of Physiology and Anatomy,
School of Medicine,
Teikyo University,
Itabashi-ku, Tokyo 173, Japan

Received 3 May; accepted 25 July 1977.

- ¹ Somlyo, A. V. & Somlyo, A. P. *Science* **174**, 955-958 (1971).
- ² Goodford, P. J. & Wolowick, M. W. *J. Physiol., Lond.* **224**, 521-535 (1972).
- ³ Jonas, L. & Zelick, U. *Expl Cell Res.* **89**, 352-358 (1974).
- ⁴ Popescu, L. M., Diclescu, I., Zelick, U. & Ionescu, N. *Cell Tiss. Res.* **154**, 357-378 (1974).
- ⁵ Somlyo, A. P., Somlyo, A. V., Devine, C. E., Peters, P. D. & Hall, T. A. *J. Cell Biol.* **61**, 723-742 (1974).
- ⁶ Popescu, L. M. & Diclescu, I. *J. Cell Biol.* **67**, 911-918 (1975).
- ⁷ Heumann, H. G. *Cell Tiss. Res.* **169**, 221-231 (1976).
- ⁸ Carsten, M. E. *J. gen. Physiol.* **53**, 414-426 (1969).
- ⁹ Batra, S. C. & Daniel, E. E. *Comp. Biochem. Physiol.* **38A**, 285-300 (1971).
- ¹⁰ Hurwitz, L., Fitzpatrick, D. F., Debbas, G. & Landon, E. J. *Science* **179**, 384-386 (1973).
- ¹¹ Komnick, H. & Komnick, U. *Z. Zellforsch. mikrosk. Anat.* **60**, 163-203 (1963).
- ¹² Legato, M. J. & Langer, G. A. *Cell Biol.* **41**, 401-423 (1969).
- ¹³ Sugi, H. & Atsumi, S. *Proc. Japan Acad.* **49**, 638-642 (1973).
- ¹⁴ Atsumi, S. & Sugi, H. *J. Physiol., Lond.* **257**, 549-560 (1976).
- ¹⁵ Mizuhira, V. *Acta histochem. cytochem.* **9**, 69-87 (1976).
- ¹⁶ Ebashi, S. & Endo, M. *Prog. Biophys. molec. Biol.* **18**, 123-183 (1968).

Tumour-specific transplantation antigen from SV40 transformed cells binds to DNA

MUCH work is being directed towards the characterisation of tumour-specific transplantation antigens (TSTA). AL/N strain mouse embryo cells transformed by SV40 virus (SV AL/N cells) are suitable for such studies, because (1) these cells are highly antigenic and are rejected at high dose ($>10^7$) by the immunologically competent syngeneic AL/N mouse^{1,2}, (2) TSTA is common to all SV40 virus-transformed cells tested but specific in that only SV40 transformed cells share a common TSTA³, and (3), the SV40 'early' or A gene is relatively small ($\sim 1.8 \times 10^6$ molecular weight), its mapping and the nucleotide sequences of the restriction endonuclease fragments are under intense study, and the various cellular antigens in the transformed cells—the TSTA, the tumour-specific surface antigen (TSSA) (ref. 4), and the nuclear T antigen—probably represent modifications of products of this early gene⁵⁻⁷. Using SV AL/N cells and a detergent technique⁸ for solubilisation, we demonstrated that immunisation by a partially purified soluble extract containing 100 μ g protein effectively protects mice against tumourigenic doses of SV40 transformed cells⁹. We report here that TSTA activity co-purifies with the T antigen in several steps, that TSTA can be recovered from a DNA column in a fraction which then effectively immunises at the 1- μ g level, and that the TSTA, like the T antigen¹⁰, binds to double-stranded mammalian DNA.

Nuclear preparations from an SV40 transformed human cell line are rich in TSTA activity³. Accordingly, fractions from murine SV AL/N cells enriched in nuclei and also containing the cytoplasmic membranes were sonicated and solubilised by the detergent technique. Details are given in Table 1. Antigen enrichment was by precipitation with $(\text{NH}_4)_2\text{SO}_4$ followed by DEAE-cellulose chromatography¹¹. A large amount of the T-antigen activity of the whole extract was recovered in the $(\text{NH}_4)_2\text{SO}_4$ precipitate (84%), and in a fraction which eluted at about 0.15 M NaCl from the DEAE column (40% of input; Table 1 and Fig. 1). This pooled fraction was enriched in TSTA activity: it gave significant ($\sim 75\%$) and specific protection at the 4- μ g level, and full protection at the 20- μ g level (Table 2).

The pooled DEAE fractions were then chromatographed on a DNA-cellulose column¹⁰. About 70% of the T-antigen activity was recovered in a sharp peak (pool III, Fig. 2),

Table 1 Quantitative analysis of elemental concentration ratios in the precipitate

Element	Line	Mean ratios	
		Resting fibres	Contracted fibres
Sb	L α	1.00	1.00
Ca	K α	0.19 \pm 0.07	0.12 \pm 0.05
K	K α	0.36 \pm 0.12	0.57 \pm 0.06
Na	K α	0.23 \pm 0.10	0.35 \pm 0.12

Values are means \pm s.e.m. Data for resting fibres from seven specimens, for contracted fibres from five specimens.

Table 1 Distribution and recovery of T-antigen activity

	CF units ($\times 10^3$)	Protein (mg)	Specific activity (CF units mg^{-1}) $\times 10^2$
Whole extract	310	82	37.5
0-50% $(\text{NH}_4)_2\text{SO}_4$ precipitate	255	60	42
DEAE column input	235		
pool recovery	95	12	50
DNA column input	59		
pool III recovery	42	3	140

Subconfluent SV40 transformed AL/N murine embryo cells (SV AL/N line) were grown as before², the cells were collected by scraping, and washed three times with Tris-buffered saline, pH 7.4. To the washed cells was added three times the pellet volume of ice-cold buffer containing 10 mM NaCl, 10 mM Tris-HCl, pH 7.8, 1.5 mM MgCl_2 , 1 mM dithiothreitol (DTT), 100 μM L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK) and 100 μM phenylmethylsulphonylfluoride (PMSF) (buffer I). The suspension was homogenised by four strokes on a Dounce homogeniser, and centrifuged at 700g for 10 min. The pellet, containing mainly nuclei and cytoplasmic membranes, was suspended in three times its volume of buffer I, given four strokes on a Dounce homogeniser and centrifuged again at 700g for 10 min. The pellet was resuspended in buffer II containing 0.02M Tris-HCl, pH 8.2, 0.08M NaCl, 1mM DTT, 10% glycerol, 0.001% Triton X-100, 100 μM each TPCK and PMSF¹¹. The suspension was sonicated for 1 min and centrifuged at 27,000g for 1 h. The pellet was resuspended in the same volume of buffer II, homogenised and centrifuged as before. The two supernatants were combined, designated as whole extract, and precipitated with ammonium sulphate to a final concentration of 50%. Further fractionation was achieved on a DEAE-cellulose column as in Fig. 1, followed by a DNA-cellulose column as in Fig. 2. T antigen was measured by standard microtitre complement fixation assay, and units were calculated as in ref. 6. Anti-T antiserum was provided by Dr J. Gruber (NCI). Protein was determined by the Lowry technique¹⁵. Values are in complement-fixing (CF) units.

with about threefold increase in specific activity (Table 1).

When the pooled fractions from the DNA-cellulose column were tested for TSTA, only those fractions which bound to calf thymus DNA had this activity. More than 50% protection was provided against 10-100-fold tumourigenic doses of mKSA-ASC tumour cell challenge by 1 μg , and full protection of 5 μg , of pool III material. Pooled fractions I and II, which did not bind to DNA and had no T antigen activity, were found to be inactive in the TSTA test at 40-60- μg level. The pooled fraction IV, which bound to the DNA column, had significant TSTA activity at the 10- μg level (Table 2).

It is significant that the T antigen and TSTA from SV40 transformed AL/N cells co-purify through ammonium

Fig. 1 Elution of T antigen from DEAE-cellulose column. The 0-50% ammonium sulphate precipitate (see legend to Table 1) was dialysed against buffer III containing 0.05M Tris-HCl pH 7.8, 1 mM DTT, 100 μM each TPCK and PMSF, and 10% glycerol. The dialysed material was chromatographed on a DEAE-cellulose (DE-52, Whatman) column ($8 \times 2 \text{ cm}$), eluted by a gradient of 0-0.4M NaCl in buffer III, as reported previously¹¹. The CF titre recovered is the reciprocal of the dilution of a 25- μl aliquot which fixes 50% of the complement⁶. The peak of T-antigen activity eluted at 0.15 M NaCl, as observed before^{11,16}. The fractions designated by the horizontal bar were pooled, and used for the DNA column fractionation (Fig. 2).

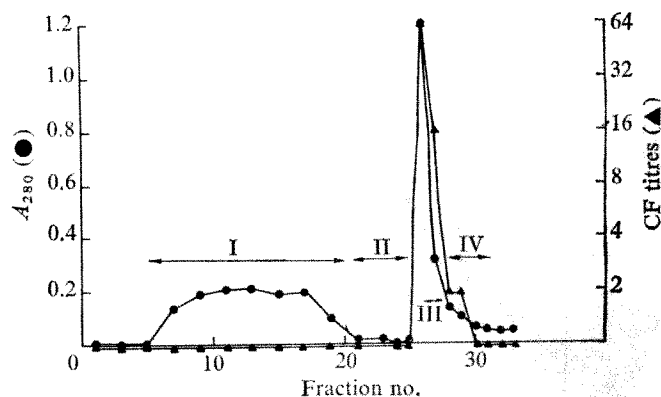
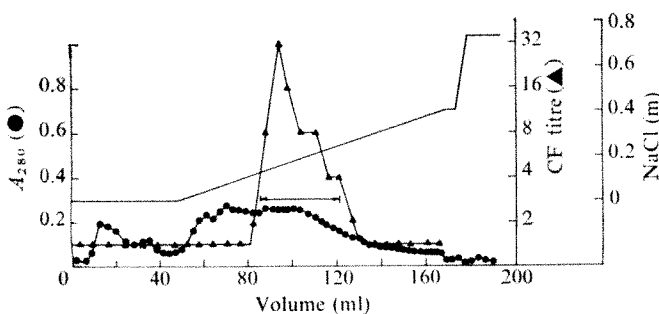


Fig. 2 Elution of T antigen from DNA-cellulose column. The pooled fractions from the DEAE-cellulose column (Fig. 1), were dialysed against 5 mM potassium phosphate buffer, pH 6.2, containing 0.1 M NaCl, 1mM DTT, 100 μM each TPCK and PMSF, and 10% glycerol. A double-stranded DNA-cellulose column ($12 \times 1 \text{ cm}$) was made from Sigma calf thymus DNA as before¹⁰. The potassium phosphate buffer was replaced, when fraction number 22 eluted, with buffer IV containing 10 mM Tris-HCl, pH 8.0, 0.6 M NaCl, 1 mM DTT, 100 μM each TPCK and PMSF, and 10% glycerol, to elute T-antigen activity¹⁰. Fractions were analysed for Δ , T antigen (CF) and \bullet , absorbance (280 nm), and pooled as defined by the horizontal bars, for TSTA determination (see Table 2).

sulphate precipitation, DEAE- and DNA-cellulose column chromatography, and that both T and TSTA specific activities were enriched through the binding to the DNA.

By studying temperature sensitive SV40 mutant viruses defective in the early A region (the tsA mutants)¹², it was found that the expression of T and TSTA is modulated coordinately^{6,13,14}. Adeno-SV40 hybrid viruses with SV40 T and/or TSTA antigen activity induce proteins with apparently overlapping regions in their polypeptide

Table 2 TSTA activity

Immunisation with	Dosage (μg protein)	Tumour cells for challenge	Tumours/total inoculated	P values
Buffer I		10^4 mKSA-ASC	10/10	
		10^4 Meth-I-A	6/10	
Whole extract	4	10^4 mKSA-ASC	5/7	
	20	10^4 mKSA-ASC	3/7	<0.015
	100	10^4 mKSA-ASC	1/7	<0.003
	20	10^4 Meth-I-A	5/10	
DEAE pool	1	10^4 mKSA-ASC	7/8	
	4	10^4 mKSA-ASC	2/8	<0.002
	20	10^4 mKSA-ASC	0/8	<0.002
Buffer IV			15/15	
DNA pool I	3	10^4 mKSA-ASC	7/7	
	15	10^4 mKSA-ASC	8/8	
	60	10^4 mKSA-ASC	8/8	
DNA pool II	10	10^4 mKSA-ASC	8/8	
	40	10^4 mKSA-ASC	7/8	
DNA pool III	1	10^4 mKSA-ASC	3/7	<0.005
	5	10^4 mKSA-ASC	0/8	<0.002
	25	10^4 mKSA-ASC	0/8	<0.002
DNA pool IV	0.2	10^4 mKSA-ASC	8/8	
	2	10^4 mKSA-ASC	7/8	
	10	10^4 mKSA-ASC	2/8	<0.002

Adult BALB/c mice were injected intraperitoneally (i.p.) twice with the indicated dose of protein at a 1-week interval. Ten days after the second injection the mice were challenged i.p. with the indicated number of the SV40 transformed BALB/c mKSA-ASC cells^{17,18} (provided by Dr L. W. Law), or intramuscularly with the methylcholanthrene transformed Meth-I-A BALB/c cells which carry their own TSTA¹⁹, for specificity control²⁰. The TD_{50} of the mKSA-ASC cell¹⁸ is 10^2 - 10^3 , the TD_{50} of the Meth-I-A is about 10^4 cells. Evidence of ascites tumour appeared by about 2 weeks in control mice, with a mean time to death of 20 d after challenge. Inoculated mice were followed for 5 weeks. The differences in the P values²¹ between immunised and non-immunised groups are significant where given.

sequences⁷. Nuclear fractions from SV40 transformed human cells are enriched in T antigen and also in TSTA⁸.

Our findings give independent confirmation of the suggested homology between T antigen and TSTA, two of the biologically important products of the early SV40 gene in SV40 transformation. Furthermore, the binding of TSTA to double-stranded mammalian DNA opens the possibility of molecular study of the paradoxical dual role of this protein: first as a tumour rejection antigen *in vivo*, and second as a candidate regulatory protein for the growth of cells in tissue culture.

CHUNG-MING CHANG
SAMUEL W. LUBORSKY
PETER T. MORA

National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 17 June, accepted 22 July 1977.

- ¹ Smith, R. W., Morganroth, J. & Mora, P. T. *Nature* 227, 141-145 (1970).
- ² McFarland, V. M., Mora, P. T., Schultz, A. & Pancake, S. J. *Cell. Physiol.* 85, 101-111 (1975).
- ³ Butel, J. C., Tevethia, S. S. & Melnick, J. L. *Adv. Cancer Res.* 15, 1-55 (1972).
- ⁴ Pancake, S. J. & Mora, P. T. *Cancer Res.* 36, 88-94 (1976).
- ⁵ Anderson, J. L., Martin, R. G., Chang, C., Mora, P. T. & Livingstone, D. M. *Virology* 76, 420-425 (1977).
- ⁶ Anderson, J. L., Chang, C., Mora, P. T. & Martin, R. G. *J. Virol.* 21, 459-467 (1977).
- ⁷ Deppert, W. & Walter, G. *Proc. natn Acad Sci U.S.A.* 73, 2505-2509 (1976).
- ⁸ Luborsky, S. W., Chang, C., Pancake, S. J. & Mora, P. T. *Biochem. biophys. Res. Commun.* 71, 990-996 (1976).
- ⁹ Chang, C., Pancake, S. J., Luborsky, S. W. & Mora, P. T. *Int. J. Cancer* 19, 258-266 (1977).
- ¹⁰ Carroll, R., Hager, L. & Dulbecco, R. *Proc. natn Acad Sci U.S.A.* 72, 3754-3757 (1974).
- ¹¹ Henderson, C. & Livingstone, D. M. *Cell* 3, 65-70 (1974).
- ¹² Martin, R. G. & Chou, J. Y. *J. Virol.* 15, 599-612 (1975).
- ¹³ Chang, C., Anderson, J. L., Martin, R. G. & Mora, P. T. *J. Virol.* 22, 281-287 (1977).
- ¹⁴ Anderson, J. L., Martin, R. G., Chang, C. & Mora, P. T. *Virology* 76, 254-262 (1977).
- ¹⁵ Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. Biol. Chem.* 193, 265-275 (1951).
- ¹⁶ Del Villano, B. C. & Defendi, V. *Virology* 51, 34-46 (1973).
- ¹⁷ Kit, S., Kurimura, T. & Dubbs, D. R. *Int. J. Cancer* 4, 384-392 (1969).
- ¹⁸ Drapkin, M. S., Appella, E. & Law, L. W. *J. natn Cancer Inst.* 52, 259-264 (1974).
- ¹⁹ McCollister, P. L. *Cancer Res.* 30, 2832-2840 (1970).
- ²⁰ Law, L. W., Henrikson, O. & Appella, E. *Nature* 257, 234-235 (1975).
- ²¹ Goldstein, A. *Biostatistics* 111 (Macmillan, New York, 1967).

Productive T7 infection of *Escherichia coli* F⁺ cells and anucleate minicells

THE development of colivirus T7 is largely independent of host cell functions and dependent on T7-encoded enzymes, for example, T7 RNA polymerase, DNA replication enzymes, and lysozyme^{1,2}. In view of this relative independence, the failure to propagate in *Escherichia coli* cells carrying an F factor^{3,4} or in anucleate minicells⁵ is particularly interesting with respect to a molecular understanding of host-virus interaction. We report here that T7 can, at a low frequency, productively infect both F⁺ cells and minicells derived from either an F⁺ or an F⁻ *E. coli* culture. The percentage of T7 permissive cells within an F⁺ population is dependent on the growth conditions of the culture but does not seem to be directly related to a specific phase of the cell cycle.

T7 produces approximately 10,000 times more plaques when plated on an F⁻ lawn of *E. coli* cells compared with F⁺ lawns. The plaques produced on an F⁺ lawn are irregular in size and do not contain selected mutant or host-cell-modified phage, as phages isolated from these plaques have no increased ability to grow on lawns of F⁺ cells. All attempts to isolate mutants of T7 capable of plating on F⁺ and F⁻ cells at the same efficiency, have so far failed. Although previous results suggest a role for a T7 early product in the exclusion of T7 by F⁺ cells⁶, we have observed exclusion of T7 deletion mutants lacking all early functions. Thus, it seems likely that the 'non-permissiveness' cannot be overcome by mutations in T7. This led us to examine factors related to the F⁺ *E. coli* cell which determine the 'non-permissiveness'.

After quantitation, we found that 2-50% of an F⁺ cell population could propagate T7, depending on growth conditions (Fig. 1a). F⁺ cells were grown in various media, infected with T7, and the infected cells plated with F⁻ indicator bacteria. A culture grown in rich medium contained the highest number of T7 permissive cells. Culture conditions therefore influence strongly the fate of T7 in F⁺ cells, by altering the ratio of permissive and non-permissive cells. The permissive F⁺ cells release a burst of T7 comparable in size with that released from F⁻ cells (Fig. 1b), indicating that once an F⁺ cell is committed to permissiveness the subsequent T7 development is normal.

In the same F⁺ cell culture, there seem to be two types of cells. This heterogeneity could not result from segregation of the F factor as identical results were obtained when growth was dependent on the presence of the F factor. As explanations for the heterogeneity we will consider the following possibilities: (1) productive infection occurs in cells which lose the plasmid during infection, for example, infection of cells in the process of cell division; (2) the permissive cells retain the plasmid but pass through a T7 permissive phase during the cell cycle; (3) each cell at any stage of the cell cycle can be infected either productively or abortively, depending on a statistical event during the infection process.

The observation that the growth rate of a culture of F⁺ cells altered the percentage of T7 permissive cells, suggested that the heterogeneity within the population for T7 permissiveness may reflect a stage within the *E. coli* life cycle. We have attempted to demonstrate an increase in the number of productively infected cells at some stage in the cell cycle by infecting *E. coli* F⁺ cells synchronised by a variety of techniques (that is, thymine and amino acid starvation,

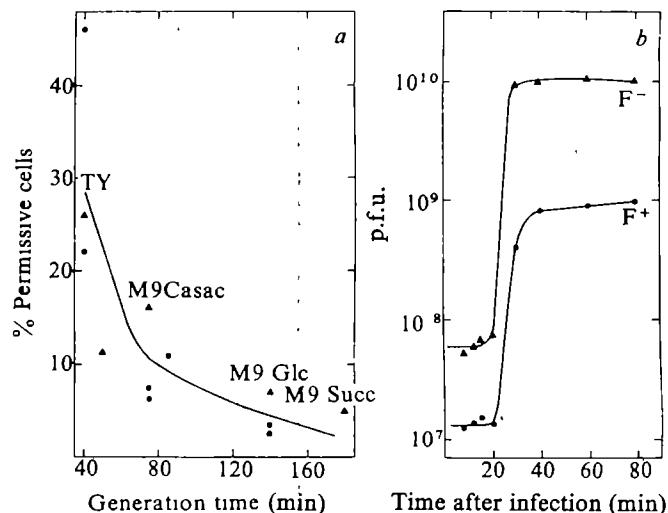


Fig. 1 Detection of T7 permissive F⁺ cells. *a*, *E. coli* 803 F⁻ and 803 F⁺ (ref. 6) were grown in various media and, at a cell density of 5×10^8 ml⁻¹, infected with T7 am 193⁺K⁺ (ref. 6) at a multiplicity of infection of 10 (30 °C). After 5 min, T7 antiserum was added and allowed to act for 3 min. Then the p.f.u.s were determined by plating aliquots with *E. coli* 803 F⁻ indicator bacteria. The p.f.u.s indicate T7 permissive cells. The p.f.u.s obtained with 803 F⁺ were plotted as a percentage of the total cell count against generation time of the bacteria. Total cell count was equal to the number of p.f.u. obtained in the F⁻ culture. Generation time varied with the culture conditions: tryptone-yeast broth, 40 min; M9 0.4% glucose supplemented with casamino acids, thymine, and thiamine¹¹, 50 min; M9 0.4% glucose supplemented with 0.03% casamino acids, 75-85 min; M9 0.4% glucose, 130 min and M9 0.4% succinate, 170 min. *b*, One-step growth curves of T7 development in *E. coli* F⁻ and F⁺. As in *a*, 803 F⁻ and F⁺ were grown in tryptone-yeast medium and infected, at a density of 5×10^8 ml⁻¹, with T7 am 193⁺K⁺. After 5 min, T7 antiserum was added and, at 8 min, the culture was diluted $1:10^4$ to dilute out the antibodies. p.f.u.s were determined at various times thereafter, by plating on 803 F⁻ bacteria.

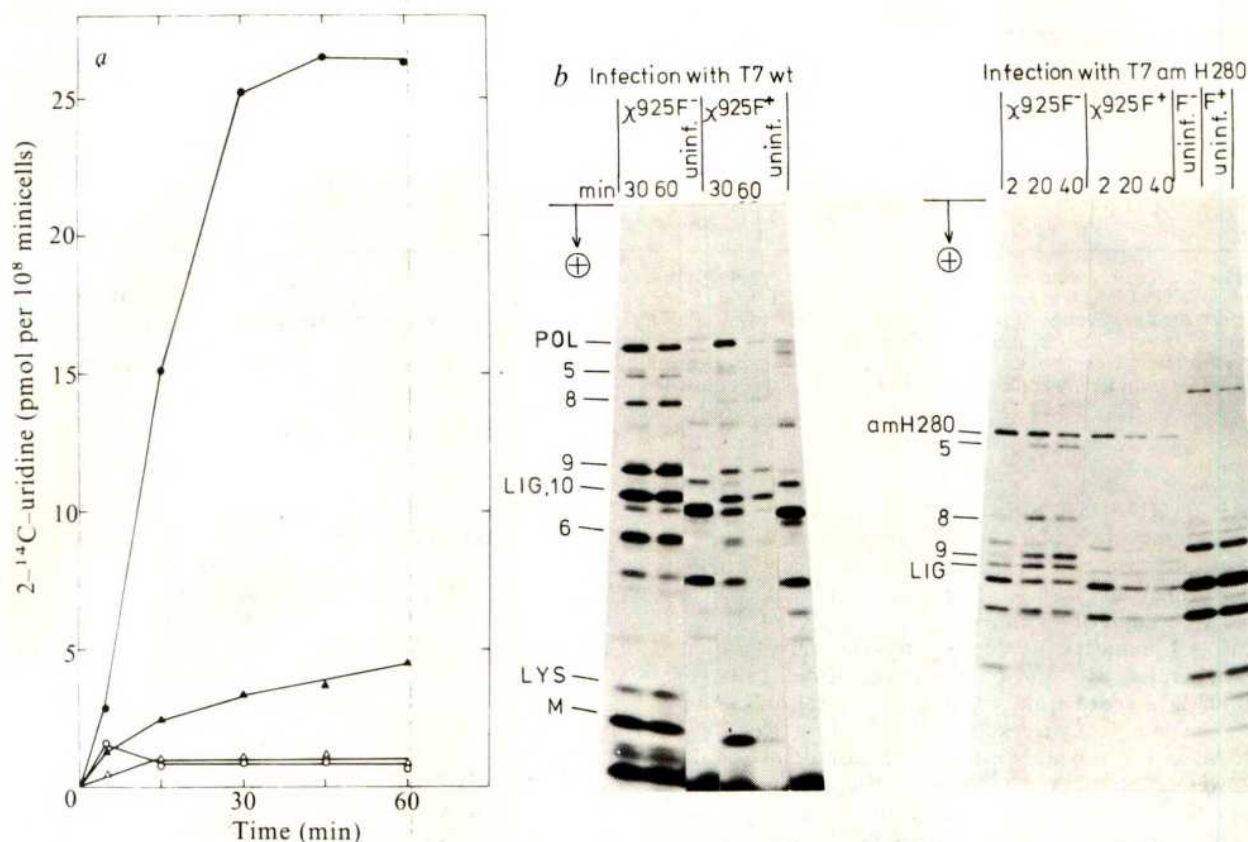


Fig. 2 T7 gene expression in anucleate minicells derived from F⁻ and F⁺ strains. The minicell producing strain χ925 (ref. 15) and an isogenic F⁺ derivative were grown in L broth to $A_{600} = 0.8$. Minicells (2×10^8 ml⁻¹) were infected with T7 at 0 °C in M9 glucose minimal medium containing 10 μg ml⁻¹ threonine and 10 μg ml⁻¹ leucine (multiplicity of infection = 20). 2-¹⁴C-uridine (0.11 μCi ml⁻¹, 59 mCi mmol⁻¹) was added and the suspensions were placed at 30 °C. Samples (100 μl) were taken at various times, mixed with 100 μl lysozyme solution (2 mg lysozyme per ml TES, that is, 0.05 M Tris-HCl, pH 7.6, 0.1 M NaCl, 0.05 M EDTA) and rapidly frozen by immersion of the tubes in liquid nitrogen. When all samples had been taken they were simultaneously placed at 37 °C for 2 min; 20 μl of 2% sarkosyl in TES was added followed by 2 ml ice-cold 5% TCA. Cold TCA-precipitable material in each sample was measured as described previously¹⁵. ●, F⁻ minicells, infected; ○, uninfected; ▲, F⁺ minicells, infected; △, uninfected. **b**, Protein synthesis. Minicells were resuspended in M9 glucose minimal medium containing 10 μg ml⁻¹ threonine and 10 μg ml⁻¹ leucine and were infected with either T7 am 193⁺K⁺ or T7 am H280 (T7 RNA polymerase⁻, protein kinase⁻) at a multiplicity of infection of 10. At various times (as indicated), ¹⁴C-amino acids were added (25 μCi ml⁻¹). After a further 20 min of incubation, the infected minicells were removed from the radioactive medium by centrifugation, and dissolved in sample buffer for polyacrylamide gel electrophoresis. Autoradiograms of 10–20% gradient gels are shown. This figure, in addition to the information discussed in the text, covers two aspects. (1) The expression of stable mRNA in minicells¹⁸ can be detected. After infection with T7, the expression of this mRNA is stopped, indicating translational control¹⁹. (2) A comparison between products made from stable mRNA in the minicells derived from F⁻ and F⁺ cells, reveals one major difference. One additional peptide of molecular weight of about 25,000 is synthesised in the F⁺ minicells. Thus, the sex factor also seems to code for an extremely stable messenger.

use of *dna*⁻ts mutants, successive fractionation of progeny cells, and fractionation of cells according to size). We have so far been unable to demonstrate any relationship between the division cycle of *E. coli* and productive T7 infection. The possibility still exists that the techniques used for synchronising cell division, did not synchronise the event responsible for T7 permissiveness (for example, the state of F factor replication). Although negative, these results argue against our first two suggestions for T7 permissiveness of F⁺ cells.

Inhomogeneity in each cell may be mediated by the cell envelope. Inhomogeneities of the cellular surface with respect to newly synthesised areas have, for example, been found using phage receptors as probes^{7,8}. The role of the membrane in the abortive infection of F⁺ cells by T7 has indeed been documented^{6,9–11}, and we know from earlier experiments that no soluble factor can be responsible for the exclusion⁶. We hypothesise, therefore, that the membrane carries 'permissive' and 'non-permissive' entry sites for T7. Depending on the growth conditions, the episome influences the ratio of the two sites. From this hypothesis we expect that after loss of the episomal DNA, cells would regain up to 100% T7 permissiveness, dependent on growth of the cell envelope, but would retain the ability to exclude

T7 if no growth occurred. These two conditions were tested using *E. coli* carrying the temperature-sensitive *F*_{L101} lac episome and using minicells derived from F⁺ parent cells.

After shift of an *E. coli* *F*_{L101} lac culture from 30 °C to 42 °C, F replication stops and the F DNA is diluted out with cell division. The re-appearance of T7 permissiveness followed the segregation kinetics with a brief lag period (not shown). These data would be consistent with the assumption of a continuous regeneration of permissive membrane sites.

Conditions in which the cellular surface is not significantly altered after loss of DNA, are found with anucleate minicells. We therefore constructed isogenic F⁺ and F⁻ strains of an *E. coli* mutant which produces anucleate minicells. The minicells formed do not contain chromosomal or episomal DNA. After infection of minicells from the F⁻ strain, uridine was rapidly polymerised into a TCA-precipitable form (RNA), whereas the infected F⁺ minicells synthesised very much less RNA (Fig. 2a). The uninfected F⁺ and F⁻ minicells did not produce significant amounts of TCA-precipitable material. This difference in the patterns of RNA synthesis is very reminiscent of the difference observed in nucleated cells^{8,12}. The initial rate of synthesis is similar between F⁻ and F⁺ nucleated cells whereas at later times the

Table 1 T7 development and recombination in *E. coli* minicells

Expt	Minicells (ml ⁻¹)	Mating type of parental cells	Plaque-forming units (p.f.u.) ml ⁻¹ resulting from infection of minicells by:				p.f.u. resulting from minicells coincidentally infected by T7 mutants and subsequently mixed		Viable cell count (ml ⁻¹)
			T7 ⁺	T7 gene 11 ⁻	T7 gene 17 ⁻	T7 gene 1 ⁻	by pairs of T7 mutants	singly infected T7 mutants	
1	2 × 10 ¹⁰	F ⁻	8 × 10 ⁷	< 10 ³	2 × 10 ⁴	—	3.3 × 10 ⁶	1.8 × 10 ⁴	1 × 10 ²
2	1 × 10 ⁹	F ⁻	4 × 10 ⁶	< 10 ³	—	< 10 ³	2 × 10 ⁴	—	1 × 10 ³
	1 × 10 ⁹	F ⁺	5 × 10 ⁶	< 10 ³	—	< 10 ³	3 × 10 ³	—	1 × 10 ³

Before infection, minicells were (expt 1) or were not (expt 2) pre-incubated in L broth containing 20 µg cycloserine ml⁻¹, at 37 °C for 1 h. The multiplicity of infection was 10 (expt 1) or 5 (expt 2). After 5 min at 37 °C, T7 antiserum was added. At 7 min, the infective centres (p.f.u.) were determined by plating with B₁ (B strain, *su*⁻) as indicator bacteria. The following viral mutants were used²⁰: T7 am 37 (gene 11⁻); T7 am 145 (gene 17⁻) and T7 am 27 (gene 1⁻). To confirm that recombination had occurred within the minicells, we mixed singly infected minicells after the antiserum treatment and plated for infective centres after additional 5 min of incubation. A 50-fold increase in the parental cell count (by addition) did not change the number of infective centres obtained.

synthesis of RNA in the F⁺ cell terminates because of the general shut-down of macromolecular syntheses^{8,12}.

An unequivocal comparison of specific viral gene products was performed at the level of protein synthesis. Whereas infected F⁻ minicells synthesised all early and late T7 proteins, F⁺ minicells produced only the early proteins, again in parallel with the process in nucleated cells (Fig. 2b). Both late genes transcribed by the viral RNA polymerase and late genes transcribed by the host RNA polymerase (as in the polymerase-protein kinase double mutant H280), were affected. At later times, early protein synthesis in F⁺ minicells was also decreased as in whole cells. From these experiments we conclude that minicells are efficiently infected with T7 and that they produce T7 products in the same order of magnitude as do nucleated cells. This provided evidence for the existence of sufficient host cell RNA polymerase in the minicells to permit transcription of infecting phage genomes as has been described previously for phage infection of minicells produced by *Bacillus subtilis*¹³. The time course of synthesis is, however, expanded by a factor of 5–10 (Fig. 2b). Thus, in minicells a factor must be rate-limiting which is not limiting in nucleated cells. In addition, infection of minicells with T7 carrying a known amber mutation led to the loss of the affected protein and production of the corresponding amber fragment (Fig. 2b), indicating that recognition of nonsense codons occurs normally in minicells.

To determine whether the F⁺ minicells were heterogeneous and contained a fraction of permissive cells, we examined the development of virus particles in F⁻ and F⁺ minicells. Although only little DNA replication was detectable by thymidine incorporation (not shown), a low but significant and reproducible number of F⁻ minicells released virus particles (0.1%). Among the infected F⁺ minicells (grown in rich medium), a fraction of about 0.01% propagated T7. This is similar to the situation in nucleated cells in which a fraction of the cells is also T7 permissive (Fig. 1a and b).

We were unable to demonstrate a one step growth curve with minicells and would suggest that this is due to a very asynchronous release of a small number of progeny phage. Because of the inherent problems in demonstrating that only one out of 1,000 or 10,000 infected minicells produced phage, the results were confirmed by recombination experiments (Table 1). A significant number of recombinant virus was detected after doubly infecting F⁻ and F⁺ minicells with T7 amber mutants.

In conclusion, we believe that the results reported here indicate that T7 development in F⁺ cells is abortive or permissive, dependent on the growth conditions of the *E. coli* cell culture. The decision as to successful or abortive infection is made at the time of infection and contained within the existing structure of the cell as minicells which

do not contain genetic information show the same properties as do whole cells.

H. PONTA*
J. N. REEVE†
M. PFENNIG-YEH
M. HIRSCH-KAUFFMANN*
M. SCHWEIGER*
P. HERRLICH

Max-Planck-Institut für Molekulare Genetik,
Berlin 33, Germany

Received 24 June; accepted 22 July 1977.

*Present address: Institute für Biochemie der Universität, Innsbruck.

†To whom correspondence and reprint requests should be sent.

- Schweiger, M. & Herrlich, P. *Curr. Topics Microbiol. Immun.* **65**, 59–132 (1974).
- Hausmann, R. *Curr. Topics Microbiol. Immun.* **75**, 77–110 (1976).
- Ryter, A., Shuman, H. & Schwartz, M. J. *Bact.* **122**, 295–301 (1975).
- Begg, K. J. & Donackie, W. D. J. *Bact.* **129**, 1524–1536 (1977).
- Ponta, H. *et al. Molec. gen. Genet.* **149**, 145–150 (1976).
- Condit, R. C. J. *molec. Biol.* **98**, 45–56 (1975).
- Britten, J. R. & Haselkorn, R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2222–2226 (1975).
- Condit, R. C. & Steitz, J. A. J. *molec. Biol.* **98**, 31–43 (1975).
- Reeve, J. N. & Cornett, J. B. J. *Viol.* **15**, 1308–1316 (1975).
- Ponta, H. *et al. Molec. gen. Genet.* **134**, 281–297 (1974).
- Frazier, A. C. & Curtiss, R., III. *Curr. Top. Microbiol. Immun.* **69**, 1–84 (1974).
- Mertens, G. & Reeve, J. N. J. *Bact.* **129**, 1198–1207 (1977).
- Reeve, J. N. J. *Bact.* **131**, 353–365 (1977).
- Levy, S. B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2900–2904 (1975).
- Herrlich, P., Rahmsdorf, H.-J., Pai, S. H. & Schweiger, M. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1088–1092 (1974).
- Studier, F. W. *Virology* **39**, 562–574 (1969).

Ratios of α -to β -globin mRNA and regulation of globin synthesis in reticulocytes

THE genomes of several mammalian species contain at least two α globin loci and a single β globin locus so that the ratio of α - to β -globin genes is 2 : 1 or more^{1–3}. Despite this difference in number of genes, reticulocytes from these species synthesise equal numbers of α - and β -globin chains indicating that compensatory regulation must occur. Cytoplasmic mRNA is an intermediate between the gene and globin protein, and it has been suggested that the cellular α/β mRNA ratio is an important factor in achieving balanced globin synthesis^{4–6}. We report here an excess of 30% α mRNA on reticulocyte polysomes of rabbits, mice, and sheep. Our data also illustrate the potential for errors in studies in which only poly(A)⁺ RNA is assayed.

Using inhibitors of polypeptide elongation in rabbit reticulocytes, Lodish has demonstrated that β mRNA initiates polypeptide synthesis 40% faster than α mRNA; and since the rate of elongation of the two globin polypeptides is equal, he inferred that a 40% excess of α mRNA on polysomes is required to achieve balanced globin

synthesis⁵. Others have inferred a functional α/β RNA ratio of 1.3:1.8 in rabbits⁶.

Attempts to measure directly the molar ratio of α to β mRNA in reticulocytes have led to conflicting results. Formamide gel electrophoresis of poly(A)⁺ RNA obtained by oligo(dT)-cellulose chromatography has yielded α to β mRNA ratios as low as 1 and as high as 1.6 (refs 7-12). Recently single determinations using hybridisation with cDNA probes of 85% specificity have given α/β RNA ratios of 1.1 and 1.5 in total RNA of mouse and poly(A)⁺ RNA of human reticulocytes^{12,13}.

Globin mRNAs of rabbit, mouse and sheep were separated by formamide gel electrophoresis⁷⁻¹². Although these mRNA templates seemed 80-90% pure by gel analysis after a single electrophoretic step, cDNAs made from these templates were 20-40% cross-contaminated by hybridisation analysis. This cross-contamination is presumably due to incomplete separation of the two mRNAs caused by heterogeneity in the poly(A) content of each. To improve mRNA separation, and thus the specificity of the cDNAs, we subjected each globin mRNA to two electrophoretic procedures (Fig. 1). The α and β cDNAs made from these purified templates were then hybridised to increasing concentrations of mRNAs (Fig. 2). The percentage specificity of a cDNA was calculated by subtracting from 1 the ratio of the amount of homologous to heterologous mRNA needed to half-saturate that probe and multiplying the answer by 100 (refs 12, 13). Heterologous and homologous

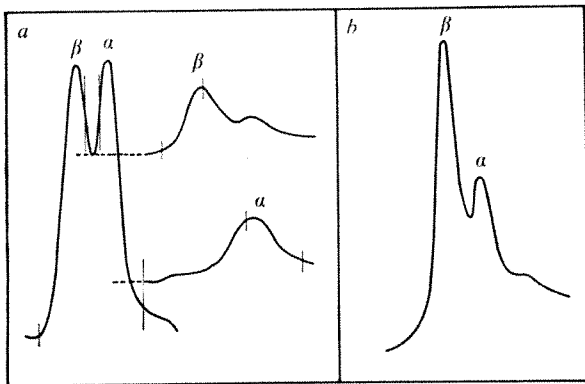


Fig. 1 Separation of α and β globin mRNAs by polyacrylamide gel electrophoresis in formamide. Total cellular RNA and polyosomal RNA of reticulocytes were isolated^{21,22} and separated into poly(A)⁺ and poly(A)⁻ fractions by oligo(dT)-cellulose chromatography²³. Poly(A)⁻ fractions were chromatographed three or four times to remove traces of poly(A)⁺ RNA. Poly(A)⁺ RNA was subjected to electrophoresis on 5% polyacrylamide cylindrical gels in 98% formamide-20 mM Na barbital and 20 mM NaCl at 3.5 mA per gel at 24 °C for 2-3 h¹². Globin mRNAs were located by scanning unstained gels at 260 nm and gel pieces containing the α and β mRNAs were excised and incorporated with the same polarity into separate 7% polyacrylamide gels. After a second electrophoresis at 3.5 mA per gel for 3-4.5 h at 24 °C, the mRNAs were again located by ultraviolet scanning and the pertinent gel fractions were excised. In some experiments, RNA was eluted electrophoretically into dialysis bags⁷. The eluate was extracted with phenol, centrifuged to remove gel particles, re-extracted twice with chloroform and made 70% in ethanol to precipitate the mRNA. In recent experiments a different procedure which resulted in a twofold increase in yield was used. The gel pieces were driven through a 25-gauge needle with 2-3 volumes of 7 M urea, 0.35 M NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 2% SDS. The resulting suspension was agitated at 45 °C for 2 h, underlayered with 60% glycerol, and centrifuged to remove gel pieces. After the aqueous layer was extracted twice with phenol and twice with chloroform, the RNA was precipitated with ethanol and purified by oligo(dT)-cellulose chromatography. (J. Ross, personal communication). *a*, Poly(A)⁺ RNA of C57 B1/6J mouse reticulocytes. After the 5% gel electrophoresis (left), each mRNA was excised and rerun in a 7% gel (right). mRNAs were excised as indicated by the vertical lines. *b*, Reticulocyte poly(A)⁺ RNA of sheep homozygous for β^H globin after 5% gel electrophoresis.

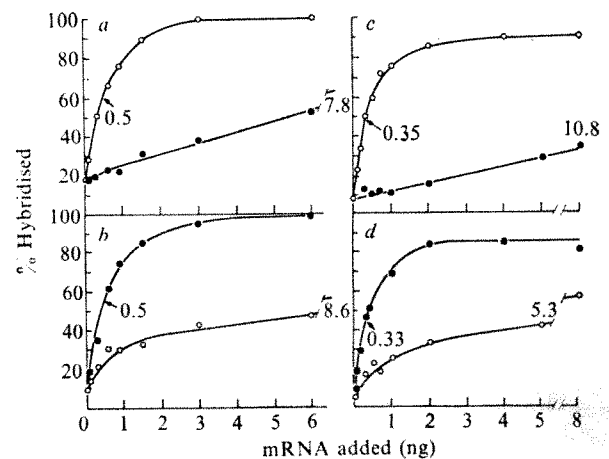


Fig. 2 Hybridisation of α and β ³H cDNAs with purified α and β mRNA templates. ³H-cDNAs were synthesised as reported²⁴ except the 100- μ l reaction mixture contained 100 μ g ml⁻¹ actinomycin D, 400 μ M each of dATP, dTTP, dCTP, and 100 μ M ³H-dGTP (10 Ci mmol⁻¹); 1-2 μ g RNA template, 0.125 μ g oligo(dT) per μ g mRNA, and 300 units per ml AMV reverse transcriptase (from NCI). After incubation at 37 °C for 1 h, 50 μ g tRNA was added, the mixture extracted with phenol, and cDNA was precipitated with ethanol. α and β cDNAs were treated for 24 h at 37 °C in 0.3 ml of 0.3 M NaOH, neutralised with 0.5 M HCl, applied to hydroxylapatite in 0.05 M Na phosphate buffer (pH 6.8) at 60 °C, and eluted in 0.14 M Na phosphate buffer (pH 6.8). The cDNAs (5-10S) were further purified by centrifugation in 10%-30% sucrose gradients (0.1 M NaCl; 10 mM Tris-HCl, pH 7.4; 1 mM EDTA; 0.5% sodium dodecyl sulphate (SDS)) at 94,000g and 24 °C for 20 h and then precipitated with ethanol. Hybridisations of ³H-cDNA to RNA were performed in sealed siliconised capillary tubes using a buffer containing 0.3 M NaCl, 30 mM Tris-HCl (pH 7), 2 mM EDTA, 0.1% SDS, and 40 μ g per ml *Escherichia coli* tRNA. Varying amounts of sample RNA and a constant amount of cDNA (500 c.p.m.) were diluted to 18 μ l, heated to 100 °C for 90 s, and then incubated 16-18 h at 68-70 °C. One-half of each sample was digested with S₁ nuclease, and the other half was an undigested control²⁵. Both samples were then precipitated with 7.5% trichloroacetic acid on to S&S nitrocellulose filters. The filters were dissolved with 0.5 M HCl at 100 °C followed by ethyl acetate at 24 °C, and Hydromix was added before counting. Half of the difference in hybridisation between zero and saturating RNA inputs is defined as half-hybridisation. Specificity was calculated as described in the text. *a*, Rabbit α cDNA = 94%; *b*, rabbit β cDNA = 94%; *c*, mouse α cDNA = 97%; *d*, mouse β cDNA = 94%; \circ , α mRNA; \bullet , β mRNA.

hybrids had identical thermal elution profiles ($\pm 1^\circ$) indicating little or no mismatching of sequence and suggesting that heterologous hybrids are due to residual contamination rather than cross-hybridisation¹². Alternatively, calculation of cDNA specificity by analysis of the shapes of curves seen in heterologous hybridisations suggests that residual contamination of the β cDNA (Fig. 2*b, d*) may be as high as 15-20%, while that of α cDNA (Fig. 2*a, c*) is near 0. But, since the contamination of β mRNA with α mRNA in Fig. 2*a* and *c* is no greater than 6% and 3%, respectively, this method of estimation may be invalid.

We have used the appropriate cDNA probes to measure the α/β RNA ratios in reticulocytes of rabbits, mice, and sheep. Typical hybridisation results are shown in Fig. 3, and the data are summarised in Table 1. In our experiments we have attempted to avoid several potential pitfalls. First, our cDNA probes, while up to 97% specific, have residual contamination. The presence of such contamination becomes more critical as one measures smaller differences in α/β mRNA ratio. Several assays using probes varying in specificity from 83% to 97% have yielded similar α/β RNA ratios in rabbit and mouse reticulocytes (Table 1). Furthermore, reconstruction experiments in

Table 1 Ratios of α -to β -globin RNA in total and polysomal RNA of rabbit, mouse and sheep erythrocytes

cDNAs	Expt no.	Minimum* specificity (%)		Total cellular RNA	α/β RNA† Polysomal RNA		
		α cDNA	β cDNA		Unfractionated	Poly(A) ⁺	Poly(A) ⁻
Rabbit	1	94	94	—	1.23	—	—
					1.38		
	2	86	90	1.25 1.36	1.26	—	—
Mouse	3	85	83	—	1.44	1.43	1.45
	1	97	89	1.28	1.29	—	—
	2	97	94	1.24	1.24	1.26	0.87
Sheep	1	90	85	0.98 0.85	1.45 1.29	0.92 0.81	1.38 1.36

Ratios were calculated from half-saturation values using α and β cDNA probes of indicated specificity. Each experiment contains data obtained with a different pair of cDNA probes and mRNA templates.

*See text for method of calculation.

†For method of calculation of α/β RNA ratios, see legend for Fig. 3.

which varying template mixtures were used demonstrated that differences in α/β mRNA ratios of 10% or more could be detected. Second, to measure molar RNA ratios while controlling for known mRNA and potential cDNA size differences, we have determined hybridisation ratios with template standards. These ratios (between 0.9 and 1.1) were used to correct observed hybridisation ratios in test samples.

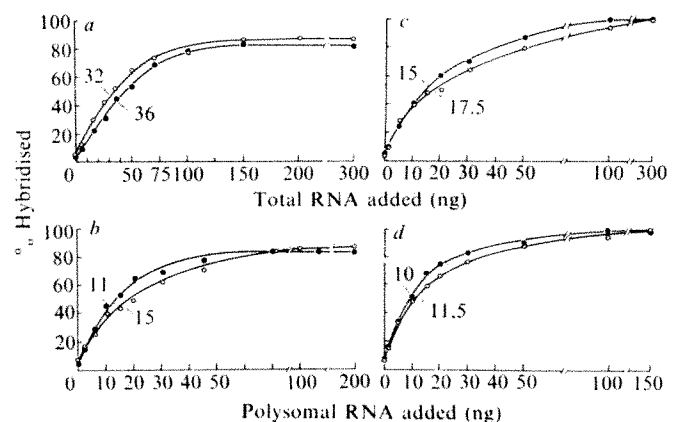
In rabbit reticulocytes, molar ratios of α to β mRNA average 1.3 in total and polysomal RNA (Table 1). Similar α/β polysomal RNA ratios were obtained using three different sets of cDNA probes with specificities ranging from 83% to 94%. While the ratios of α/β mRNA in total and polysomal RNA were equal, the α and β mRNA concentrations (calculated by comparing template and sample RNA amounts needed for half-hybridisation) were greater in polysomal ($\alpha, \beta = 1.5\%, 1.2\%$) than in total RNA ($\alpha, \beta = 1.1\%, 0.8\%$) because of the presence of 4S RNA in total RNA samples. When polysomal RNA was fractionated by oligo(dT)-cellulose chromatography, both poly(A)⁺ and poly(A)⁻ fractions (see legend to Fig. 1) had the same α/β ratio, and 95% of each mRNA was in the poly(A)⁺ fraction. By subtraction of the α and β globin mRNA amounts in polysomal RNA from those in total RNA, we calculate that 9% of α mRNA and less than 1% of β mRNA are present in non-polysomal RNA of rabbit reticulocytes, in agreement with previous estimates¹⁴.

In C57 B1/6J mice, both the total and polysomal RNA of reticulocytes have average α/β RNA ratios of approximately 1.3 (Table 1, Fig. 3c and d). Again, globin mRNA concentrations are higher in polysomal ($\alpha, \beta = 1.6\%, 1.3\%$) than in total RNA ($\alpha, \beta = 1.2\%, 0.9\%$). Note, however, that poly(A)⁺ and poly(A)⁻ fractions of mouse polysomal RNA have different α/β RNA ratios (1.26 and 0.87, respectively). Also, the α/β RNA ratio in polysomal RNA calculated from the poly(A)⁺ and the poly(A)⁻ data agrees closely with the observed ratio (1.20 compared with 1.24). Since the ratio of α/β in the poly(A)⁺ RNA is 45% greater than in the poly(A)⁻ fraction, α mRNA may contain greater average poly(A) length than β mRNA in these mouse samples. Mouse and human mixed globin mRNAs are known to have different size classes of poly(A)¹⁵⁻¹⁷. The existence of different α/β RNA ratios in poly(A)⁺ and poly(A)⁻ fractions of mouse polysomal RNA suggests that such poly(A) size classes may have different distributions in α and β mRNAs. Measurement of poly(A) length in the poly(A)⁺ and poly(A)⁻ fractions should verify these differences.

Results obtained from reticulocyte RNA of homozygous β^B sheep contrast with the rabbit and mouse results. First, while the α/β ratio in reticulocyte polysomal RNA of sheep is similar to that of rabbit and mouse, about 1.4, the α/β

RNA ratio in total RNA is only 0.9 (Table 1, Fig. 3a, b). In addition, α and β mRNAs make up 1.8% and 1.5% of polysomal RNA, but only 0.62% and 0.70% of total RNA, respectively. Second, the results obtained by assays of poly(A)⁺ RNA differ greatly from those obtained for unfractionated polysomal RNA. By gel electrophoresis, the α/β RNA ratio of sheep poly(A)⁺ RNA is about 0.5, or half that of rabbit and mouse poly(A)⁺ RNA, implying an excess of β mRNA in poly(A)⁺ RNA of sheep (Fig. 1b). By hybridisation analysis, an excess of β RNA was found in polysomal poly(A)⁺ RNA ($\alpha/\beta = 0.86$), while an excess of α RNA was present in polysomal poly(A)⁻ RNA ($\alpha/\beta = 1.37$). But, the ratio of α/β RNA in polysomal RNA calculated from the poly(A)⁺ and poly(A)⁻ data does not agree well with the observed polysomal α/β ratio (1.0 compared with 1.29), possibly due to the lower specificities of the sheep cDNA probes. It is unlikely that the observed β mRNA excess in poly(A)⁺ polysomal RNA of these β^B sheep is β^c mRNA, since Benz has demonstrated with β^c specific cDNA that β^B homozygotes essentially lack β^c genes¹⁸. The proportions of sheep mRNAs in polysomal poly(A)⁻ RNA were greater than those observed in rabbit or mouse reticulocytes, but the percentages varied depend-

Fig. 3 Hybridisation of reticulocyte total and polysomal RNA to α (●) and β (○) cDNAs of sheep (a, b) and mouse (c, d). Relative concentrations of α and β mRNA are determined by comparing amounts of sample RNA needed to half-saturate each cDNA probe. Using homologous templates as reference standards, the amounts needed to half-saturate each set of α and β cDNAs usually differed by less than 10% ($\alpha, \beta = 0.27, 0.28$ and $0.35, 0.33$ ng, respectively, for sheep and mouse cDNAs shown). The ratios obtained using template standards were used to correct observed sample ratios. For example in c, $a/\beta = (17.5/15) \times (0.35/0.33) = 1.24$.



ing on whether the observed concentration of globin mRNAs in total polysomal RNA or the calculated concentration (sum of poly(A)⁺ and poly(A)⁻ RNA fractions) was used (poly(A)⁺ α = 41.25%, β = 13.17%). These results suggest a substantial difference in the α/β RNA ratio between the poly(A)⁺ and poly(A)⁻ fractions in these sheep samples.

Since poly(A) length may affect mRNA stability and/or transport from nucleus to cytoplasm, differential adenylation of globin mRNAs could be important in balancing globin chain synthesis^{19,20}. Thus, if sheep α mRNA has a shorter average poly(A) length than β mRNA, as suggested by our data, any pool of α mRNA not associated with ribosomes in early erythroid cells could be degraded at a faster rate than β mRNA, thereby accounting for the low α/β ratio observed in total RNA of sheep reticulocytes (Table 1, Fig. 3a).

Thus, our data indicate that α/β mRNA ratios in poly(A)⁺ RNA may not reflect the actual α/β ratio in unfractionated total or polysomal RNA. In this regard we have recently re-examined the accumulation of globin mRNAs in erythropoietic mouse spleen cells. Gel analyses of globin mRNAs in poly(A)⁺ total RNA of spleen had yielded α/β ratios of 0.55–0.8 at different times after phenylhydrazine injection⁹. By hybridisation, we now find a constant α/β RNA ratio of 1.25 in unfractionated total RNA at all times. In contrast, poly(A)⁺ total RNA of spleen cells have α/β RNA ratios by hybridisation which parallel those obtained previously by gel analysis (J. A. P., in preparation). Thus, while a changing α/β ratio and β excess in poly(A)⁺-selected RNA is seen with both methods, in total spleen RNA an α RNA excess is observed using specific cDNA probes.

In addition, by hybridisation we have found α/β RNA ratios in polysomes of 1.2–1.4 in rabbit, mouse and sheep reticulocytes in agreement with theoretical predictions of functional α/β mRNA ratios^{4,5}. Our results suggest that balanced synthesis of α and β chains in these species depends on an excess of α mRNA on polysomes, regardless of the relative poly(A) length of the two messages. In these animals, the α/β mRNA ratios of 1.2 to 1.4 differ significantly from the α/β gene ratios. Thus, a further regulation of gene expression presumably occurs at transcription and/or mRNA processing.

This work was supported by USPHS grants GM 00261 and AM 13983.

J. A. PHILLIPS III
P. G. SNYDER
H. H. KAZAZIAN, JR

Department of Pediatrics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

Received 26 May; accepted 18 July 1977.

- 1 Kitchen, H. *Ann. N.Y. Acad. Sci.* **241**, 12–24 (1974).
- 2 Hilse, K. & Popp, R. A. *Proc. natn. Acad. Sci. U.S.A.* **61**, 930–936 (1968).
- 3 Wilson, J. B., Wrightstone, R. N. & Huisman, T. H. J. *Nature* **226**, 354–355 (1970).
- 4 Lodish, H. F. *J. biol. Chem.* **246**, 7131–7138 (1971).
- 5 Lodish, H. F. & Jacobsen, M. J. *J. biol. Chem.* **247**, 3622–3629 (1972).
- 6 Boyer, S. H., Smith, K. D., Noyes, A. N. & Mullen, M. A. *J. biol. Chem.* **249**, 7210–7219 (1974).
- 7 Kazazian, H. H., Jr, Snyder, P. G. & Cheng, T.-C. *Biochem. biophys. Res. Commun.* **59**, 1053–1061 (1974).
- 8 Morrison, M. R., Brinkley, S. A., Gorski, J. & Lingrel, J. B. *J. biol. Chem.* **249**, 5290–5295 (1974).
- 9 Hamlyn, P. H. & Gould, H. J. *J. molec. Biol.* **94**, 101–109 (1975).
- 10 Kazazian, H. H., Jr, Ginder, G. D., Snyder, P. G., Van Beneden, R. J. & Woodhead, A. P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 567–571 (1975).
- 11 Cheng, T.-C. & Kazazian, H. H., Jr *Proc. natn. Acad. Sci. U.S.A.* **73**, 1811–1815 (1976).
- 12 Forget, B. G., Housman, D., Benz, E. J. & McCaffrey, R. P. *Proc. natn. Acad. Sci. U.S.A.* **72**, 984–988 (1975).
- 13 Orkin, S. H., Swan, D. & Leder, P. *J. biol. Chem.* **250**, 8753–8760 (1975).
- 14 Jacobs-Lorena, M. & Baglioni, C. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1425–1428 (1972).
- 15 Lingrel, J. B., Morrison, M., Gorski, J. & Merkel, C. G. *Ann. N.Y. Acad. Sci.* **241**, 156–169 (1974).
- 16 Gorski, J., Morrison, M. R., Merkel, C. G. & Lingrel, J. B. *J. molec. Biol.* **86**, 363–371 (1974).
- 17 Cann, A., Gambino, R., Banks, J. & Bank, A. *J. biol. Chem.* **249**, 7536–7540 (1974).
- 18 Nienhuis, A. W., Benz, E. J., Jr, Steggle, A. W., Turner, P. & Geist, C. E. *Fedn Proc.* **36**, 662A (1977).

- 19 Darnell, J. E., Jelinek, W. R. & Malloy, G. R. *Science* **181**, 1215–1221 (1973).
- 20 Marbaix, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 3065–3067 (1975).
- 21 Evans, M. J. & Lingrel, J. B. *Biochemistry* **8**, 3000–3005 (1969).
- 22 Natta, C., Banks, J., Niazi, G., Marks, P. A. & Bank, A. *Nature new Biol.* **244**, 280–281 (1973).
- 23 Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1408–1412 (1972).
- 24 Verma, I. M., Temple, G. F., Fan, H. & Baltimore, D. *Nature new Biol.* **235**, 163–167 (1972).
- 25 Ross, J., Gielen, J., Packman, S., Ikawa, Y. & Leder, P. *J. molec. Biol.* **87**, 697–714 (1974).

Evidence for alkali-sensitive linkers in DNA of African green monkey kidney cells

MITOCHONDRIAL DNA possesses ribonucleotide linkers^{1–3} and although double-stranded eukaryotic chromosomal DNA seems to be continuous throughout the length of the chromosome⁴ it is possible that single-stranded DNA possesses physical (nicks) or chemical (non-deoxynucleotide linkers) discontinuities. To distinguish between an *in vivo* nick and a nick introduced by shear forces during DNA extraction is very difficult with the presently available techniques. The detection of non-deoxynucleotide linkers is, however feasible, providing that denatured DNA can be sedimented in a chemical environment in which the linker is not destroyed. In these conditions, the comparison between sedimentation profiles of DNA, previously treated or not with a reagent that attacks the linker, may reveal the discontinuity. An obvious such reagent is a strong base, thus precluding the use of alkaline gradients in these experiments, and so we have attempted to use sucrose gradients in non-aqueous formamide. In addition, the

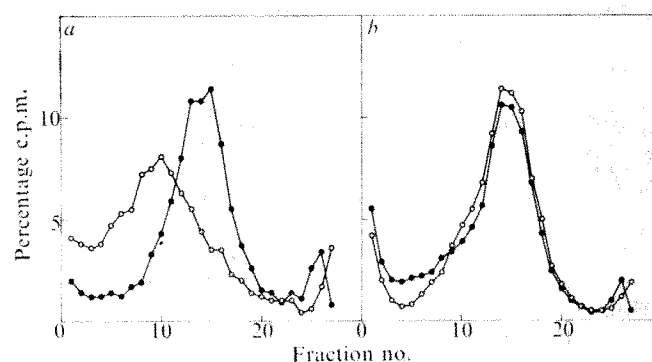


Fig. 1 Alkali-sensitive sites in green monkey kidney cell DNA. The cells were grown in glass Petri dishes, in Dulbecco's modified medium containing 10% calf serum, in a 5% CO₂ humidified atmosphere at 37 °C, and labelled for 8 h with ³H-thymidine (2 µCi ml⁻¹). The cells were washed with saline-EDTA (100 mM NaCl, 10 mM EDTA, pH 8.0) and incubated with 2.0 ml of 0.5% Triton X-100 in saline EDTA for 1 min. The nuclei remained attached to the bottom of the plate and were lysed by incubating for 30 min at 37 °C with 0.75% Sarkosyl, 1 M NaCl, 10 mM EDTA, pH 8.0. The proteins were extracted by mixing the lysate gently with equal volume of 80% phenol at 4 °C. The aqueous phase was separated by centrifugation and gently mixed again with equal volume of chloroform-isoamyl alcohol (20:5, v/v). The final aqueous phase was dialysed overnight against 2 l of 10 mM Tris-HCl, pH 7.5. Aliquots (0.25 ml) of DNA were layered on the top of 4.0-ml gradients which were 0–15% sucrose (w/v) in formamide. Centrifugation was at 32,000 r.p.m. at 30 °C in an SW-50.1 rotor (Spinco) for 4 h. A total of 26–27 equal volume fractions were dripped on to strips of Whatman No. 17 paper and radioactivity was measured as described elsewhere¹⁴. **a**, Percentage ³H-DNA from monkey cells; **b**, percentage ³H-DNA from T4 phage; ○, 100 µl of DNA solution were mixed with 50 µl of 1 M Tris-HCl, pH 7.0 and 100 µl of 0.225 M NaCl, before layering on the gradients; ●, 100 µl of the DNA solution were mixed with 50 µl of 0.45 M NaOH, incubated for 10 min at 20 °C, mixed with 50 µl of 1 M Tris-HCl followed by 50 µl of 0.45 M HCl, and then layered on the gradients. The number average molecular weights (M_n) were calculated as described elsewhere¹⁴, except that in Studier's expression the exponent 0.400 was replaced by 0.312 which was better fitted to our conditions, as determined with T4 and λ DNA. **a**, M_n (○) = 68.2; M_n (●) = 45.6; **b**, M_n (○) = 46.2; M_n (●) = 49.8.

DNA had to be extracted by a very gentle method in order to keep its average molecular weight in the range of $50\text{--}80 \times 10^6$. Using these conditions, we were able to detect alkali-sensitive linkers in chromosomal DNA of green monkey kidney cells.

A typical result is shown in Fig. 1a. DNA which has been sedimented without any previous treatment has a number average molecular weight (M_n) equal to 68.2×10^6 daltons. Exposure of DNA to 0.15 M NaOH for 5 min at 20 °C, before sedimentation causes M_n to drop to 45.6×10^6 daltons and the profile becomes less heterogeneous. Figure 1b shows that the same treatment on T4 DNA produces no change in the sedimentation profile; T7 DNA displays exactly the same behaviour (results not shown). The results of several experiments similar to that shown in Fig. 1a are given in Table 1. The average number of alkali-sensitive linkers for five different determinations (a–e) was 0.87 ± 0.29 per 10^8 daltons. This means that an average of one linker is found for every 115×10^6 daltons. In one case (Table 1e) the NaOH treatment was more drastic, without introducing comparatively more breaks, therefore, the more gentle reaction condition seems to give a full account of the linker disruption. In two other experiments (Table 1f,g) DNA was pulse-labelled for 25 min and the number of breaks observed was larger than in DNA labelled for several hours; other experiments are needed to define whether these results reflect a modification of the frequency of linkers as the DNA matures.

In opposition to the linker hypothesis, it could be argued that formamide does not render the DNA completely denatured, and that this is only accomplished after treatment with NaOH. This seems not to be the case, however, since T4 DNA displays the same sedimentation profile with or without NaOH treatment. Further evidence that DNA is already denatured before NaOH treatment is given by the experiment of Fig. 2. In this experiment, we intended to produce a DNA in which the opposite strands were labelled differently and had different sizes. If, on centrifugation in formamide, we obtained separation of the two sedimentation profiles we could conclude that the DNA was denatured. Cells were therefore prelabelled with ^{14}C -thymidine for 48 h and then pulse-labelled with ^3H -thymidine for 25 min. In these conditions, long strands of ^{14}C -labelled parental DNA should be hydrogen bound to strands of ^3H -labelled, newly synthesised DNA. This latter should be more heterogeneous in size, because it corresponds to growing strands of all sizes between zero and the size of the replicating unit^{5,6} (about 10^8 daltons). Denaturation is thus denoted in this experiment by the separation of the two profiles, in a situation essentially identical to that obtained in alkaline sucrose gradients⁹.

We conclude from these results that the DNA molecules from African green monkey kidney cells contain linkers that are sensitive to alkali and that they are, on average, located 115×10^6 daltons apart. The nature of these linkers is now being investigated and two main possibilities are

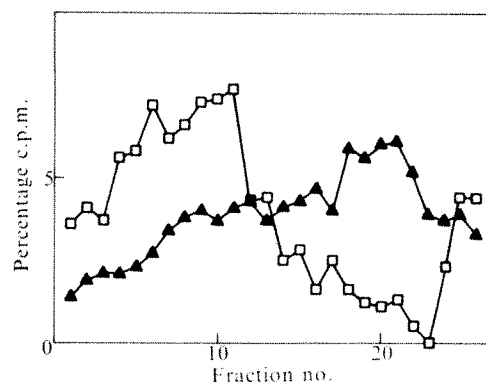


Fig. 2 Strand separation in sucrose-formamide gradients. The cells were incubated in ^{14}C -thymidine ($0.1 \mu\text{Ci ml}^{-1}$) for 48 h. The medium was withdrawn and the cells incubated for 25 min in $50 \mu\text{Ci ml}^{-1}$ ^3H -thymidine. The DNA was extracted, centrifuged, fractionated and counted as described in the legend of Figure 1. \square , Percentage of ^{14}C -DNA, $M_n = 62.8$; \blacktriangle , percentage of ^3H -DNA, $M_n = 20.5$.

being pursued, namely, a ribonucleotide linker and an apurinic and/or apyrimidinic site.

Ribonucleotides have been detected in mature closed-circular mitochondrial DNA¹⁻³. Their presence could be envisaged as due to insertion into DNA during replication by a relatively non-stringent DNA polymerase or that they are remnants of RNA primers which have not been completely excised. Substantial evidence has been presented from several systems that an RNA polymerase product provides the 3'-hydroxy primer required by all known DNA polymerases⁷⁻⁹. Apurinic sites are known to be formed spontaneously in DNA at a rate corresponding to 5×10^{-2} depurinations per 10^8 daltons per h at 37 °C (ref. 10). Table 1 shows that the linkers are already present at a frequency of 1.5 per 10^8 daltons in short pulse-labelled DNA, therefore, apurinic/apyrimidinic sites could only have been introduced catalytically, such as by a base alkylation mechanism.

Several laboratories have reported that treatment of cell lysates with proteases before centrifugation in alkaline sucrose gradients decreased the size of the DNA molecules¹¹⁻¹³. After the treatment, single-strand pieces of 60×10^6 , 45×10^6 and 6×10^6 daltons were observed in CHO (ref. 11), mouse L cells¹² and Ehrlich ascites tumour cells¹³, respectively. The connection between these results and ours is not clear as yet. A preliminary experiment in which NaOH was replaced by proteinase K did not show any reduction in the molecular weight. It is possible that the protease treatment of the cell lysates removed protein from the DNA rendering the linkers accessible to the alkali. In our experiments, these proteins would have been extracted by the phenol-chloroform treatment and so

Table 1 Number of single-strand breaks produced in DNA on treatment with sodium hydroxide

Experiment	M_n untreated	M_n treated	No. of breaks per 10^8 daltons	Treatment
a	71.9	49.7	0.62	0.15 M NaOH, 10 min, 20 °C
b	66.7	42.4	0.86	0.15 M NaOH, 5 min, 20 °C
c	78.3	38.4	1.32	0.15 M NaOH, 5 min, 20 °C
d	68.2	45.6	0.73	0.15 M NaOH, 5 min, 20 °C
e	71.9	45.2	0.82	0.50 M NaOH, 1 h, 37 °C
f	42.7	26.7	1.40	0.15 M NaOH, 10 min, 20 °C
g	42.7	25.3	1.61	0.50 M NaOH, 1 h, 37 °C

a–e, DNA was labelled with radioactive thymidine for periods varying from 8 to 48 h. In f and g DNA was pulse-labelled for 25 min only. Determination of M_n is described in the legend to Fig. 1. Alkali treatment varied as indicated. The number of single-strand breaks was calculated using the expression $(M_n \text{ treated}/M_n \text{ untreated}) - 1$. The values were normalised to 10^8 daltons.

the alkali-sensitive sites would already be accessible to attack.

This work was supported by a grant from project BIOQ/FAPESP. E.F. was supported by a fellowship from FAPESP.

EUSTRATIOS FILIPPIDIS
ROGERIO MENECHINI

Department of Biochemistry,
Institute of Chemistry,
University of São Paulo,
C.P. 20780, São Paulo, Brazil

Received 13 May; accepted 4 August 1977.

- 1 Grossman, L. I., Watson, R. & Vinograd, J. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3339–3443 (1973).
- 2 Miyaki, M., Koide, K. & Ono, T. *Biochem. biophys. Res. Commun.* **50**, 252–258 (1973).
- 3 Wong-Staal, F., Mendelsohn, J. & Goulian, M. *Biochem. biophys. Res. Commun.* **53**, 140–148 (1973).
- 4 Kavenoff, R. & Zimm, B. H. *Chromosoma (Berl.)* **41**, 1–27 (1973).
- 5 Huberman, J. A. & Riggs, A. D. *J. molec. Biol.* **32**, 327–341 (1968).
- 6 Lehmann, A. R. *J. molec. Biol.* **66**, 319–337 (1972).
- 7 Brutlag, D., Schekman, R. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2826–2829 (1971).
- 8 Sugino, A. & Okasaki, R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 88–92 (1973).
- 9 Magnusson, G., Pigiet, V., Winnacker, E. L., Abrams, R. & Reichard, P. *Proc. natn. Acad. Sci. U.S.A.* **70**, 412–415 (1973).
- 10 Lindahl, T. & Nyberg, B. *Biochemistry* **11**, 3610–3618 (1972).
- 11 Hozier, J. C. & Taylor, J. H. *J. molec. Biol.* **93**, 181–201 (1975).
- 12 Fujiwara, Y. *Cancer Res.* **35**, 2780–2789 (1975).
- 13 Hershey, H. V. & Werner, D. *Nature* **262**, 148–150 (1976).
- 14 Meneghini, R. *Biochim. biophys. Acta* **425**, 419–427 (1976).
- 15 Studier, F. W. *J. molec. Biol.* **11**, 373–390 (1965).

Chloramphenicol-sensitive labelling of protein in microsomes of *Neurospora crassa*

SEVERAL peptides are synthesised on mitochondrial chloramphenicol-sensitive ribosomes¹. In the presence of cycloheximide ($\sim 200 \mu\text{g ml}^{-1}$), amino acids are incorporated exclusively into mitochondrial proteins in *Neurospora crassa*² and the flight muscle of *Locusta migratoria*³, leading to the generally accepted idea that mitochondrially synthesised proteins remain in the mitochondria. It is possible, however, that the high concentration of cycloheximide used in those experiments could inhibit any potential export of such proteins to other parts of the cell, particularly since the drug has been shown to affect several vital cellular processes independently of its effect on protein synthesis; for example, it stimulates the catabolism of nucleic acids and decreases intracellular ATP (ref. 4). This may explain some published indications of export of mitochondrially synthesised proteins in experiments in which cycloheximide was not used, such as the continued synthesis of myelin proteolipid in crude mitochondrial fractions of rat brain homogenates treated with RNase⁵; and the cytoplasmic location of the mutation in a line of *Zea mays* responsible for the susceptibility of its root plasma membrane K^+ -stimulated ATPase to the toxin of *Helminthosporium maydis* race T.⁶ We describe here observations on the incorporation of radioactivity into membranes of the post-mitochondrial supernatant of homogenates of *N. crassa* which had been pulsed with radioactive amino acids in the presence of cycloheximide ($25 \mu\text{g ml}^{-1}$) then grown for 2 h in drug- and label-free medium. We have been unable to account for this solely on the basis of mitochondrial fragmentation. Of the membranes present in the postmitochondrial supernatant, we have investigated in detail a microsomal fraction enriched for endoplasmic reticulum, as a first step in determining the nature of this incorporation.

N. crassa 74A was cultured and pulse-chase labelled with amino acids in the presence of selective inhibitors of pro-

tein synthesis. Microsomes and mitochondria were isolated as described in the legend to Fig. 1, and the two fractions characterised by marker enzyme assays. The microsomal fraction was enriched 10-fold over the mitochondria in glucose-6-phosphatase activity measured according to de Duve *et al.*¹⁰, and no haem *a* was detectable in reduced minus oxidised difference spectra¹¹. The assays indicated that the microsomes were enriched in endoplasmic reticulum and virtually devoid of mitochondrial inner membrane.

Four proteins whose synthesis is chloramphenicol-sensitive are normally obtained from pulse-labelled *N. crassa* mitochondria. Their molecular weights are roughly 10,000, 20,000, 30,000 and 40,000. The 10,000 molecular

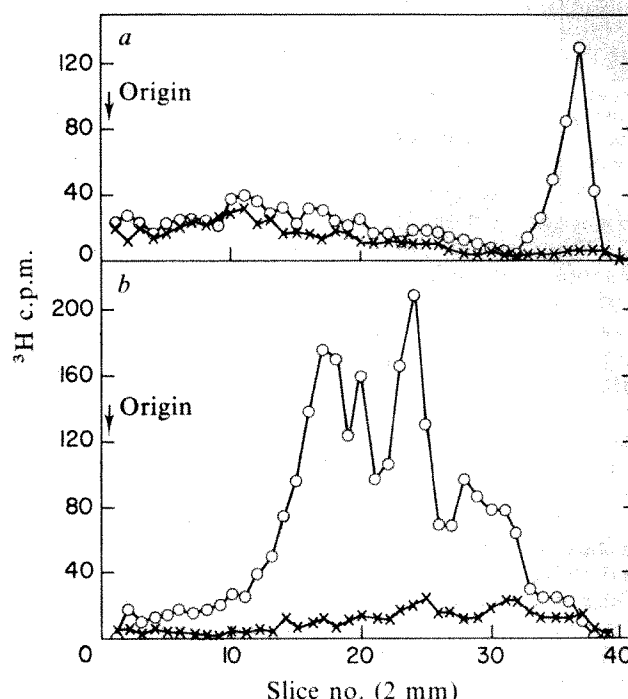


Fig. 1 Distribution of radioactivity in *a*, microsomes and *b*, mitochondria. *N. crassa* was labelled as described previously⁷, except that 200 μCi of $[\text{G-}^3\text{H}]\text{L-phenylalanine}$ (Schwarz-Mann, 10 Ci mmol⁻¹), per 500 ml of medium was routinely used in place of leucine as the labelling agent. In one series of experiments the cultures were labelled with 500 μCi ^3H -amino acid mixture (Amersham/Searle code TRK.440) per 600 ml of medium. The cells were labelled in the presence of cycloheximide ($25 \mu\text{g ml}^{-1}$) or cycloheximide ($25 \mu\text{g ml}^{-1}$) plus chloramphenicol (4 mg ml^{-1}). Mycelial pads were collected by vacuum filtration and disrupted in a mill (B. Braun, Melsungen) with 0.5-mm glass beads in the following buffer: 0.5 M sucrose, 1 mM EDTA, and 50 mM Tris-HCl, pH 7.5. To inhibit protease activity, 0.5 mM phenylmethylsulphonyl fluoride was added to all buffers. Nuclei and the bulk of the cell wall were removed by centrifugation at 2,000g for 10 min, and a heavy mitochondrial fraction was obtained by centrifugation of the supernatant at 8,000g for 15 min. The mitochondria were washed by homogenising in the same buffer, and repeating the above centrifugations. Light mitochondria and lysosomes were removed from the postmitochondrial supernatant by centrifugation at 50,000g for 30 min, and discarded. Microsomes were then prepared by centrifuging the supernatant at 105,000g for 1 h. The pellet was washed by resuspension in the same buffer, followed by centrifugations at 50,000g and 100,000g. All operations were carried out at 0–4 °C. Protein was measured by the method of Lowry *et al.*⁸. Microsomes and mitochondria were assayed by SDS-polyacrylamide gel electrophoresis in sodium dodecyl sulphate, using 11.5% gels as described by Fairbanks *et al.*⁹, as modified by Lansman *et al.*⁷. The radioactivity in 2-mm gel slices was measured by liquid scintillation counting after swelling in 0.4 ml NCS Tissue solubiliser (Amersham/Searle) for 2.5 h at 45 °C followed by addition of toluene plus Omnifluor (Packard). *a*, 100 μg sample of microsomes; *b*, 100- μg sample of mitochondria; \circ , cycloheximide; \times , cycloheximide plus chloramphenicol.

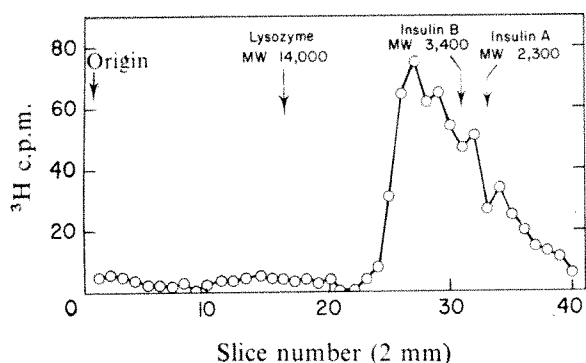


Fig. 2 Determination of the apparent molecular weight of CSMP. Microsomes (650 μ g), labelled in the presence of cycloheximide as described in Fig. 1, were extracted with chloroform-methanol (2:1) centrifuged at 5,000g for 10 min and the supernatant dried under nitrogen. The residue was electrophoresed in SDS-urea polyacrylamide gels according to Swank and Munkres¹². Radioactivity was determined as described in Fig. 1. The position of markers was determined after staining with Coomassie Blue¹².

weight (MW) peak disappears from mitochondria after a 2-h chase period⁷. In experiments reported here, equal quantities of mitochondrial and microsomal protein (100 μ g per gel) from cells pulse-chase labelled in the presence of cycloheximide or cycloheximide plus chloramphenicol were compared by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. The distribution of radioactivity in mitochondria and in microsomes from cells pulse-chase labelled in the presence of cycloheximide alone showed distinct differences (Fig. 1). The mitochondria had little protein of MW about 10,000 and none smaller than this, as expected for *N. crassa* mitochondria labelled in this manner⁷. In contrast, the majority of the radioactivity in the microsomes appeared in a low MW protein which was smaller than the 10,000 MW protein seen in mitochondria after a pulse-label experiment, and is therefore unlikely to be derived from mitochondrial membrane fragments. The synthesis of the microsomal protein was reproducibly sensitive to chloramphenicol. The apparent MW of this chloramphenicol-sensitive microsomal protein (CSMP) as determined by polyacrylamide gel electrophoresis in the presence of SDS-urea was 5,000 to 8,000 (Fig. 2). CSMP was readily extractable from microsomes with 20 volumes of 80% acetone or with chloroform-methanol (2:1), the latter property suggesting it is a proteolipid.

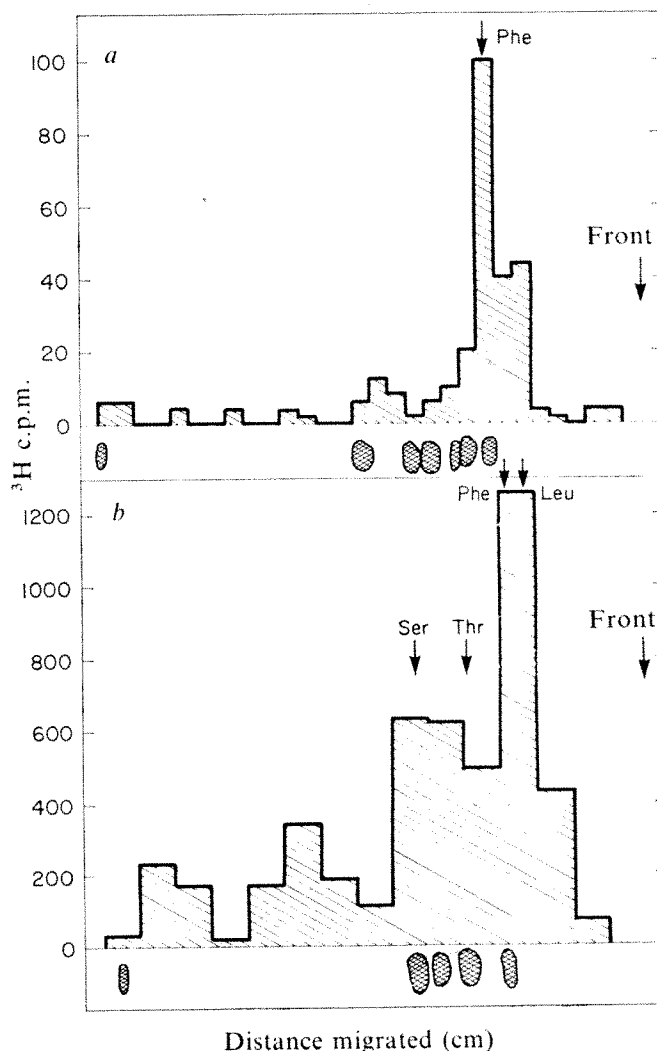
Saponification of chloroform-methanol extracts of microsomes labelled in the presence of cycloheximide, which should release any amino acids present as amino-lipids, revealed negligible amounts of radioactivity recoverable as amino acids, suggesting that the bulk of the radioactivity was incorporated into protein. Only 6% of the radioactivity was recovered in petroleum ether extracts after saponification of ³H-phenylalanine-labelled CSMP, indicating a low level of amino acid conversion to lipid. Total acid hydrolysis of CSMP was obtained only after saponification, however, suggesting that the peptide chain may have been protected to some degree by lipids. Labelled phenylalanine (as assayed by thin-layer chromatography) was recovered from total acid hydrolysates of CSMP isolated from cells pulse-labelled with ³H-phenylalanine (Fig. 3a). Several different amino acids were detected by ninhydrin visualisation and radioactivity in total hydrolysates of CSMP isolated from cells labelled with a mixture of ³H-amino acids (Fig. 3b).

Thin-layer chromatography of chloroform-methanol extracts of microsomes or mitochondria in a system that separates a variety of lipids gave a band of radioactivity whose synthesis was chloramphenicol sensitive. This band

migrated with an R_f similar to that reported for a low molecular weight proteolipid synthesised by isolated rat liver mitochondria^{15,16}, and which is similar to the R_f of phosphatidyl choline (Fig. 4a and b). The acid hydrolysis data described above, however, indicated that the radioactivity was recovered in amino acids, not in phosphatidyl choline. Phosphatidyl serine, the most common amino-lipid, migrates with an R_f close to 0.4 in the first developing system. Thus, the radioactivity in CSMP could not be due to label converted to phosphatidyl serine.

All the above data suggest very strongly that CSMP is indeed a protein, albeit with somewhat unusual properties.

Fig. 3 Analysis of total hydrolysates of chloramphenicol-sensitive cycloheximide-insensitive material by thin-layer chromatography. *N. crassa* microsomes labelled in the presence of cycloheximide with: a, [³H] phenylalanine (see Fig. 2) or b, ³H-amino acid mixture (as described in the text) were extracted in chloroform-methanol (2:1) and dried under nitrogen. The dried residues were saponified in 95% methanol, 0.5 M NaOH at 30 °C for 15 h, acidified to pH 3 with 6 M HCl and extracted three times with 2 vol. petroleum ether which removed non-saponifiable lipids. The methanolic layer was neutralised, dried under N₂ and hydrolysed in 6 M HCl at 110 °C for 72 h. HCl was removed in a vacuum dessicator over NaOH and concentrated H₂SO₄. The residues were dissolved in water and desalted, using a Dowex-2 (AG-2 X-10, BioRad) anion exchange resin (which also removed ethanolamine and choline)¹³ followed by removal of HCl and acetic acid by vacuum desiccation as above. Amino acids were separated on silica gel plates (EM Laboratories), with ethanol-water (7:3), and visualised with ninhydrin (shown as cross-hatched spots below the graphs). The radioactivity was determined by liquid scintillation counting of 0.5-cm bands in Bray's solution¹⁴.



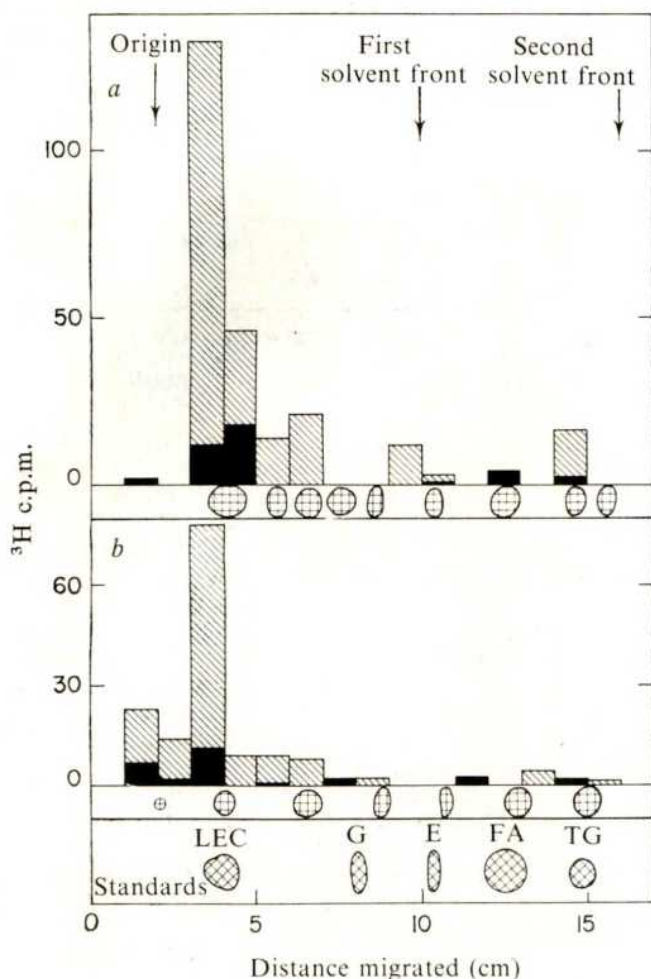


Fig. 4 Thin-layer chromatography of chloroform-methanol extracts of subcellular fractions. *a*, Microsomes and *b*, mitochondria were isolated from *N. crassa* pulse-chase labelled in the presence of cycloheximide or of cycloheximide plus chloramphenicol. Membrane suspensions (146 μ g protein) were extracted with 1 ml of chloroform-methanol (2:1) at room temperature for 10 min followed by centrifugation at 5,000g for 10 min. The supernatants were dried under nitrogen, dissolved in a small volume of the same solvent, and separated by thin-layer chromatography on silica gel HR (Merck). Phospholipids and neutral lipids were separated by successive developments in chloroform-methanol-glacial acetic acid-water (65:25:8:4) (ref. 17) and then petroleum ether-diethyl ether-glacial acetic acid (75:25:1) (ref. 18). Lipids were visualised with iodine vapour (shown as cross-hatched spots below the graphs). Bands (0.5 cm) were scraped from the plate into counting vials, and radioactivity was determined by counting in toluene-liquifluor (New England Nuclear) using a Packard Tri-Carb liquid scintillation counter. Standards were: LEC, dipalmitoyl lecithin; G, gramicidin D; E, ergosterol; FA, oleic acid; TG, triolein. Cycloheximide, hatched; cycloheximide plus chloramphenicol, solid.

Purification of this protein is in progress. After gel filtration of the chloroform-methanol soluble fraction of *N. crassa* microsomes on LH-20 columns, a single radioactive ninhydrin-positive spot is observed on paper chromatography.

The appearance of the protein CSMP in the microsomal fraction could be explained in several ways. As shown above, it is unlikely to be due to simple cross-contamination from mitochondrial inner membranes. The results could be accounted for by CSMP escaping from the mitochondria during the isolation procedure, but a very hydrophobic protein such as this is unlikely to be removed from mitochondrial membranes in the isolation conditions used. It is unlikely that CSMP is the result of labelling of microsomal protein(s) by a non-ribosomal-dependent enzyme

system insensitive to cycloheximide but sensitive to chloramphenicol as has been reported for *in vitro* preparations of myelin¹⁹. In the conditions used for *in vitro* incorporation of radiolabelled amino acids into a myelin fraction¹⁹, no chloramphenicol-sensitive incorporation of ³H-phenylalanine by a 20,000g supernatant of an *N. crassa* homogenate was found. The only labelling observed was sensitive to cycloheximide and insensitive to chloramphenicol, suggesting normal *in vitro* cytoplasmic ribosomal-dependent incorporation of amino acids. These observations are in direct contrast to the *in vivo* labelling pattern of CSMP, supporting our contention that CSMP is synthesised on chloramphenicol-sensitive ribosomes and not by an unusual enzymatic reaction sensitive to chloramphenicol. It is possible that there are hitherto undescribed cycloheximide-insensitive, chloramphenicol-sensitive ribosomes outside the mitochondria. It is also possible that CSMP is a product of mitochondrial protein synthesis which has been processed during or after export. In summary, the evidence is most consistent with CSMP being synthesised on mitochondrial ribosomes and exported from the mitochondria.

Preliminary experiments with isolated rat hepatocytes have indicated that export of low molecular weight proteolipids from mitochondria to the endoplasmic reticulum and also to the plasma membrane occurred.

The role or roles of CSMP in the cell are unknown. Several investigators have concluded mitochondrial export to the cytoplasm must exist. Some of these reports suggest a repressor function for this protein. Barath and Kuntzel²⁰ observed that in the presence of chloramphenicol or ethidium bromide, an increased biosynthesis of certain nuclear gene products associated with the *Neurospora* mitochondrial transcriptional and translational apparatus occurred. A model was proposed for a mitochondrial repressor which regulates the synthesis of these proteins. Such a repressor has also been proposed by Edwards *et al.*²¹ to explain a similar chloramphenicol effect on the induction of the cyanide-insensitive alternate oxidase of *Neurospora*, an enzyme that is synthesised in the cytoplasm. This repressor model could also explain the induction of the alternate oxidase in the mitochondrial mutants *mi-1* and *mi-3* of *Neurospora*²².

Export of mitochondrial protein could be the functional basis for a mitochondrial linkage to cellular transformation. This is suggested by the protection of BALB 3T3 cells from viral transformation by pretreatment with ethidium bromide²³, by the close correlation of the severity of granulocytic leukaemia with the frequency of circular dimers of mitochondrial DNA in leukocytes²⁴ and by the distinct and specific changes in the synthesis of mitochondrial DNA in chick embryo fibroblasts transformed by Rous sarcoma virus²⁵.

This work was supported by grants GM 10067 and 5 TO1 GM 00158 from the US NIH. We thank W. Dorsey Stuart for helpful discussions.

W. B. MACKLIN
D. J. MEYER
D. O. WOODWARD

Department of Biological Sciences,
Stanford University,

S. K. ERICKSON

Department of Medicine,
Stanford University School of Medicine,
Stanford, California 94305

Received 13 May; accepted 18 July 1977.

- ¹ Schatz, G. & Mason, T. L. *A. Rev. Biochem.* **43**, 51-87 (1974).
- ² Sebald, W., Schwab, A. J. & Bucher, T. H. *FEBS Lett.* **4**, 243-246 (1969).
- ³ Sebald, W., Hofstötter, T. H. & Hacker, D. *FEBS Lett.* **2**, 177-180 (1968).
- ⁴ McMahon, D. *Pl. Physiol.* **55**, 815-821 (1975).
- ⁵ Klee, C. B. & Solokoff, L. *Proc. natn. Acad. Sci. U.S.A.* **53**, 1014-1021 (1965).
- ⁶ Tipton, C. L., Mondal, M. H. & Uhlig, J. *Biochem. biophys. Res. Commun.* **51**, 725-728, (1973).
- ⁷ Lansman, R. A., Rowe, M. J. & Woodward, D. O. *Eur. J. Biochem.* **41**, 15-23 (1972).

- ⁸ Lowry, E. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. Biol. Chem.* **193**, 265–275 (1951).
⁹ Fairbanks, G. T., Steck, L. & Wallach, D. F. H. *Biochemistry* **10**, 2606–2617 (1971).
¹⁰ de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. *Biochem. J.* **60**, 604–617 (1955).
¹¹ van Gelder, B. F. *Biochim. biophys. Acta* **118**, 36–46 (1966).
¹² Swank, R. T. & Munkres, K. D. *Analyt. Biochem.* **39**, 462–477 (1971).
¹³ Neuner, M., Niederwieser, A. & Pataki, G. in *Thin Layer Chromatography* (ed. Egon Stahl) 737 (Springer, New York, 1969).
¹⁴ Bray, G. A. *Analyt. Biochem.* **1**, 279–285 (1960).
¹⁵ Kadenbach, B. *Biochem. biophys. Res. Commun.* **44**, 724–730 (1971).
¹⁶ Dianoux, A.-C., Bof, M., Cesarini, R., Reboul, A. & Vignais, P. V. *Eur. J. Biochem.* **67**, 61–66 (1976).
¹⁷ Skipski, V. P., Peterson, R. F. & Barclay, M. J. *Lipid Res.* **3**, 467–475 (1962).
¹⁸ Bragden, J. H. in *Lipids and the Steroid Hormones in Clinical Medicine* (eds Funderman, F. W. & Funderman, F. W., Jr) (Lippincott, Philadelphia, 1960).
¹⁹ Johnson, J. D., King, N. L. & Benkley, F. J. *Neurochem.* **26**, 361–367 (1976).
²⁰ Barath, Z. & Kuntzel, H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1371–1374 (1972).
²¹ Edwards, D. L., Rosenberg, E. & Maroney, P. A. *J. biol. Chem.* **249**, 3551–3556 (1974).
²² Jagow, G., Weiss, H. & Klingenberg, M. *Eur. J. Biochem.* **33**, 140–157 (1973).
²³ Roa, R. C. & Bose, S. K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4337–4340 (1975).
²⁴ Clayton, D. A. & Vinograd, J. *Nature* **216**, 652–657 (1967).
²⁵ D'Agostino, M. A. & Nass, M. M. K. *J. Cell Biol.* **71**, 781–794 (1976).

A cholesterol-isopropanol gel

IN the course of measuring solubilities of cholesterol in pure and mixed solvents we have observed the formation of a transparent gel in the cholesterol+isopropanol system. Because of the historical importance of liquid crystals of cholesteryl esters¹ and the biological importance of cholesterol, we are reporting here some observations on the properties of this gel. Liquid crystals of cholesterol with fatty alkanols (C₁₂–C₁₈) are known^{2,3}, but have not been reported for smaller alcohols. The transparent gel of cholesterol and isopropanol seems to be stable at 4 °C for concentrations of 4.6–5.3% (w/w) cholesterol. At higher concentrations and temperatures (up to 10.2% at 22 °C), the transparent gel can be observed for a day or more, then white 'snowflakes' appear throughout the gel and grow slowly into a translucent or opaque mass. On warming, the transparent gels liquify, and the translucent gels seem to go directly to the liquid state. The transparent gel is unstable to vigorous shaking; samples with less than 5.5% cholesterol liquify then return to the transparent gel state on standing at 4 °C. Samples with larger concentrations form the translucent or opaque state on shaking and do not return to the transparent state over a period of several weeks. Observations of the transparent gel under a polarising microscope at room temperature showed the sample to be mostly isotropic with some small points of optical activity.

Cholesterol (Fisher Certified Reagent Grade) originally with a melting point of 147–148 °C. After several recrystallisations from ethanol the melting point was 148–149 °C. Isopropanol (Matheson, Coleman and Bell Spectroquality) was stored over molecular sieves and twice distilled, retaining the middle one-third each time. In all of the measurements reported here, these purified materials were used, but very similar results were obtained with untreated materials. Samples were prepared by weight, sealed in glass ampoules under reduced pressure, warmed to dissolve the solid, and then allowed to stand without shaking in four different environments ranging from –10–22 °C, all with observed temperature fluctuations of about 1 °C. Four physical states were observed: liquid, transparent crystalline plates, transparent gel, and the white material which seems to be an amorphous solid dispersed throughout the gel. Our observations are shown in Table 1.

After initial preparation, the gel took 2–3 d to form. If the gel was broken by shaking or slight warming, it reformed in a matter of minutes to hours, but if warmed excessively gel formation required several days. The upper surface of the gel was always coated with a small amount of liquid, presumably due to temperature fluctuations and handling. In one case, however, (4.62% cholesterol at 4 °C) substantial amounts of the liquid and gel phases can coexist.

Table 1 Coexisting states in the cholesterol-isopropanol system

% Cholesterol (w/w)	–10 °C	4 °C	18 °C	22 °C
4.03	L+P	L	L	L
4.16	L+P	L	L	L
4.62	L+P	L+P+G	L	L
5.29	L+P	P+G	L	L
5.49	L+P	P+G+W*	L	L
6.69	W	W	W	L
7.61	W	W	W	W
11.66	W	W	W	W†

L, Liquid; P, crystalline plates; G, transparent gel; W, white mass.

*Small flakes of white appeared in the transparent gel after approximately one week, and seemed to be slowly increasing in size.

†This sample liquified at 43 °C.

Samples with 6.69% or more cholesterol form the white mass at –10 °C, but it is unlikely that this is an equilibrium state. There seems to be kinetic competition between formation of the white material and formation of the crystalline plates. Samples of 5.49% and 6.69% cholesterol were allowed to gel at 4 °C, both with a small amount of white 'snowflakes'; the 5.49% sample also contained some transparent plates. When these samples were moved to the –10 °C environment, the less concentrated sample formed plates with liquid, and the more concentrated sample formed the white mass, possibly containing some crystallites.

To find out more about the white materials we have observed, we have begun measurements of the weight gain of cholesterol on exposure to isopropanol vapours. We have observed a gain corresponding to 1.9 molecules of isopropanol per molecule of cholesterol above pure liquid isopropanol, and some of our measurements indicate that the ratio of 0.5 isopropanol molecules per cholesterol molecule may be a stable configuration.

WILLIAM E. ACREE, JR

GARY L. BERTRAND

Department of Chemistry,
University of Missouri-Rolla,
Rolla, Missouri 65401

Received 25 May; accepted 3 August 1977.

¹ Reinitzer, F. *Monatsch. Chem.* **9**, 421 (1888).

² Młodziejowski, A. Z. *Phys. Chem. (Leipzig)* **135**, 129–146 (1928).

³ Lawrence, A. C. S. *Liquid Crystals and Ordered Fluids* (ed. Johnson, J. F. & Porter, R. S.) 289–291 (Plenum, New York, 1970).

Errata

In the letter 'Upper limits for the radio pulse emission from exploding black holes' by W. P. S. Meikle, *Nature* **269**, p. 41, in paragraph 3, line 15, for t_p read t_0 ; in paragraph 3, line 17, for t , read t_p ; in paragraph 4, line 10, for $\nu \approx 200$ MHz, read $\nu \lesssim 200$ MHz; in paragraph 4, equation (II) for $R_m \approx 5 \times 510^{21}$, read $R_m \approx 5 \times 10^{21}$; references 6 and 7 should read ⁶ Colgate, S. A. & Noerdlinger, P. D. *Astrophys. J.* **165**, 509–521 (1971) and ⁷ Colgate, S. A. *Astrophys. J.* **198**, 439–445 (1975).

In the letter 'Electrical stimulation of denervated muscle reduces incorporation of methionine into the ACh receptor' by C. G. Reiness & Z. W. Hall, *Nature* **276**, p. 655, the correct order of authors' name was transposed.

Corrigenda

In the letter 'Aflatoxin B-oxide generated by chemical or enzymic oxidation of aflatoxin B₁ causes guanine substitution in nucleic acids' by C. N. Martin & R. C. Garner, *Nature* **267**, p. 865, the legend to Fig. 1, line 14 should read . . . To this two-phase system was added 1.25 mg 3-chloro-perbenzoic acid (4 molar excess) and the whole stirred for 25 h at room temperature in the dark in a stoppered flask.

In the letter 'On the optical identifications of five X-ray sources' by H. V. Bradt *et al.*, *Nature*, **269**, p. 21, the last line of the abstract should read . . . suggested class of Be-star X-ray emitters.

reviews

Reproductive physiology in the male

B. P. Setchell

The Process of Spermatogenesis in Animals. By Edward C. Roosen-Runge. Pp. viii+214. (Cambridge University: Cambridge, London and New York, 1977.) £15.50.

THIS book is an extremely welcome addition to the literature on reproduction in the male. It brings together an absolute wealth of information on the formation of spermatozoa in lower animals and then relates this to the much greater body of knowledge about testis function in mammals. The book begins with a historical introduction, important in a subject which has developed explosively in the last ten years. The author then works systematically through the Animal Kingdom, collating and tabulating the widely scattered information, and more importantly, discussing its relevance to the situation in other classes of animals. As a scientist working on the physiology and biochemistry of the testis in mammals, I found the book extremely interesting and stimulating.

The invertebrate species, in particular, provide so many extraordinary variants on the basic theme that I will look with new eyes at my own data to see whether things in mammals are as simple as they have seemed till now. I found the description of the 'carrier' spermatozoa in the marine snail *Janthina* particularly intriguing, as was Professor Roosen-Runge's own work on the marine hydrazoon *Phialidium*. This animal releases spermatozoa twice daily at sunrise and sundown for 3-4 months and its testes continue to produce and release spermatozoa *in vitro* although in these conditions spermatogonia are not renewed. This animal should obviously be studied in greater detail in view of the failure so far to maintain spermatogenesis, particularly the meiotic divisions, in cultures of fragments of mammalian testes.

I am not quite so sure whether a zoologist, who wanted to obtain a general picture of reproduction in male mammals to relate to the animals he was studying, would find all the information he needed. Professor Roosen-Runge is one of the most imaginative of the still-active older scientists in this field and he has worked for many years on spermatogenesis in mammals, although his contribution is often underrated. It is therefore a pity that

he feels that this field "has been reviewed almost to excess" and does not attempt a detailed exposition of his view of the present state of knowledge of mammalian spermatogenesis.

The book is very well produced, although I do find it irritating that the plates are grouped together rather than spaced through the text, but I suppose that this is a consequence of rising costs. There seem to be very few spelling mistakes (even fewer if the 'bettle' on p70 is not meant to be a 'beetle'). Some of the line drawings could be clearer and several are inadequately labelled, for example Fig. 3

(p7), in which the numbers are undefined and cannot be reconciled easily with the text.

In summary, this is a book which every library interested in the physiology of reproduction should have; and almost all workers in this field would learn something new by reading it. Professor Roosen-Runge is to be congratulated. I do not know of anyone else who could have done it as well. □

B. P. Setchell is a Senior Principal Scientific Officer at the ARC Institute of Animal Physiology, Babraham, Cambridge, UK.

Human variation and adaptation

Population Structure and Human Variation. International Biological Programme. Vol. 11. Edited by G. A. Harrison. Pp. 342 (Cambridge University: New York, London and Cambridge, 1977.) £17.50.

THIS monograph consists of a selection of studies of human populations carried out during the International Biological Programme. Each of the 12 chapters attempts a broad review of investigations concerned with population genetics, demography and ecology as they relate to human variation and adaptation.

In the past, physical traits (such as head shape and skin colour) have been largely used for defining population groups, but the value of such traits is somewhat limited because they are polygenically determined, and at least some are significantly affected by the environment. For these reasons anthropologists and population geneticists have increasingly turned their attention in recent years to monogenically determined biochemical markers (blood groups, haemoglobins, serum proteins and enzymes) and it is with the distribution of these markers, as well as disease incidences and various physiological parameters, that this book is also concerned.

The world distribution of genetic markers is reviewed and also the findings in various "isolates" in North Asia, the Yanomama Indians of Brazil and the Solomon Islanders. The nature of specific factors which may influence

human variation and adaptation are also approached through the study of migration of genetically similar groups into different environments (Tokelau Islanders of the Pacific into New Zealand, the African savanna dwellers into the equatorial rain forests) the study of genetically different groups living in similar environments (various Jewish communities in Israel) and physiological adaptations to extreme environments (the semi-arid regions of Central Africa and the tropical forests of Papua New Guinea).

There are those who might question the merit of studying the frequencies of various traits in different populations which, at their lowest level, Mall the anatomist might somewhat irreverently have referred to as 'brick-counting-research'. But in defence of such studies it can be asked how else can human evolution and adaptation be investigated? If there is a defect perhaps it lies not so much in the collection and analysis of such data but rather in their interpretation.

This is essentially a postgraduate text, and in any event is far too expensive for possible recommendation to undergraduates. It is well edited and presented, and should prove particularly valuable for research workers. It is also an excellent sourcebook of relevant data for teachers of human biology, population genetics and anthropology.

Alan E. H. Emery

Alan E. H. Emery is Professor of Human Genetics at the Medical School, University of Edinburgh, Scotland, UK.

Crystal structure compendium

Three-Dimensional Nets and Polyhedra.

By A. F. Wells. Pp. 268. (Wiley Interscience: New York and London, 1977.) £22; \$36.

WE are much indebted to A. F. Wells for his massive volumes of *Structural Inorganic Chemistry* which systematise the spatial arrangements of atoms in crystalline inorganic solids. Professor Wells has now taken the topological material involved in these structures and examined it in greater detail.

He considers primarily nets which are periodic in three dimensions. These are characterised topologically with symbols derived from those of Schläfli: the indices (p, q) meaning that q links meet at each point and that the shortest circuits including any pair of links from a point have p edges. This symbol he finds to be inadequate for complete description, and further numbers are added, although it is not perhaps easy enough for the reader to grasp the significance of the sub- and superscripts, especially when dipping into the book. Perhaps about a hundred different nets are considered so that the subject is a considerable test of the spatial imagination. As Wells admits, the various structures which he describes are difficult to apprehend and the building of models is recommended to supplement the stereo photographs provided. The latter are not very successful because the screen pattern becomes intrusive when they are magnified by a stereo-viewer.

The most interesting section is on infinite polyhedra, the simplest of

which were discovered by Petrie and Coxeter in 1926. These can be obtained by inflating the links in a network until they become tubes. The resulting surface is then tiled with polygons. Such polyhedra divide space into two regions. Some molecular sieve crystals are of this type. It is clear that these figures are just the first of a hierarchy, and they begin to hint at the development of 'biogeometry', which may describe structures such as membranes, micelles, butter, the frameworks observed by electron microscopy in wood, moving up to cell organelles, and so on.

There is scope for the development of the notation and presentation of data on these networks and there are further topics, especially that of random networks, which we would like to hear about, but which the author has consciously eschewed.

The constraints which sheer topology imposes on spatial structure are remarkably strong, both in detail for atomic networks and statistically, as in the phase rule of physical chemistry. Wells has made a start on the systematisation of important structural invariants and it is to be hoped that this will eventually lead to their more complete characterisation, such as has been done for the symmetry groups by the International Tables for X-ray Crystallography. Until such time, Wells' compendium will be of value to those dealing with crystal structures and suggestive in many other areas concerned with three-dimensional structure.

A. L. Mackay

A. L. Mackay is Reader in the Department of Crystallography at Birkbeck College, University of London, UK.

Computational fluid dynamics

Numerical Methods in Fluid Dynamics. By Maurice Holt. Pp 253. (Springer: Berlin and New York, 1977.)

THIS monograph is based on lectures from a course for graduate engineers. The emphasis is on methods developed in the USSR. For problems governed by hyperbolic partial differential equations, methods attributable to Godunov, the BVLR method, and methods using the theory of characteristics are described. The number of application studies is noticeably smaller in chapters dealing with hyperbolic problems than is the case for the later chapters dealing with the method of integral relations, Telenin's method, and the method of lines.

More than half of the book is devoted to applications of the method of integral relations, Telenin's method, and the method of lines to flows described by elliptic, parabolic, or mixed elliptic/hyperbolic partial differential equations. Two-dimensional incompressible flow problems, problems associated with blunt bodies at incidence, three-dimensional cone-field problems, and viscous laminar boundary layer problems are amongst those considered. The detailed explanations of the implementation of the methods and the many specimen results, should make it relatively easy for the inexperienced to use the methods described.

The careful presentation of variations on methods for the successful solving of particular problems, and the many references ensure that the monograph will be a welcome addition to the computational fluid dynamics literature. The text can readily be understood by anyone having a basic knowledge of the equations governing fluid flow. It will be useful both to graduate students in engineering or applied mathematics and to research workers.

Professor Holt's monograph is, however, not a model work. More authoritative criticisms of methods, together with results demonstrating the breakdown of methods, would have been desirable to avoid giving the reader the unfortunate impression that he has been provided with infallible recipes which can be used to solve any given problem.

F. Walkden

F. Walkden is Director of the Fluid Mechanics Computation Centre at the University of Salford, where he is Professor of Applied Mathematics.

Arab survey

Scientific Results of the Oman Flora and Fauna Survey 1975. A Journal of Oman Studies Special Report. Introduced by David L. Harrison. Pp. 267. (Michael Rice: London; Ministry of Information and Culture: PO Box 600, Muscat, Sultanate of Oman, 1977.) \$10; Rials Omani 5.

THE change in fortune of the peoples of Arabia are the talk of the world today: less well known are the peninsula's untrampled desert plains and mountains which are among the few wilderness areas left on earth for scientists to explore. For example the Sultanate of Oman in south-eastern Arabia, with its backbone of mountains up to 3,000 m high, offers unique oppor-

tunities for naturalists to record plant and animal life in habitats which until a few years ago were largely inaccessible.

Oman is in a critical zoogeographical position between Eurasia and Africa and also offers in the cooler highlands of its northern parts an environment in which relict forms have survived from more temperate conditions. This useful survey, the first of its kind to be carried out in the country, has provided many species and forms new to science and has laid the foundations for conservation programmes in the Jabal Akhdar and Jabal Aswad ranges where the rare Arabian tahr (*Hemitragus jayakari*) still survives. **Sarah Bunney**

Sarah Bunney, a zoologist, recently returned from Oman, where she spent two years working as librarian for the Omani Ministry of Health.

Control of eye movements

The Theory of Binocular Vision. By E. Hering. Edited by B. Bridgemann and Lawrence Stark. Pp. 218. (Plenum: London and New York, 1977.) \$29.40.

THIS admirable translation of Hering's monograph on the control of eye movements will be of unfaded value to present-day workers in the field; but it will also interest historians of science, for Hering prepared his monograph largely in response to Helmholtz's *Physiological Optics*, and it illustrates the nature of the systematic controversy between the two physiologists. The translation will help to dispel the two textbook myths, that Hering eschewed detailed experiments and that he adopted a rigidly nativist position; and it serves to emphasise how much Helmholtz and Hering shared—common problems, common assumptions, common experimental techniques and common criteria for the acceptability of evidence.

Central to the monograph are the

idea that the two eyes should be treated as a single organ (the *Doppel-auge*) and the principle that corresponding muscles are always equally innervated, whatever the initial position of the eyes before a movement (Hering's Law). In opposing the Helmholtzian view that the two eyes come to be used together only by habit, Hering is led to discuss the movements of occluded, suppressed and blinded eyes; asymmetrical vergence movements; cases of unilateral paresis of eye muscles; and the coordination of the eyes in infants.

B. Bridgemann's translation is both careful and readable (although some obscurities survive in chapter 17) and he has added a helpful Introduction. L. Stark has independently provided summaries and commentary interleaved in the main text. Internal evidence suggests a curiously strained relationship between the two editors and this is to be regretted, for the task was a very worthwhile one. **J. D. Mollon**

J. D. Mollon is Lecturer in Experimental Psychology at the University of Cambridge, UK.

Inhomogeneous optical waveguides

Inhomogeneous Optical Waveguides. By M. S. Sodha and A. K. Ghatak. Pp. x+269. (Plenum: New York and London, 1977.) \$35.40.

ELECTROMAGNETIC THEORY as formulated by Maxwell has been with us for a good hundred years but the interest in it has shown no signs of abating. New potential applications (in this case the promise of optical communications) have brought the need for new analyses which after a period of digestion could be profitably presented in book form. The authors of this book are particularly suited for the task of writing on inhomogeneous optical waveguides since they have themselves written no less than twenty papers on the subject. The product is a book which anyone interested in the mathematical approaches is well advised to study. Apart from a few omissions (for example, the complex ray method) the authors provide a detailed and interesting review.

The strength of the book lies in giving copious answers to the question 'how to solve it?', its weakness is that the equally important question of 'what is it good for?' is sadly neglected. After all, the sole justification for the existence of these waveguides

is that they can perform some useful function. An engineer would like to know why certain structures have been chosen, what are their comparative advantages and disadvantages, and would like to see the conclusions presented in simple form. Unfortunately, this kind of discussion is entirely missing and 25-page appendix on manufacturing techniques is hardly a compensation for it. The descriptions are so sketchy and the links with the calculations so tenuous that they could have been replaced by a list of references.

There are also some minor inconsistencies in the presentation. For example, under the heading of "General Theoretical Considerations" in section 2.2, the particle current is taken as zero and the solution in section 2.3 is obtained on that assumption. In section 2.4, however, complex dielectric constants suddenly appear, and the reader is told without any explanation that the real part of the dielectric constant of a metal is negative. Such minor flaws are likely to confuse undergraduates and less well equipped graduate students. On the whole, however, the book may be recommended to those already familiar with a wide range of electromagnetic problems and wishing to move into this new field.

L. Solymar

L. Solymar is Lecturer in Engineering Science at the University of Oxford, UK.

the
tins
opener
is on
page
ix

Circle No. 18 on Reader Enquiry Card.

BOOKS

ON PURE
AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £7.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Circle No. 19 on Reader Enquiry Card.

Cell and tissue proteinases

Proteinases in Mammalian Cells and Tissues. Edited by A. J. Barrett. Pp. xx + 735. (North-Holland: New York, Oxford and Amsterdam, 1977.) Dfl. 196; \$79.95.

THE scope of this long and expensive book is somewhat wider than is indicated by its title because two of its sixteen chapters deal with proteinase inhibitors and another is devoted to synthetic substrates and inhibitors. In addition, the properties of the plasminogen activators of plasma, tissue and urine are described in an interesting chapter by Christman, Silverstein and Acs, who emphasise that neither the source of the plasminogen activators of the plasma, nor the identity of these enzymes with their tissue counterparts, can be stated with certainty.

Evidently, it is difficult to decide on all the enzymes which should be considered to be either cell or tissue proteinases. Undoubtedly, however, the decision to include consideration of certain plasma constituents has proved fruitful because, without data of this kind, discussion of the physiological roles of the cell and tissue proteinases would be difficult. The discussion of some aspects of how the tissue proteinases may be expected to function *in vivo*, such as whether plasminogen activators are important in tumorigenesis and wound healing, is speculative, but the inclusion of these concepts adds greatly to the interest of the book.

The rapidity at which information on tissue proteinases has accumulated since 1975 is mentioned in the preface. It is indeed remarkable that, whereas only six enzymes were dealt with in a similar book published in that year, details of seventy-one are to be found in the present volume. The introductory chapter by Barrett on the history and classification of the tissue proteinases helps to clarify the subject. By way of an extra, it is interesting to learn that Darwin in 1875 was the first to pose the question of the evolutionary relationship of the proteinases of plants and animals.

Perhaps because of the importance of elastase and Cathepsin G in inflammation the first chapter by Starkey is devoted to the methods of isolation and properties of these two enzymes which together have been shown to be capable of hydrolysing all the major tissue proteins. Particularly when extracellular (as, for instance, at the start of inflammation and thus not subject to the same degree of inhibition by α_2

macroglobulin or α_1 antitrypsin), these enzymes have been shown to be capable of digesting immune complexes and probably of playing a part in combating bacterial infection. These findings are mentioned mainly as an example of the mass of detailed information which has been brought together in this book. Undoubtedly the efforts of the many contributors have resulted in a volume which is both stimulating and will remain as a useful source book.

It is a pleasure to be able to report that there are few errors: in fact only one of any importance has been detected by the reviewer. This concerns the α_1 and α_2 macroglobulins of rabbit and rat. Unfortunately on p670, lines 7 and 8, the subscripts have been transposed. This is of some importance because as printed the impression is

given that α_2 macroglobulin of rabbit and α_1 macroglobulin of rat increase in concentration during inflammation. In fact, it is the other way round.

It will be an added recommendation to potential readers that more than half of the chapters have been written either by the editor himself or by other members of the staff of the Strangeways Laboratory. In an important sense, the appearance of this volume will make more easily available much of the research on tissue proteinases for which the Strangeways has become an acknowledged centre.

A. H. Gordon

A. H. Gordon is a member of the scientific staff at the National Institute for Medical Research, Mill Hill, London, UK.

Asymmetric reactions

Stereo-Differentiating Reactions: The Nature of Asymmetric Reaction. By Yoshiharu Izumi and Akira Tai. Pp. ix + 334. (Kodansha/Academic: Tokyo, New York and London, 1977.) \$29.50; £20.95.

FROM the introduction, it would seem that this book has two main aims: (1) to provide an introductory text with the basic physics and mathematics appropriate to asymmetric reactions; and (2) to present a new system of classification of asymmetric reactions developed by the authors and previously published in Japanese. One wonders what kind of reader the authors had in mind, since they have written a book which anyone unfamiliar with the field would have considerable difficulty in following and and which therefore cannot be regarded as an introductory text. A research worker interested in the mechanism of asymmetric reactions, on the other hand, will hardly want a research monograph to contain a chapter "Basic Principle of Optical Activity" or even one on experimental methods.

The key chapters are those in which the authors give their new nomenclature and classification. The authors' exposition is sometimes difficult to follow. They write: "A stereoselective reaction is defined as one in which one specific stereoisomer is produced to a greater extent than the other", but give as an example, the conversion of maleic and fumaric acids to maleic anhydride! They then go on to introduce terms like enantiozeroplane, enantiotopos, enantioface, or diastereotopos-differentiating reaction, and the

reader is bound to wonder if these terms are necessary. Are they really useful? Does their use clarify problems in reaction mechanisms?

The new classification with six reaction types could have some advantages when considering mechanistic problems. The chapters which discuss these in detail, and which form the bulk of the book are, however, disappointing. They contain a mass of information about organic reactions and about mechanisms proposed in the literature. The extensive discussion of the authors' own work on modified Raney nickel catalysts provides an interesting test case, and it must be said that nothing has been written which could not have been written as clearly or indeed more clearly without recourse to their terms.

One is left with the feeling that the authors have made a brave effort to think about problems which are conceptually notoriously difficult, and that their ideas are not without value. Their own exposition, however, is unconvincing and it would have been far better to have written a much shorter book with fewer examples to try and make their concepts clear.

The occasional absence of the definite article indicates that the translator was Japanese; on the whole, the translation is good, but there are quite a lot of misprints. The index is inadequate; for example, one could not discover from it that Grignard reactions are twice discussed, the only entry being a subheading under "enantioface-differentiating reaction".

T. L. V. Ulbricht

T. L. V. Ulbricht is Scientific Adviser (Special Duties) at the Agricultural Research Council, London, UK.

announcements

On the Move

T. J. Martin, Professor of Chemical Pathology, University of Sheffield, to Professor of Medicine, University of Melbourne, Repatriation General Hospital, Australia, from 1 October 1977.

N. H. Hunt, Department of Chemical Pathology, University of Sheffield, to Department of Experimental Pathology, John Curtin School of Medical Research, A.N.U., Australia.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Appointments

Dr D. F. Hornig, President-emeritus of Brown University to Professor of Chemistry in Harvard Faculty of Public Health to direct development of a proposed University-wide undertaking, Interdisciplinary Programs in Health.

Mr L. R. Hanton, University of Otago, New Zealand, to a Rutherford Scholarship for three years from 1 October 1977 to work in the department of organic and inorganic chemistry, University of Cambridge.

Awards

Dr D. Schofield, chief of the Defence Research Establishment at Dartmouth, N.S. to deputy chief of laboratories, responsible for all Department of National Defence research establishments. Dr Schofield will be replaced by F. A. Fergusson, Deputy Chief in Ottawa.

Dr S. Weinberg, Higgins Professor of Physics at Harvard University and Senior Scientist at the Smithsonian Astrophysical Observatory, is the 1977 winner of the American Institute of Physics-United States Steel Foundation Science-Writing Award (\$1,500) for his book, 'The First Three Minutes', relating the theory of elementary particles to the problem of the very early moments of the Universe.

The British Council East European Exchange Visits

Cultural agreements with the Soviet Union, Czechoslovakia, Hungary, Romania and Bulgaria provide for short visits (usually 2 weeks) and medium term research visits (2-6 months) for British scientists. Scholarships are also available for periods of up to one academic year. For full

details and application form, contact Higher Education and Science Department, The British Council, 10 Spring Gardens, London SW1, UK.

Meetings

21-22 October, **International Symposium on Chromogenic Substrates**, London (Thrombosis Research Unit, Department of Surgery, Denmark Hill, London SE5, UK).

2-4 November, **Symposium on Advanced Ozone Technology**, Toronto (A. Netzer, Head, Physical Chemical Processes, Wastewater Technology Center, Canadian Center for Inland Water, PO Box 5050, Burlington, Ontario, Canada).

2-5 November, **Symposia on Perspectives in Biochemistry, and Isoenzymes**, Cordoba, Argentina (R. Caputto, Departamento Quimica Biologica, Facultad de Ciencias Quimicas, Universidad Nacional de Cordoba, Ciudad Universitaria, 5000-Cordoba, Argentina).

9 November, **Liquid Crystals**, London, UK (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

9 November, **Symposium on Humidity Measurement**, London, UK (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

Person to Person

Mathematician seeks family accommodation in Oxford, January to December 1978, or exchange house in Newcastle, N.S.W., Australia. Contact W. Brisley, Mathematics Department, University of Newcastle, N.S.W. 2308, Australia.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

10-12 November, **Open Conference on the Implications of Recombinant DNA Research**, Bloomington, Indiana (Professor R. P. Bareikis, The Poynter Center 410 North Park Street, Bloomington, Indiana 47401).

14-15 November, **New Developments in Immunoassays**, Düsseldorf (M. A. Andreopoulos, Conference Director, Robert S. First, Inc., Avenue Marnix 19A, Box 6, 1050 Brussels, Belgium) (also in Chicago, 5-6 December).

14-15 November, **Symposium on Mass Spectrometry and Combined Techniques in Medicine, Clinical Chemistry**

and Clinical Biochemistry, Tübingen (Medizinische Klinik, Universität Tübingen, Otfried-Müller-Strabe 10, 7400 Tübingen, GFR).

14-17 November, **International Symposium on the Pineal Organ**, Jerusalem (I. Nir, Faculty of Medicine, Hadassah Medical School, Jerusalem, Israel).

14-18 November, **The Pan American Conference on Forensic Applications of Anthropology, Dentistry, Medicine and Palaeopathology**, Mexico City (W. G. Eckert, Laboratory, St Francis Hospital, Wichita, Ks. 67214).

17-18 November, **Symposium on Nutritional Management of Genetic Disorders**, New York (The National Foundation-March of Dimes, 622 Third Avenue, New York, N.Y. 10017).

21-26 November, **Fifth International Conference on Global Impacts of Applied Microbiology**, Bangkok (Chairman, GIAM V, National Organising Committee, Mahidol University, GPO Box 4-130, Bangkok, Thailand).

22-23 November, **Symposium on Photography in the Laboratory**, London (The Secretary, Scientific Symposia Ltd, 42-43 Gerrard Street, London W1, UK).

Reports and Publications Other Countries—July

United States Department of the Interior: Geological Survey. Professional Paper 655-M: Effects of Phreatophyte Removal on Water Quality in the Gila River Phreatophyte Project Area, Graham County, Arizona. By R. L. Laney. Pp. iv+23+2 plates. (Washington, DC: US Government Printing Office, 1977.) [117]

CERN — European Organization for Nuclear Research. CERN 77-10: Selection of Formulae Concerning Proton Storage Rings. By G. Guignard. Pp. x+108. (Geneva: CERN, 1977.) [117]

Deutsches Hydrographisches Institut, Hamburg. 29./30. Jahresbericht 1974/1975. Pp. 134. (Hamburg: Deutsches Hydrographisches Institut, 1977.) [127]

Bayer 1976. Pp. 103. (Leverkusen: Bayer Aktiengesellschaft, 1977.) [127]

National Multiple Sclerosis Society. 1976 Annual Report. Pp. 32. (New York: National Multiple Sclerosis Society, 205 East 42nd Street, 1977.) [137]

CERN — European Organization for Nuclear Research. CERN 77-11: Future Photomultiplier Assemblies and Associated Electronics in Large Experiments. By P. Duteil, R. Hammarström, P. G. Innocenti, A. Michelini, B. Smith and F. Soso. Pp. vi+35. (Geneva: CERN, 1977.) [147]

United States Department of the Interior: Geological Survey. Bulletin 1422-B: The Maudslowi and Sedan Formations of the Upper Cretaceous Livingston Group on the West Edge of the Crazy Mountains Basin, Montana. By Betty Skipp and L. W. McGrew. Pp. iv+68. Professional Paper 608-B: General Geology and Petrology of the Precambrian Crystalline Rocks, Park and Jefferson Counties, Colorado. By C. C. Hawley and R. A. Wobus. Pp. v+77+plate 1. Professional Paper 984: Geology of an Upper Cretaceous Copper Deposit in the Andean Province, Lassiter Coast, Antarctic Peninsula. By Peter D. Rowley, Paul L. Williams and Dwight L. Schmidt. Pp. vi+36. (Washington, DC: US Government Printing Office, 1977.) [147]

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 174: Gaschromatographie der Pflanzenschutzmittel—Tabellarische Literaturreferate VI; Kumulative Indices I-VI. Von Dr. Winfried Ebing. Pp. 102. (Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirtschaft, 1977.) DM 17.50. [187]

Journal of Mechanical Working Technology, Vol. 1, No. 1, July 1977. Published Quarterly. Pp. 1-114. Annual Subscription: Dfl. 155; \$58.95. (Amsterdam: Elsevier Scientific Publishing Company, 1977.) [187]

UK & Ireland—August

- Department of the Environment. Lead in Drinking Water: a Survey in Great Britain, 1975-1976. (Report of an Interdepartmental Working Group.) Pp. ix + 47. (London: HMSO, 1977.) £1.15 net. [18]
- The Electricity Council Annual Report, 1976-1977. Pp. 24. (London: The Electricity Council, 30 Millbank, SW1, 1977.) [18]
- Chinese Astronomy*, Vol. 1, No. 1, June 1977. (A selected translation of *Acta Astronomica Sinica*, Vols. 15-17, 1974-1976.) Pp. 1-196. Annual subscription for 1977: \$105. (Oxford and New York: Pergamon Press, 1977.) [18]
- Writing a Scientific Paper. By Vernon Booth. Fourth edition. Pp. 32. (London: The Biochemical Society, 1977. Published by permission of Koch-Light Laboratories, Ltd.). £1; \$2. [38]
- Office of Population Censuses and Surveys. Mortality Statistics: Cause. (Review of the Registrar General on Deaths by Cause, Sex and Age, in England and Wales, 1975. Series DH2, No. 2.) Pp. vi + 82. (London: HMSO, 1977.) £2 net. [38]
- The Determination of Vinyl Chloride: a Plant Manual. (Analytical Methods Required for the Control of Vinyl Chloride Concentrations in and Around Manufacturing and Process Plants.) Compiled by specialists convened by the Vinyl Chloride Committee of the Chemical Industries Association, Ltd., and edited by W. Thain. Third edition. Pp. 154. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, SE1, 1977.) CIA members £15; non-CIA members £20. [48]
- The Malaysian Rubber Producers' Research Association. Thirty-ninth Annual Report, June 1977. Pp. 28. (Brickendonbury, Hertford: The Malaysian Rubber Producers' Association, 1977.) [48]
- The Natural Environment Research Council. Postgraduate Training in the Sciences of the Natural Environment. Pp. 1-9. Research Grants in the Natural Environmental Sciences—NECR Research Grants Current on 1st October 1976. Pp. v + 60. (London: The Natural Environment Research Council, 1977.) [48]
- Department of Industry. Research and Development Requirements Boards, Reports 1976-1977. Pp. 40. (London: Department of Industry, 1977.) [48]
- International Planned Parenthood Federation. Occasional Essay No. 4: Defining Family Health Needs, Standards of Care and Priorities, with particular reference to Family Planning. By Fred T. Sai. Pp. 32. (London: International Planned Parenthood Federation, 18-20 Lower Regent Street, SW1, 1977.) 85p; \$1.45. [48]
- Gesellschaft für Strahlen- und Umweltschutz, München. Jahresbericht 1976. Pp. 312. (München: Gesellschaft für Strahlen- und Umweltschutz, 1977.) [187]
- National Research Council Canada. Report of the President, 1976/1977. Pp. 108. (Ottawa: National Research Council Canada, 1977.) [187]
- Smithsonian Contributions to Zoology. No. 244: A Review of the Troglobitic Decapod Crustaceans of the Americas. By Horton H. Hobbs, Jr., H. H. Hobbs, III, and Margaret A. Daniel. Pp. v + 183. No. 248: Redescription of *Echinoderes dujardini* (Kinorhyncha) with Descriptions of Closely Related Species. By Robert P. Higgins. Pp. iii + 26. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [207]
- World Health Organization. Technical Report Series, No. 608: Criteria for the Evaluation of Learning Objectives in the Education of Health Personnel—Report of a WHO Study Group. Pp. 47. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 6; \$2.40. [217]
- Economic and Social Commission for Asia and the Pacific. Committee for Co-ordination of Joint Prospecting for Mineral Resources in Asian Offshore Areas (CCOP). Technical Bulletin, Vol. 10. Pp. v + 212. (Tokyo: Sumitomo Printing and Publishing Co., Ltd., 3-2 Kanda Jinbo-cho, Chiyoda-ku, 1976.) [217]
- World Health Organization. Technical Report Series, No. 605: Chemotherapy of Solid Tumours—Report of a WHO Expert Committee. 106. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 10; \$4. [257]
- Massachusetts Institute of Technology: Lincoln Laboratory. Technical Note 1977-24: Station Magnitude Bias—Its Determination, Causes, and Effects. By R. G. North. Pp. v + 62. (Lexington, Mass.: Lincoln Laboratory, MIT, 1977.) [257]
- World Health Organization. Technical Report Series, No. 607: WHO Expert Committee on Leprosy—Fifth Report. Pp. 48. (Geneva: WHO; London: HMSO, 1977.) Sw. fr. 6; \$2.40. [267]
- Unesco. Intergovernmental Oceanographic Commission. Technical Series No. 12: Oceanographic Products and Methods of Analysis and Prediction. Pp. 172. (Paris: Oecd, 1977.) [267]
- United States Department of the Interior: Geological Survey. Professional Paper 1014: Late Quaternary Depositional History, Holocene Sea-Level Changes, and Vertical Crustal Movement, South San Francisco Bay, California. By Brian F. Atwater, Charles W. Hedel and Edward J. Helley. Pp. v + 15 + plate 1. (Washington, DC: US Government Printing Office, 1977.) [267]
- Annual Report of the Inter-American Tropical Tuna Commission, 1976. Pp. 180. (La Jolla, California: Inter-American Tropical Tuna Commission, c/o Scripps Institution of Oceanography, 1977.) \$2. [277]
- Lawrence Livermore Laboratory. First Annual Report to the High Altitude Pollution Program. Frederick M. Luther: Principal Investigator. Pp. 48. (Washington, DC: US Department of Transportation, Office of Environmental Quality, 1977.) [277]
- Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 32: Some Aspects of Genesis of a Red Earth Profile at Townsville, Queensland. By R. Brewer and R. J. Coventry. Pp. 21. (Melbourne: CSIRO, 1977.) [287]
- Government of India. Ministry of Health and Family Planning. Report of the Central Drugs Laboratory, 1st April 1971 to 31st March 1974. Pp. i + 60. (Calcutta: Central Drugs Laboratory, 1976.) Rs. 14; £1.64; \$5.04. [287]
- Israel. The National Council for Research and Development Advances in Cloud Physics and Precipitation Stimulation in Israel. (2nd Israeli Symposium.) Pp. 97. Advances in Israel Research on Water Desalination. (Lectures given by the Israeli Scientists at the International Desalting and Environmental Association Conference, held in Mexico City, Mexico, 24-19 October, 1976.) Pp. 73. (Jerusalem: National Council for Research and Development, 1977.) [287]
- Lautäusserungen und Lauterkerne bei Insekten (Grillen). Von Franz Huber. (Rheinisch-Westfälische Akademie der Wissenschaften: Natur-, Ingenieur- und Wirtschaftswissenschaften, Vorträge, N. 265.) Pp. 66. (Wiesbaden: Westdeutscher Verlag, GmbH., 1977.) DM 19.80. [297]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 280, No. 970: The Formation of Muscle Scars in Articulate Brachiopods. By D. I. MacKinnon. Pp. 1-12 + plates 1-11. UK £4.25; Overseas £4.40. Vol. 280, No. 971: Cranial Morphology of the Loxomatidae (Amphibia: Labyrinthodontia). By Eileen H. Beaumont. Pp. 29-101 + plates 1 and 2. UK £5.50; Overseas £5.70. (London: The Royal Society, 1977.) [48]
- The Radiochemical Centre Limited, Amersham. Sixth Annual Report, for the year ended 31st March 1977. Pp. 18. (Amersham: The Radiochemical Centre Limited, 1977.) [48]
- Philip Harris, Ltd. Science Education Equipment and Materials—Supplement and Price List, 1977. Pp. 60. (Shenstone, Staffordshire: Philip Harris, Ltd., 1977.) [58]
- National Health Service Act 1966. Report of the General Practice Finance Corporation for the period 1st April, 1976 to 31st March, 1977. Pp. 18. (London: HMSO, 1977.) 35p net. [88]
- Rowett Research Institute. Annual Report of Studies in Animal Nutrition and Allied Sciences, Vol. 32, 1976. Pp. 125. (Bucksburn, Aberdeen: Rowett Research Institute, 1977.) £1.50. [88]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1337: The Six Roll Mill: Unfolding an Unstable Persistently Extensional Flow. By M. V. Berry and M. R. Mackley. Pp. 1-16 + plates 1-5. (London: The Royal Society, 1977.) UK £1.85; Overseas £1.95. (London: The Royal Society, 1977.) [98]
- Town and Country Planning Act 1971. Control of Office Development, Annual Report by the Secretary of State for the Environment and the Secretary of State for Wales for the year ended 31st March 1977. Pp. 8. (London: HMSO, 1977.) 25p net. [118]
- Second Report from the Select Committee on Science and Technology. Session 1976-77. A Case Study of a Machine Tool Development at the Cranfield Institute of Technology. Pp. xxiv + 132. (London: HMSO, 1977.) £3.60 net. [118]
- Computers and Chemical Engineering, Vol. 1, No. 1, 1977. Edited by Richard R. Hughes. Published quarterly. Pp. 1-100. Annual subscription: \$64. Specially reduced rates to individuals whose institution takes out a library subscription: \$30. (Oxford and New York: Pergamon Press, 1977.) [118]
- Department of Energy. Freight Transport: Short and Medium Term Considerations. (Energy Paper No. 24.) (Advisory Council on Energy Conservation.) Pp. iv + 22. (London: HMSO, 1977.) £1.50. [158]
- National Radiological Protection Board. NRPB-R62: Insignificant Levels of Dose—a Practical Suggestion for Decision Making. By G. A. M. Webb and A. S. McLean. Pp. 16. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1977. Obtainable from HMSO, London.) £1. [158]
- National Radiological Protection Board. Protection Against Ultraviolet Radiation in the Workplace. Pp. 16. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1977. Obtainable from HMSO, London.) 40p. [178]
- Council for National Academic Awards. Directory of First Degree Courses. Pp. 175. Directory of Postgraduate Courses, 1977-78. Pp. 84. (London: Council for National Academic Awards, 344/354 Grays Inn Road, WC1, 1977.) [178]
- Annals of the ICRP, Vol. 1, No. 1: Radiation Protection in Uranium and Other Mines. (ICRP Publication No. 24) Pp. 28. (Oxford: Pergamon Press, Ltd., 1977. Published for the International Commission on Radiological Protection.) £3.25. [178]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 280, No. 972: The Changing Environmental Conditions in Great Britain and Ireland During the Devensian (Last) Cold Stage. A Discussion organised jointly for the Royal Society and the Royal Irish Academy by G. F. Mitchell, FRS, and R. G. West, FRS. Pp. 103-374 + 1 plate. (London: The Royal Society, 1977.) UK £18.75; Overseas £19.25. [178]
- Meteorological Office. Annual Report for 1976. (Met.0.907) Pp. xiv + 145. (London: HMSO, 1977.) £2.75 net. [188]
- Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 77, No. 3: Constructing Smooth Measures on Certain Classes of Paved Sets. By P. McGill. Pp. 31-34. 98p. Section B: Biological, Geological and Chemical Science. Vol. 77, No. 4: Breccia-Pipes Associated with the Ardara Pluton. County Donegal. By W. J. French. Pp. 101-117. 84p. Vol. 77, No. 5: Contributions to the Lichen Flora of South-East Ireland—II. By M. R. D. Seaward. Pp. 119-134. 78p. Vol. 77, No. 6: The Genetics of Hatching in the Potato Cyst-Nematode *Heterodera rostochiensis* Woll. By H. El-Shatoury. Pp. 135-141. 47p. Vol. 77, No. 7: Palaeontological Evidence for the Age of the Lower Palaeozoic Rocks of the Slieveanmont Inlier, County Tipperary. By J. R. J. Colthurst and D. G. Smith. Pp. 143-158 + 2 plates. 96p. (Dublin: Royal Irish Academy, 1977.) [198]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 287, No. 1338: Damage Tolerant Fibre Reinforced Sheet Metal Composites. By I. R. McColl and J. G. Morley. Pp. 17-43. (London: The Royal Society, 1977.) UK £1.60; Overseas £1.65. [198]
- The Neutron Bomb. By Prof. Eric Burhop. Pp. 8. (London: The Campaign for Nuclear Disarmament, 29 Great James' Street, WC1, 1977.) [228]
- Potato Marketing Board. Maincrop Potato Production in Great Britain, 1975-6. (An Investigation by the Potato Marketing Board into the Farm Types, Production Techniques and Materials Used in Growing Maincrop Potatoes in Great Britain.) Pp. 68. (Cwely, Oxford: Potato Marketing Board, 4 Between Towns Road, 1977.) 75p. [228]
- Monitoring and Assessment Research Centre of the Scientific Committee on Problems of the Environment, International Council of Scientific Unions. MARC Report No. 4: The Utility of the Nigerian Peasant Farmer's Knowledge in the Monitoring of Agricultural Resources. By David Barker, Julius Oguntuyinbo and Paul Richards. (A General Report.) Pp. 53. £1; \$2. MARC Report No. 5: Monitoring Tropical Forests—a Review with Special Reference to Africa. By Timothy J. Synnott. (A Technical Report.) Pp. 45. £1; \$2. (London: MARC, Chelsea College, 1977.) [228]
- Rutherford Laboratory. RL-77-072/A: Quark-Parton Model Relations for the Hadronic Production Lepton Pairs and γ 's with Different Beam Particles. By F. Halzen and S. Matsuda. Pp. 15. (Chilton, Didcot, Oxon: Rutherford Laboratory, 1977.) [228]
- The Lister Institute of Preventive Medicine. Report of the Governing Body, 1977. Pp. 12. (Elstree, Herts: Lister Institute of Preventive Medicine, University of London, 1977.) [228]
- The Acceptability of Risks: The Logic and Social Dynamics of Fair Decisions and Effective Controls. Pp. 104. (Chichester: Barry Rose (Publishers), Ltd., in association with the Council for Science and Society, London.) [228]
- In Defence of the NHS. Pp. 40. (London: Radical Statistics Health Group, c/o BSSRS, 9 Poland Street, London, W1, 1977.) 50p + 15p p&p. [258]
- Factors which Influence the Success of Supervisory Training. By Don Bates and Dian Hosking. (Occasional Paper No. 5.) Pp. 64. (Watford: Engineering Industry Training Board, 54 Clarendon Road, 1977.) £1.50. [228]
- Progress in Crystal Growth and Characterization, Vol. 1, No. 1, 1977. Pp. 1-91. Published as one volume of four parts per year. Editor-in-Chief: Dr Brian R. Pamplin. 1977 Subscription rate: \$45 (including postage and insurance). Two year subscription (1977/78): \$85.50. (Oxford and New York: Pergamon Press, 1977.) [238]
- The Handling, Storage, Use and Disposal of Unsealed Radionuclides in Hospitals and Medical Research Establishments. (A Report of Committees 3 and 4 of the International Commission of Radiological Protection.) (Annals of the ICRP, Vol. 1, No. 2.) Pp. 46. (Oxford and New York: Pergamon Press, 1977.) £4.50. [258]
- Tobacco Research Council. Research Paper 14, Part 1: Report on a Second Retrospective Mortality Study in North-East England. Factors Related to Mortality from Lung Cancer, Bronchitis, Heart Disease and Stroke in Cleveland County, with particular emphasis on the Relative Risks Associated with Smoking Filter and Plain Cigarettes. By G. Dean, P. N. Lee, G. F. Todd and A. J. Wicken. Pp. 95. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1977.) [258]
- Annual Report for 1976 of The Medicines Commission, the Committee on Safety of Medicines, the Veterinary Products Committee, the British Pharmacopoeia Commission, the Committee on the Review of Medicines, and the Committee on Dental and Surgical Materials. Pp. 95. (London: HMSO, 1977.) £1.60 net. [268]
- Ministry of Agriculture, Fisheries and Food. Agricultural Development and Advisory Service. Technical Bulletin No. 18: Manual of Veterinary Parasitological Laboratory Techniques. Pp. v + 129. (London: HMSO, 1977.) £3.50 net. [268]

Other Countries—August

- International Agency for Research in Cancer. Annual Report for 1976. Pp. 159. (Lyon: International Agency for Research on Cancer, 1976. Obtainable from WHO, Geneva; HMSO, London.) Sw. fr. 12; \$4.80. [18]
- US Department of Transportation: Federal Aviation Administration. Aircraft Emissions: Potential Effects on Ozone and Climate: a Review and Progress Report. By R. C. Oliver, with E. Bauer, H. Hidalgo, K. A. Gardner and W. Wasylkiwskyj. (Report No. FAA-EQ-77-3. Final Report.) Prepared for High Altitude Pollution Program. (Washington, DC: US Department of Transportation, Federal Aviation Administration, Office of Environmental Quality, 1977.) [18]
- Spreading Deserts—The Hand of Man. By Erik Eckholm and Lester R. Brown. Pp. 40. (Worldwatch Paper No. 13.) (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, N.W., 1977.) \$2. [18]
- Siemens Forschungs- und Entwicklungsberichte/Siemens Research and Development Reports, Bd. 7 (1977), Nr. 4. Edited by Hans Suchlandt. Pp. 191-262. (Berlin and New York: Springer-Verlag, 1977.) [48]
- Publications of the United States Naval Observatory. Second Series, Vol. XXIV, Part 3: Fourth Catalog of Trigonometric Parallaxes of Faint Stars. By C. C. Dahm, R. S. Harrington, B. Y. Riepe, J. W. Christy, H. H. Guetter, A. L. Behall, R. L. Walker, A. V. Hewitt and H. D. Ables. Pp. 27. (Washington, DC: US Government Printing Office, 1976.) [48]

nature

6 October 1977

Facing up to demography

THE United States is gradually, if painfully, coming to grips with the problems of discrimination within its society. Most liberal-minded people have gone along with much that has been done in the past fifteen years, even when initial efforts to reduce discrimination have not been sophisticated or quick-footed enough to avoid charges of reverse discrimination, as in the case of Allan Bakke, a white student with good grades excluded from the medical school of the University of California at Davis. Racial minorities, women, the handicapped—these are causes with which very few would disagree. But scientists, in particular, are having very mixed feelings about the plight of those most recently claimed to be discriminated against—the elderly.

A bill that has just passed through the US House of Representatives with negligible opposition would prohibit mandatory retirement in private employment before the age of 70, and would prohibit mandatory retirement in the federal sector at any age (with exceptions such as the police and firefighters). At present private employers may retire their staff at 65, the federal government at 70. These moves came about for two reasons, both connected with demographic changes which are progressively peopling the United States with older citizens (23 million are 65 or over at present, and 31 million are expected to be so by the end of the century). First, the burden of social security payments is going up, yet the numbers of new workers is going down as the birthrate declines, so there are good actuarial reasons for wishing to postpone retirement. Second, the elderly are rapidly becoming a potent political force, of which politicians are becoming increasingly aware.

The bind for scientists is this: that deep concern was already being expressed before the retirement bill ever surfaced over the way that university faculty employment was moving towards a crisis. In 1979 there will be 4.3 million American eighteen-year-olds, 60% more than in 1960. The course of university expansion in the 1960s ensured that these young people would be well catered for. But after 1979, the numbers will steadily decline until in 1990 there will be fewer than 3.5 million in this age bracket. Many universities will presumably have to go out of business and elsewhere faculty will have to be trimmed. Things look particu-

larly bad in the physical sciences; an NSF projection puts the number of faculty positions in 1985 at 25% less than the number in 1972. Nor can this attrition be taken care of solely by retirement. Vigorous recruitment over the past thirty years has ensured that there is a predominance of young to middle-aged faculty members at present. So the prospects for the post-doctoral worker looking to get a toehold on the academic ladder already seemed bleaker than ever before. Nor should the position of the scientist working for the government be reckoned to be very different. Federal agencies have often followed very similar employment policies to those of the universities.

The new retirement legislation would obviously compound these problems very seriously, so it is small wonder that academic employers have been lobbying hard for exemption this past week. They have so far met with some success in that the Senate Human Resources Committee, in endorsing the bill (which now goes to the Senate), has excluded universities from its provisions. But no one seems yet to have spoken up for the federal government laboratories, where scientists will, presumably, be able to go on working to any age they choose.

Even if universities do emerge from the legislation relatively unscathed, there will still remain the urgent question of diminishing job prospects for the young. Richard Atkinson, Director of NSF, has recently made some fairly radical proposals (*Chronicle of Higher Education*, 28 March). These are that the government should facilitate more mid-career shifts for those who wish to depart early; that research institutes should be established close to or within universities to which senior academics could move, devoting more of their time to research and passing their teaching load on to newly employed junior faculty; and that industry should take greater advantage of the basic-research skills of senior academic scientists in some form of joint venture.

All of these proposals are doubtless open to many and varied criticism, but we simply cannot afford the luxury of a lengthy and hair-splitting debate on the subject. Action is needed in the very near future, otherwise demography will be upon us and we will be responding to the crisis in an arbitrary way. That would lead to an even worse form of discrimination. □

The long journey into the end of the artificial night

Sargun A. Tont, staff research associate at Scripps Institution of Oceanography, University of California at San Diego, offers a view on the study of solar eclipses

ALTHOUGH the occurrence of a solar eclipse is one of the most precisely predicted phenomena in the entire solar system, the chances of its coinciding with clear skies are about as good as Mr Buckley's inviting Mr Vidal for a transatlantic cruise on his yacht, or Bobby Fischer's opening with a queen's pawn.

Although unfriendly weather is an important obstacle for eclipse lovers, it is far away from being the only one. Getting to the path of an eclipse usually turns out to be a problem that itself exercises a good bit of human ingenuity. For the past of a total solar eclipse seldom favours highly populated regions. There is no reason that it should, of course, yet the number of eclipse paths which run toward far-away places almost appears designed to test the limits of human curiosity. Not surprisingly, then, the traditional vehicles for eclipse expeditions have been mules, donkeys, camels, sledges drawn by Alaskan huskies, trains and, of late years, even chartered jets. In 1972, a novelty was added: a huge, luxurious ocean liner, the *T.S.S. Olympia*, carried 800 eclipse freaks 900 miles out into the cold Atlantic.

The following paragraph from the *New York Times* of 30 July 1972 explains my presence on that ship of fools, as it appeared to me at the start:

A team from the Scripps Institute [*sic*] of Oceanography in La Jolla, California, would conduct an eclipse experiment possible only at sea; they would lower a 300-pound echo chamber over the *Olympia's* bow to determine whether the layer of microscopic ocean animals called plankton rises at night merely because it gets dark or because of an internal biological clock that would not be triggered by the extraordinary, sudden darkness of the eclipse.

In short, an eclipse provides a sort of artificial night for testing this unique hypothesis.

The ships may be more luxurious and larger, but the age-old problem of not being able to avoid undesirable situations seems to remain the same. The problem in my case was 350 freelance writers who were looking for interesting angles, which primarily meant interviewing me, the expedition leader, and my colleague and friend Dr Gerald Wick. However, as the big day approached, we were pretty much left alone, and I was able to take a closer look at my fellow passengers. They were a most interesting bunch. Astronaut Scott Carpenter, along with several members of New York high society, were there. So were secretaries, nurses, and several professional astronomers.

People have responded to solar eclipses in a variety of ways. One king, Louis of Bavaria, was frightened to death by it. A solar eclipse stopped a battle between the Lydians and the Medes, and, as late as 1948, the Korean national elections were postponed, again because of an eclipse. American Indians have described eclipse as "the sun being very sick and going to bed"; and India's Indians have interpreted them as the sun being devoured by evil spirits. Our fellow passengers might have lost some of the fear which beset their early ancestors, but definitely not their reverence and enthusiasm. When I asked several of them how it felt to witness an eclipse, the answers were invariably the same: "There is nothing like it". Like what? Few people seemed to know, or were prepared to say. Yet Leif Robinson, the associate editor of *Sky and Telescope* and a fellow passenger

on board the *Olympia*, probably summed up best what it is about eclipses that forces us to follow them wherever they take us: "Every solar eclipse unfolds in a predictable way, only the details changing. It is the manner in which these subtleties of colour, brightness, and form are blended that gives each eclipse a flavour as distinct as vintage wine". Conrad would have liked that.

It did not take me long to discard my idea that the passengers were on this cruise to have a good time, in addition to watching the eclipse. It was the eclipse and the eclipse alone that they were after, and many, I am sure, would have swum the distance if that were the only transportation possible. Nevertheless, the entrepreneur of the cruise, Phil Siegler, a social scientist from New York, had also insured a full house by offering something called 'Science at Sea'. Courses ranging from astronomy to bird-watching were taught by astronomers, atmospheric scientists, Scott Carpenter and Leif Robinson.

Nor, as it turned out, was our group the only one mixing pleasure with business. Milner Schaefer, the inventor of cloud seeding and a most charming man, was geared to record cosmic rays; and Dr Williams, of Yale University, passed out photocopied notices inviting all interested passengers to share their eclipse-related dreams with him as a part of his research project¹. However, the most important scientist on board was the meteorologist whose primary function was to guide the ship to the most favourable (that is, cloud-free) location. Outfitted in a Scottish tweed jacket and impeccably shiny shoes, he entered into Ahab-like trances which never failed to command our closest attention, and his least utterance about the weather was speedily relayed to the farthest corner of the vessel.

Then it happened. It wasn't what I expected. What surprised and fascinated me was not the sudden darkness which swooped down on us with the grace of a hammer-head entering a South-Sea lagoon, but the grasshopperesque chatter of cameras, all 799 of them. I suddenly realised that I might be the only one who was watching it—the rest were busy recording it for posterity, be it for a lecture demonstration for an undergraduate astronomy class at Harvard, or for a slide show especially prepared for a maiden aunt and her boyfriend in Galesburg, Missouri. What a pity! Thanks to the camera, most people became the lovers of an illusion of reality, rather than of the reality itself.

It is that time again and the faithful are getting ready to watch, on 12 October, the total solar eclipse which, according to reports, will be best viewed off the coast of central America (13° 42'N 123° 07'W). In order to accommodate the large number of applicants, Sitmar Lines is offering two ocean liners, along with such luminaries as Margaret Mead and Isaac Asimov. If you happen to be in the neighbourhood, take a look at it. It is some sight.

As for our experiment², in the finest scientific tradition, the results were rather ambiguous. Even though some of the organisms responded to the eclipse by migrating vertically, some did not. One of the results was totally unexpected: increased bioluminescent activity during totality. One can offer several hypotheses to explain this phenomenon, but I am sure, to the eclipse lover, this was nothing more than the little bugs celebrating the joyous occasion.

¹ For the various scientific projects conducted during solar eclipses, see *Solar Eclipse Bulletin 1-4* (National Science Foundation, Washington, D.C.; 1973).

² For the results of our experiment, see *Deep-Sea Research* 20, 769-771; 1973.

Two decades of Soviet space

Vera Rich looks at the Soviet space programme, twenty years old this week

ON 4 October 1957 the first artificial satellite, later to be known as *Sputnik 1*, was launched as part of the Soviet contribution to the International Geophysical Year. From the Soviet point of view, the date fell auspiciously close to the 40th anniversary of the October Revolution, and also in the centenary year of the birth of Konstantin Eduardovich Tsiolkovskii, 'the father of cosmonautics'. During the subsequent decade the Soviet space programme notched up an impressive number of 'firsts'—first animal in space, first lunar impact, first circum-lunar probe, first man in space, first (and to date only) woman in space, first interplanetary probe, first automatic docking and separation. The USSR could even claim the first 'space baby', Elena, the daughter of cosmonauts Tereshkova and Nikolaev, whose healthy arrival did much to disperse alarmist fears of the genetic hazards of space flight.

It was not until the late 1960s that the unbroken run of Soviet 'firsts' slowed down. Like the Apollo 8 accident in the USA, the tragic death on 24 April 1967 of Vladimir Komarov, whose re-entry parachutes failed to open properly, put an end to the euphoria. The death a month before of Yuri Gagarin in a flying accident not only deprived the Soviet Union of its greatest living hero, but also appears to have caused a major change in policy. During her post-mission world tour, Tereshkova had told a press conference in Cuba that the Soviet moon team had already been picked and that Gagarin was to lead it. Since his death, no mention has been made of a manned lunar mission in the foreseeable future and, with the success of the US Apollo missions, the Soviet planners have preferred to stress the considerable saving (up to 50%) effected by using unmanned rather than manned lunar probes.

This stress on saving is not simply a face-saving excuse. The actual cost of the Soviet space programme is a closely guarded secret, but from the very beginning there was a strong body of feeling that whatever the cost might be, it was too high. A whole series of black jokes circulated, based on the resemblance of the word for meat (*myaso*) and the archaic name for the moon (*mesyats*), suggesting that the shortages of the one were not unconnected with the rockets to the other. By 1960, *Komsomolskaya Pravda* had to launch a special campaign to quash

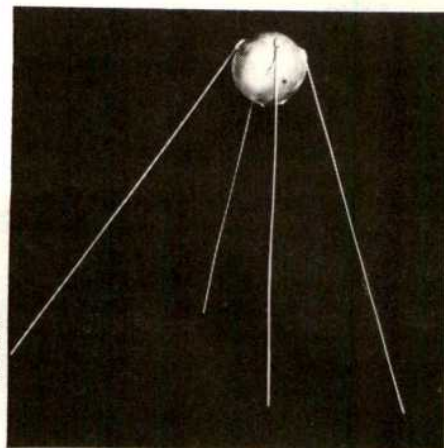
such criticism. But public opinion had made its point; the comrade-in-the street was not prepared to accept pie-in-the-sky as a substitute for consumer goods on earth. Gradually it became standard practice to announce all space-missions as being "in the interests of the national economy"—a phrase which seems somewhat hard to justify (except in terms of serendipity) when applied to a Venus or Mars probe.

Terrestrial applications

A considerable proportion of Soviet launches do, of course, have terrestrial applications. The *Meteor* meteorological satellites, and the *Molniya* communications satellites have proved invaluable in the opening up of Siberia, not the least of the advantages of *Molniya* being that it provides the solace of TV for workers on remote construction sites. Visual and infrared photography, from manned and unmanned satellites, has found a wide range of applications from oil-prospecting to fish-spotting. The *Kosmos* series of satellites, initiated in 1962 and now approaching the 1,000 mark, covers a number of such applications. The vagueness of the series title makes it an ideal cloak for any purpose the planners may wish to conceal. In the early days unsuccessful lunar or interplanetary probes were assigned a *Kosmos* number, while *Kosmos 47* was probably an unmanned trial run for the manned *Voskhod 1*, which followed a week later.

Initially one of the basic missions of the *Kosmos* programme was stated to be the monitoring of high-altitude nuclear explosions. *Kosmos 5*, in particular, measured the decay of high-altitude radiation following the American 'Starfish' nuclear test of 9 July 1962. Happily the 1963 atmospheric test ban treaty rendered this part of the programme obsolete. There is little doubt, however, that the *Kosmos* series is not without its military applications; and the term *Kosmos* has also covered various 'occasional' experiments, including automatic docking, and tests on biological specimens.

With the abandonment of any immediate plans for a manned moon mission, the Soviet manned space programme became centred on the concept of orbital space stations—the approach which had been favoured by Tsiolkovskii himself. The first such station, *Salyut 1*, was launched in 1971. Although the success of the mission was overshadowed by the death of the second-shift crew during re-entry, the



Where it all began: Sputnik 1



Where the USSR is now: Soyuz 24 about to take off

series continues, and this week's anniversary is marked by the launch of *Salyut 6*, as well as by the publication by the Crimean Astrophysical Observatory of a 'catalogue' of solar radiation of various wavelengths and a detailed spatial distribution of the temperature of solar flares, based on films taken aboard *Salyut 4*.

According to Academician Georgii I. Petrov, the *Salyut* programme is a three-fold one—astrophysical observations, surveying earth resources, and physical experiments associated with weightlessness. Already a number of interesting results have been reported on crystal growth and the cooling of liquid metals in weightlessness; in a recent *Pravda* interview Petrov suggested also that a space station would be a suitable base for producing specimens of ultra-high purity.

He further suggested that such space stations could well provide a base for a long-term moon expedition. He envisaged a lunar-orbital station with a crew aboard; they would send down automatic probes and sampling devices to the surface of the moon, to make observations or bring back specimens as required. "I can see no reason", Petrov remarked, "that would require man to make a long-term stay on the

moon". He then proceeded, not surprisingly, to rule out any possibility of a manned Mars mission, largely on the grounds that the biological effect of long-term weightlessness is still unknown. If weightlessness really presents such a danger, it seems a little remarkable that a Soviet manned 'lunar' mission would preferably be kept in orbit rather than allowed to benefit from the reduced but at least perceptible gravity of the moon.

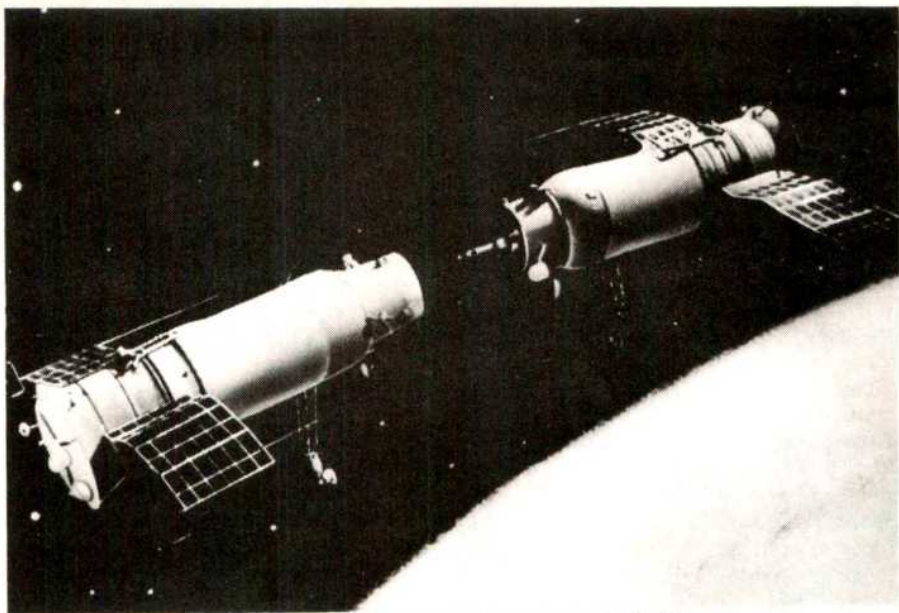
No further

For the moment, it would appear, Soviet man will venture no further than a circumterrestrial orbit. Not surprisingly, the "most interesting" future experiments which Petrov outlines will all be carried out by unmanned probes—the collection of highly rarefied 'undamaged primary meteorite material', and the recovery of solid material from interplanetary space. Other exciting projects for robot probes include flights over the poles of the sun, and a deep-level descent into the atmosphere of Jupiter. And, said Petrov, the only sure way to solve the age-old problem of whether there is life on Mars is the automatic recovery of a soil specimen from the planet. It is here, incidentally, that Soviet expertise gained during the automatic moon programme will pay off. The specimens of lunar soil recovered by the *Luna* probes were far smaller than those brought back by the Apollo crews, a fact which the US media were not slow to note and which the Soviet publicists played down by stressing the technological prowess behind automatic sampling. For Mars, however, automatic sampling will probably antedate any manned mission by a number of years, while in the case of Venus, automatic sampling would appear essential. Similarly, a roving vehicle of the *Lunokhod* type could easily be redesigned as a *Marsokhod*, with only minor modifications to the Martian environment.

To launch such missions, there are, according to Petrov, "lively discussions" about the possibility of a "reusable" launch craft similar, presumably, to the US Space Shuttle but possessing, if necessary, two returnable launch stages.

International cooperation

One aspect of the space programme was not mentioned by Petrov—international cooperation. That is the responsibility of his namesake, Academician Boris N. Petrov, the head of the *Interkosmos* programme. *Interkosmos* itself is the space research organisation of the Comecon block, launching the astrophysical *Interkosmos* satellites (17 to date) and the *Vertikal* high-altitude rockets. However, the Soviet Union also has programmes of space cooperation with other countries,



1967: Kosmos 186 and Kosmos 188 approach for automatic docking



Training in capsule recovery in water: normal Soviet style is over land

notably France, India, Sweden, and the USA. The type of cooperation varies from country to country.

To date the Franco-Soviet programme has been the broadest based, and has included geodesy, aurora borealis/australis, and interplanetary particle flux experiments, as well as laser ranging to the moon, using French reflectors on *Lunokhods* 1 and 2. A Swedish experiment on ultraviolet solar radiation was carried by *Interkosmos* 16 under an agreement with the Soviet Academy of Sciences, and a new cooperation agreement signed last month will provide for Swedish magnetosphere-plasma experiments to be carried by Soviet high-apogee satellites.

The most significant cooperation agreement of the whole 20 years, how-

ever, was that between the USA and the Soviet Union, which culminated in the Apollo-Soyuz link-up of July 1975. The space age began in an atmosphere of mutual doubt between the superpowers—the Soviet triumphantly, the USA fearfully assessing the propaganda advantage of *Sputnik* 1. It took several years for even so innocuous a matter as the exchange of high-altitude meteorological photographs to become a matter of practical politics. The political and psychological implications of space-flight are too large a subject to be dismissed in a paragraph; nevertheless it may well be that *détente* on the one hand, and the growing concern with conservation and ecology on the other, are themselves as much a product of the space age as an Apollo capsule or a Venus descent craft. □

CANADA

Digesting the food problem

David Spurgeon writes from Ottawa on the impact of a symposium held in August to consider how Canada could best contribute in the future to the alleviation of world food shortages

A SYMPOSIUM on the theme "Canada and World Food" has had the paradoxical effect of bringing to the fore some deep-seated concerns about the state of Canadian agriculture itself. Sponsored by the Royal Society of Canada and the Agricultural Institute of Canada, and featuring some leading figures of Canadian and US agriculture and foreign aid programmes, the meeting also brought into question some of the country's official food aid policies.

Some consensus was reached at the meeting. There seemed to be general agreement that the world food supply was precarious, but that world agriculture was capable of producing enough food to meet demand in the near term. There also appeared to be general agreement with the idea that the world's food security was a political and not an economic or scientific question. Too little emphasis was seen to be placed on agriculture by developing countries.

But the symposium also brought out wide differences of opinion on the effects of food aid and its consequent value to developing nations. On the Canadian position, for example, Gordon A. MacEachern, president of the Agricultural Economics Research Council of Canada, had alarming things to say. He maintained there had been "a serious deterioration in Canada's agriculture-food industry output, trade, productivity performance, and a serious decline in our relative contribution to world food, compared to earlier periods and the performance of other net food exporters, most notably the United States".

Though others pointed out that Canada currently is the world's second largest supplier of food aid, MacEachern said there seemed

little benefit in addressing the relatively attractive world food demand prospects, or the economic potential for Canada expanding its agriculture-food capacity to play a greater role in world food supplies, when its cost-price structure says it can't compete. Pervasive voices from almost all segments of Canada's agriculture and food industries are echoing these sentiments, as are senior agricultural officials in Canada, suggesting that what is presently at stake is the very survival of our agriculture-food industries.

Canada continues to enjoy a favourable trade balance in food, as it always has. But over the past 20 years the country's food and feed exports have not kept pace with the growth of either the Canadian or the world economy or the growth in world food and feed trade. In the past five years, Canada has maintained a net export position for only a few commodities such as grain and oilseeds, and in these output and export performance have remained more or less static.

One reason for this, said MacEachern, is the steady growth in Canada's food imports, two-thirds of which are similar to or the same as foods that can be grown in Canada. Other reasons included the artificial support of the Canadian dollar by the federal government, the high level of wage rates in the industry in Canada, and an effective level of protection of 20-40% for manufacturing industry, which further raised agricultural costs.

Len Shebeski, dean of the University of Manitoba's faculty of agriculture, quoted a pessimistic comment made in June by Dr Fred Bentley of the University of Alberta's soil science department, who is chairman of the board of

trustees of the International Centre for Research in the Semi-arid Tropics (ICRISAT) in India. Without a massive and well-coordinated effort to meet the needs of agriculture, he had said, Canada could become a food deficit nation before the end of this century. Shebeski disputed this. He believed that Canada's agricultural potential was even greater than the 65 and even 100% increase over present production others had suggested. He based his prediction on the possibility of doubling the land area devoted to agriculture and a 60% increase in yield per acre through improved management. Agricultural lands in Canada had the potential—with present technology—for "more than three times present production of field crops and more than ten times the present ruminant livestock production". And these estimates could be revised upwards "if adequate moisture supplies in the more arid regions of western Canada could be assured."

Shebeski wondered, however, whether Canada ought to reach such production figures. "Because Canada, territorially, is the second largest country in the world, there has developed a popular belief—often voiced—that it is the bread basket of the world," he said.



Indian wheat harvest: but how to assure supplies?

MacEachern acknowledged that

Those of us involved in agriculture know that this is a myth. An examination of a world map depicting the agriculture lands of the world shows that Canada's component of the world's present 3.6 billion acres of crop land is approximately 5% . . . Canada's strategic position in the current world food situation does not arise from a high tonnage of food outputs, but rather from the large proportion of her total production available for off-shore consumption.

This production, he intimated, should not be relied upon freely by developing countries to fill their food shortages. The costs of claiming the 20 million hectares of potentially arable land of low fertility would be enormous. Crops produced would be modest in quantity and marginal or submarginal in profitability. The food produced would be thousands of miles from where it would be needed.

As an alternative, Shebeski proposed that the Canadian government, through the International Development Research Centre (IDRC) or the Canadian International Development Agency, make available to developing countries the money it would cost to bring large areas of potential Canadian land into food production. This money could then be used to bring into production land areas with the agricultural potential of the Indus-Ganges-Brahmaputra plain of North India, or parts of Brazil, where experiments have shown production possibilities of more than 70 bushels per acre. He had struck on a note that was echoed in much of the conference: investments in food production to meet shortages in developing countries should be made in those countries rather than in developed ones.

David Hopper, president of the IDRC, said food assistance should not be denied in cases of genuine emergency or hardship. But imports had in some cases in the past held domestic farm prices below levels that would have prevailed in the recipient countries had the imports not been available, and this had destroyed the incentives to generate local increases in food production. Where the food aid was essentially free, its sale within the country by the recipient government had directly augmented national treasury resources, reducing the need to tax more heavily or to encourage larger savings for domestic investment programmes. That blunted incentives for local development, and removed any urgency to look further into measures to promote the more rapid development of indigenous farming.

Fred Bentley proposed that, as a minimum, every food-deficient less-developed country should be required to increase the budget for agriculture by an amount at least equal to the value of food aid received, without any diminution in the percentage of the

national budget already being allocated to the agricultural sector. Alternatively, recipients could add the funds to their population control programmes. But T. K. Warley, of the School of Agricultural Economics and Extension Education at the University of Guelph, Ontario, defended food aid, saying it had served many purposes beyond providing increased food supplies to hungry people in poor countries, including the general transfer of resources to developing countries, support of the balance of payments and thus maintenance of high rates of economic growth, and promotion of political stability and allegiances. Food aid, he said, should be used to accelerate agricultural and rural development, alleviate hunger in specific target groups, and help build minimum stocks of basic foodstuffs.

Frank Shefrin, director of the Canada Department of Agriculture's international liaison service, summed up the magnitude of the world problem. He said that it was not enough to increase the production of food. There had to be increases in food production in the poorer countries and by the small farmers, so that imports of food would not exceed their means of paying. The food had to be produced where it was needed and get to those who need it. Just to stay at the present per capita food production, developing countries could require an annual investment in agriculture of about \$20 billion, of which 60-70% would have to come from the countries themselves unless there was a substantial increase in capital assistance.

Findings of the latest report of the International Food Policy Research Institute (IFPRI) in Washington were also revealed. Projecting food shortfalls of some 82 developing countries (containing roughly half the earth's population) by comparing prospective food production if performance continues in the next 15 years as it has in the past 15, IFPRI has come up with the following forecasts:

- A total food grain deficit among those countries with less than \$300 GNP per capita in 1973 of 70-85 million tons by 1990 (12 million tons in 1975).
- A need for some 35 million metric tons over and above the projected production in 1990 just to provide for population increases in these countries (maintaining per capita consumption at 1975 levels).
- Potential shortfalls by 1990 among all developing countries included in the study of 120-145 million tons, a figure which approaches the total volume of world grain trade in recent years.
- A potential shortfall of 21-25 million tons by 1990 in India, which has almost half the people in low-income



Onloading in Canada



Offloading in India

food deficit countries. To meet this, an increase in production of $3\frac{1}{2}\%$ a year would be required, compared to the historical $2\frac{1}{2}\%$.

- Deficits of 6-8 million tons each for Bangladesh and Indonesia. For Bangladesh, production would need to increase almost $4\frac{1}{2}\%$ a year compared to a historical rate of $1\frac{1}{2}\%$. For Indonesia, the rate should be $4\frac{1}{2}\%$ instead of the historical 3%.
- A deficit of 18-21 million tons facing Nigeria, which would require $5\frac{1}{2}\%$ increase a year (historic rate $\frac{1}{2}\%$).
- An increase to $3\frac{1}{2}$ -4% required in Sahel countries to avoid a shortage of $3\frac{1}{2}$ million tons (historic rate $-\frac{1}{2}\%$).
- To meet nutritional needs, most low-income food-deficit countries need an additional $\frac{1}{2}$ -1% growth rate over and above those necessary to meet market demand. □

USA

Department store

David Davies reports from Washington on the new US Department of Energy

THE Department of Energy, the twelfth Cabinet agency of the United States, comes into being on 1 October. Proposed by President Carter on 1 March of this year, the department will be headed by Secretary James Schlesinger. It will have almost 20,000 employees and a first-year budget of \$10,400 million. An amalgam of several agencies, most notably the Energy Research and Development Administration (ERDA) and the Federal Energy Agency, the new department's structure is a little unusual in that it is divided along functional lines rather than technological lines. Thus technologies will move through different parts of the department as they progress from basic research through development and into commercial implementation.

Responsibility for basic research will lie, in large measure, in the Office of Energy Research, which will manage basic science programmes both within the department's own laboratories and in the universities. The Director of this office will be Professor John Deutch, a physical chemist from the Massachusetts Institute of Technology. Deutch, 39, is no stranger to the Washington scene. In the 1960s he worked as a systems analyst in the Department of Defense, and consulted for the Bureau of the Budget. He has been a part-time member of the Defense Science Board, most recently as its Vice-Chairman. But he declares, indeed promises, that in several years time he will be back doing chemistry.

In the meantime he will play four major roles. He will provide Schlesinger with technical advice on basic science issues. He will co-ordinate

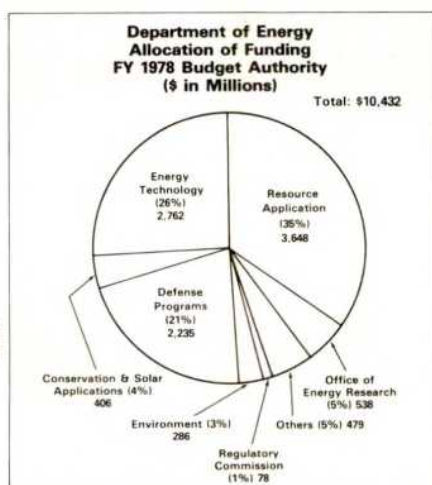
all the department's basic research, some of which will obviously be done outside the immediate orbit of his office. He will be responsible for the well-being of all the department's 144 laboratories (excluding the weapons laboratories). And he will be responsible for the financial support of basic research.

A very substantial fraction of the basic research money at present goes to high energy and nuclear physics, and clearly the future of this research is not too closely tied to immediate questions of energy policy. But in fiscal year 1977, \$125 million was spent in basic energy research, divided roughly 7:3 between governmental laboratory and university recipients. There is little doubt that this sum will rise sharply in the next few years; already federal financial obligations to energy R&D as a whole in fiscal year 1978 are seen as rising by at least 10% in real terms over the 1977 figure.

The department has an Under Secretary and six Assistant Secretaries on the technical side. Subject to confirmation, the Under Secretary, who will oversee all the programmes but have a special brief for energy conservation, is Dale Myers, at present a Vice-President of Rockwell International. Apart from basic research the Assistant Secretaries also cover energy technology; resource applications, with responsibility for the commercial introduction of new technology; conservation and solar applications, a contact point for individual inventors and small businesses; environment, with responsibility for ensuring that programmes comply with environmental health and safety regulations and with the authority to conduct related research; and defence programmes, directing nuclear weapons research and investigations into laser fusion. □



Research director: John Deutch



DNA bill delay

RECENT moves on Capitol Hill have created further confusion in the already contorted story of the various pieces of pending legislation to control recombinant DNA research in the United States. In the House of Representatives, the bill drafted by Rep. Paul Rogers' Health subcommittee was scheduled for consideration by the Commerce Committee last week and was not expected to run into much trouble. Commerce chairman Harley Staggers postponed consideration of the bill, however, and suggested that important amendments might be required. Further amendments have been proposed by other committee members. The chances that the bill will be approved by the committee and reach the floor of the House before the end of the legislative session have now diminished almost to zero.

In the Senate the situation is more confusing. The Kennedy bill which was already running into trouble with Senators concerned about its bureaucratic provisions has been removed from the legislative calendar. Senator Kennedy now proposes to set up a Study Commission composed of interested members of lay and scientific organisations to consider the best form of legislation. As a stopgap, a simple measure to give the NIH guidelines the force of law would be enacted. This would apparently contain no enforcement provisions but would apply to industry as well as government-financed research. The position of the bill recently drafted by Senator Gaylord Nelson, which is similar in many respects to the House bill (see *Nature*, 1 September) is not clear, although it does not now appear to be gathering sufficient support for passage this session.

In addition Senator Adlai Stevenson III, as chairman of the Subcommittee on Science, Technology and Space of the Senate Commerce Committee, has announced hearings on the effect of the proposed legislation; at the same time he expressed strong misgivings about the pending bills and their effect on basic research and freedom of scientific enquiry.

All these moves owe something to the lobbying efforts of some scientists and scientific societies, together with statements on the relative risks and benefits of recombinant DNA research from a number of scientific meetings over the summer. More battles are in store before legislation finally emerges.

Sandy Grimwade

IN BRIEF

UK sweetener review

The UK Food Additives and Contaminants Committee is to conduct a full review of the regulations controlling the use of artificial sweeteners in food. The committee, which falls under the aegis of four governmental departments, will thus extend its present studies of saccharin and of Aspartame; it has published two reports on cyclamates in the past.

Waste now, power later

A three-member panel of the White House Council of Environmental Quality has, according to the *New York Times*, reported that an expansion of nuclear power in the United States should be made conditional upon a demonstrably safe method of containing nuclear waste. The verdict is seen less as a counter to President

Carter's energy plan than as an attempt to encourage work on waste disposal.

IAEA reprocessing view

Though it was generally accepted that the number of nuclear fuel reprocessing plants should be kept to a minimum, to prohibit them would probably lead to a result opposite of that intended, according to Dr Sigvard Eklund, the Director General of the International Atomic Energy Agency (IAEA). He was speaking at the IAEA General Conference's 21st Regular Session in Vienna last week. Dr Eklund, who was appointed to his fifth consecutive term as director general, was also critical of those who wanted to eliminate the nuclear option.

Accelerator approval

Approval has come for the construction of Brookhaven National Laboratory's 200 GeV superconducting

intersecting storage ring accelerator Isabelle. A joint US House-Senate committee last week authorised expenditures totalling nearly \$200 million. The device will enable protons to be fired at each other with centre of mass energies well in excess of those available elsewhere, providing detail on how and if the weak and electromagnetic interactions are unified.

HSC report

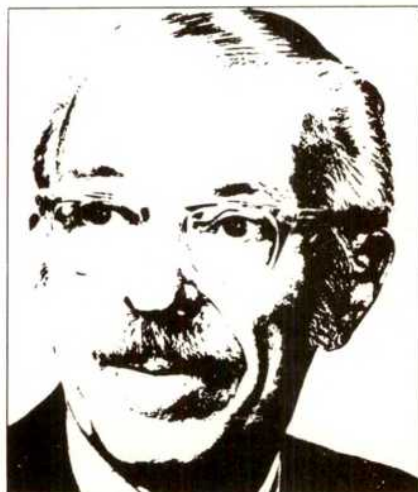
A broad-ranging 51-page report from the UK Health and Safety Commission, published this week, details the work of the Commission and its Executive between their establishment in October 1974 and January 1975 respectively and March last year. The Commission emphasises that legislation can play only a part in achieving the objectives of the Health and Safety at Work Act, and indicates in its discussion of future strategy what more can be done.

THE field of molecular evolution deals largely with comparisons of the sequences of 'informational macromolecules'—DNA, RNA and proteins. The first sequences that were available were of proteins: cytochromes *c* and haemoglobins, from different animals. The story is now familiar: the number and location of differences in corresponding amino acids were roughly proportional to the taxonomic separation of two species. For example, human and rhesus monkey haemoglobins differ in 12 locations; human and horse haemoglobins in 42; rhesus monkey and horse in 43. The differences between human and horse are not all in the same places, or of the same kind, as the differences between monkey and horse, thus showing that the three species have followed separate evolutionary pathways. These findings expanded and accumulated for various families of proteins, and were extended into comparisons between insects, green plants, yeasts and moulds. Various authors constructed large 'phylogenetic trees' based on such findings. It became evident that a 'molecular evolutionary clock', ticking away through the aeons, left its imprint in terms of divergence between sequences. The clock did not always run at the same rate, but, in the main, the divergences roughly corresponded to the passage of time as judged by other criteria, such as the fossil record.

But wait! The differences between proteins were merely the expression of changes in the base sequences of

'codes'. Quite often, two to four DNA molecules as reflected by the genetic code. And the genetic code, or amino acid code, is ambiguous in certain respects, because 18 of the 20 amino acids have from two to six

Molecular evolution



THOMAS H. JUKES

codes for the same amino acid differ only in the third base of the nucleic acid codon that specifies the amino acid. So a change of one amino acid, as measured in a protein, could reflect more than one change in a gene. Various 'educated guesses' were proposed to calculate the actual numbers of such unknown changes.

The discovery of procedures for

rapidly determining the sequences of long segments of DNA molecules is going to revise all this. One such method came from Sanger's group at Cambridge, and, more recently, the field has been galvanised by a chemical procedure devised by Maxam and Gilbert at Harvard. Such sequences can be matched against a protein sequence, even a partial one, and the exact code for each amino acid can be 'read off'. Furthermore, comparisons of DNA sequences obtained from homologous regions in related organisms tell precisely which nucleotides have undergone change during the period of evolutionary separation. There are preliminary indications that the synonymous 'third bases' of codons may undergo such changes more frequently than the first two bases, which specify amino acids.

New techniques, such as automated Edman degradation, are improving the accuracy of measuring amino acid sequences in proteins. A sequence between the 23rd and 30th residues in cytochrome *c* of *Neurospora crassa*, previously listed as -Gly-Glu-Gly-Gly-Asn-Leu-Thr-Gln- was found to be -Thr-Leu-Glu-Glu-Gly-Gly-Asn-. This was a bit of a shocker, and it called for some revision of evolutionary comparisons, but it is probably an extreme case. Other re-evaluations, and an expansion of ideas on evolution, will stem from forthcoming studies of DNA molecules. These studies should shed new light on the question of 'neutral' or 'near-neutral' evolutionary changes that occur in genes.

news and views

Molecular basis of plant tumour induction

from James A. Lippincott

THE long sought tumour-inducing principle (TIP) produced by the bacterium *Agrobacterium tumefaciens* and responsible for the transformation of normal plant cells to tumour cells (crown gall) seems now to be largely identified. This bacterium is known to cause transformation in a wide variety of plants when it infects wounded areas. Unlike cells of the nitrogen-fixing nodules induced by rhizobia, however, these tumour cells do not contain bacteria, and the bacterium needs to be present for only hours to a few days to induce tumours which will continue to proliferate in their absence. This growth autonomy is maintained in tissue culture where the tumours do not require hormones for growth, as do most normal tissues. The tumours also continue their characteristic growth when the bacteria-free cultures are grafted on normal plants. This evidence suggested that a TIP was produced by the bacterium and could alter the growth and developmental potential of plant cells in an essentially permanent fashion.

A major break in the problem came with the demonstration that tumorigenic strains of *Agrobacterium* carried a large ($>110 \times 10^6$ daltons) circular extrachromosomal DNA element or plasmid, whereas several non-tumorigenic strains did not (Zaenen *et al. J. molec. Biol.* **86**, 109; 1974). One strain of *Agrobacterium* readily loses virulence when grown at 37 °C (Hamilton & Fall *Experientia* **27**, 229; 1971) and this was shown to be accompanied by plasmid loss (Van Larebeke *et al. Nature* **255**, 742; 1974; Watson *et al. J. Bact.* **123**, 255; 1975). Genetically marked non-virulent strains of *Agrobacterium* had been found by Kerr (*Nature* **223**, 1175; 1969; *Physiol. Plant Pathol.* **1**, 241; 1971) to

acquire virulence after several weeks when co-inoculated on plant hosts along with virulent bacteria. This gain of virulence was also shown to depend on the plasmid and results from the transfer of a plasmid *in planta* from the virulent to the non-virulent strain (Watson *et al. op. cit.*, Van Larebeke *et al. Nature* **255**, 742; 1975). The necessity of the plasmid for tumorigenicity was thus firmly established. More conventional methods of plasmid transfer have since been developed and results from these tests fully support the initial findings (Levin *et al. J. Bact.* **127**, 1331; 1976; Chilton *et al. Genetics* **83**, 609; 1976; Kerr *et al. Nature* **265**, 560; 1977; Genetello *et al. Nature* **265**, 561; 1977).

Direct evidence that the *Agrobacterium* plasmid is the TIP or at least a major essential element of this concept has now been obtained. Chilton *et al. (Cell* **11**, 263; 1977) have isolated DNA from normal and crown gall tumour tissue cultures and hybridised it with different restriction nuclease fragments of the *Agrobacterium* plasmid. Most plasmid fragments do not hybridise with either normal or tumour DNA. Two plasmid pieces, however, hybridise with tumour DNA but not normal DNA, indicating that the tumour cells have acquired plasmid DNA sequences as a consequence of the transformation process. About 20 copies of a $3.7-6 \times 10^6$ dalton plasmid segment are estimated to be present in each tumour cell, sufficient information to code for about 10 different proteins.

Further support for these results, obtained by hybridising RNA isolated from tumour cells and normal cells with nuclease fragments of the plasmid, is reported by Drummond *et al.* in this issue of *Nature* (page 535). RNA transcripts of one plasmid segment are found in the tumours but not in normal tissues and this segment is the same as one detected by DNA-DNA

hybridisation experiments. This transcription of the transposed plasmid segment by the plant tumour cells suggests that it codes for one or more proteins which could have functional significance in inducing and/or maintaining the tumour syndrome. A major role of the bacterial plasmid, therefore, is to contribute a segment of new genetic information to the plant cell, and the translation of these plasmid genes in the plant, as suggested by the RNA transcripts, may be essential to the process. In many respects, therefore, the crown gall transformation process seems to parallel the events which occur in the induction of animal tumours by viruses.

Several traits in addition to that essential for virulence have been localised on the *Agrobacterium* plasmid. The ability of the bacterium to utilise either octopine or nopaline, unique N^α substituted derivatives of arginine, was shown to be highly correlated with virulence (Lippincott *et al. J. Bact.* **116**, 378; 1973) and loss or gain of the plasmid is accompanied by corresponding changes in utilisation of these compounds (Watson *et al. op. cit.*; Van Larebeke *et al. op. cit.*). Typically, the tumours synthesise one or the other of these arginine derivatives and the derivative synthesised is the same as can be utilised by the strain which induced the tumour (Petit *et al. Physiol. Veg.* **8**, 205; 1970; Bomhoff *et al. Molec. gen. Genet.* **145**, 177; 1976). A second plasmid gene is responsible for the ability to induce tumour synthesis of these compounds (Klapwijk *et al. J. gen. Microbiol.* **96**, 155; 1976; Montoya *et al. J. Bact.* **129**, 101, 1977).

This remarkable ability of agrobacteria to induce plants to synthesise compounds which the bacteria can utilise specifically as both a carbon and nitrogen source may well provide the *raison d'être* of this pathogen-host relationship. The finding that plasmid transfer

is greatly promoted *in vitro* by octopine (Kerr *et al. op. cit.*; Genetello *et al. op. cit.*) suggests that these compounds function similarly in the bacterium-tumour complex, promoting the plasmid exchange observed here. The tumours may thus provide both the stimulus and the nutritional requirement to accomplish gene transfer in the agrobacteria. It is also proposed that the enzymes responsible for octopine or nopaline synthesis in these tumours might be coded by bacterial genes (Petit *et al. op. cit.*) and recent plasmid results suggest that one of the plasmid genes which is transcribed in the tumour could be involved. No agreement exists, however, as to whether these arginine derivatives are normal plant products or are present only in these tumours.

Exclusion of *Agrobacterium* phage AP1 (Van Larebeke *et al. op. cit.*) and, in some nopaline-utilising strains, sensitivity to a unique bacteriocin of *Agrobacterium* strain K84 (agrocin 84, Roberts *et al. Nature* **265**, 379; 1977) is determined by the plasmid (Engler *et al. Molec. gen. Genet.* **138**, 345; 1975; Watson *et al. op. cit.*). Selection for resistance to agrocin 84 frequently results in plasmid loss or a deletion of portions of the plasmid essential for virulence. This has found practical application through use of strain K84 to control *Agrobacterium* infections in horticultural conditions (New & Kerr *J. appl. Bact.* **35**, 279; 1972). The plasmid also carries a gene which determines the ability of the bacterium to adhere to infection sites, an essential step in tumorigenesis (Whatley, dissertation, Northwestern Univ; 1977).

At least two plasmid genes seem to be functional in the transformation process in addition to adherence, because certain non-virulent strains which also carry a plasmid can increase tumour initiation when inoculated with virulent bacteria in conditions where bacterium-to-bacterium plasmid transfer is not observed (Lippincott *et al. J. Bact.* in the press). Indirect evidence suggests that the virulent bacteria produce or induce a diffusible product other than the intact plasmid which permits tumour initiation by the non-virulent strain. Obviously, much has to be learned about the nature of the genes localised on that part of the plasmid which is replicated in the tumours, as well as the genes and functioning of the much greater part of the plasmid which is excluded. The need for such a large plasmid is in itself an enigma. The possibility that *Agrobacterium* chromosomal genes are also directly involved in tumorigenesis is neither proven nor disproven.

Tumorigenic agrobacteria may now be counted the first cellular organisms demonstrated to accomplish in nature

the genetic engineering feat of transfer and stable incorporation of foreign DNA into a eukaryotic cell. These newer results also provide the first convincing indication that DNA from a prokaryotic cell may contain the appropriate base sequences to permit replication, transcription and possibly translation by the normal cellular machinery of eukaryotic cells. While important details of this transforma-

tion have yet to be established, the exciting prospects of genetic engineering for the production of beneficial changes in crop plants assures that they will be forthcoming. The transfer of nitrogen-fixing genes of *Rhizobium* to the closely related agrobacteria and subsequently to various plant hosts is but one example of the possibilities which are already receiving attention (*Science* **196**, 640; 1977). □

Are galactic γ rays point sources or diffuse emission?

from J. J. Quenby

A WEALTH of positive results from the SAS 2 and COS B satellites has firmly established the science of γ -ray astronomy, although as recently as 1970 the usefulness of making comprehensive searches for these high energy cosmic photons was actively debated. However, while the information acquired is clearly significant, what is still not clear is exactly which branch of astrophysics is being served by the measurements. A letter by Hermesen *et al.* in this issue of *Nature* (page 494) illustrates the difficulties involved.

γ -Ray emission is expected from the galactic disk by virtue of the interaction of cosmic rays with the residual matter in the interstellar medium. Although these charged, relativistic, cosmic ray particles may fill an extended galactic halo region (see *Nature* **268**, 401; 1977), it is only in the galactic disk that sufficient hydrogen atoms, at a density of about 1 cm^{-3} , are encountered for detectable γ -ray emission to occur. The π^0 mesons arising from the cosmic ray interactions decay into two γ rays producing a broad spectrum of photons. Any variation in the galactic longitude distribution of the resulting emission from the Milky Way should yield information on the spatial distribution of cosmic rays, provided the matter density is well established from other astronomical evidence.

The surprise arising from the earliest detection of γ rays from near the galactic centre by Kraushaar *et al.* (*Astrophys. J.* **177**, 341; 1972) on board OSO-111 was the unexpectedly high intensity, implying that cosmic rays are much more numerous at the centre than far out in the disk, near the Sun.

J. J. Quenby is in the Blackett Laboratory at Imperial College, London.

Maybe there are more cosmic-ray sources at the centre of the galaxy, but particle diffusion along the spiral arm magnetic fields needs to be well constrained to maintain this apparent gradient in number density. The SAS 2 γ -ray detector provided the first comprehensive survey of the galactic plane emission (Fichtel *et al. Astrophys. J.* **198**, 163; 1975) and the results show a reasonable correlation of γ -ray intensity with matter density in the spiral arms lying between us and the galactic centre. Furthermore, some of the discrepancy between the observed flux and that expected if cosmic rays were uniformly distributed in the Galaxy has been removed by including in the matter density the amount of molecular as opposed to atomic hydrogen, as estimated in an analysis performed by Scoville and Solomon (*Astrophys. J.* **187**, L67; 1974). However, an increase of a factor of two in the cosmic ray intensity is still needed in the inner spiral arm regions (Stecker *Phys. Rev. Lett.* **35**, 188; 1975). Moreover, at the Cosmic Ray Conference this August, several workers were concerned whether or not cosmic rays could actually easily penetrate the expected high magnetic fields within the dense clouds where molecular hydrogen is important (Cesarsky *et al.* and Strong *et al. Plovdiv C.R. Conf.* **1**, 61, 55; 1977).

The ESA satellite COS B is devoted entirely to a γ -ray detector constructed by six West European groups and data from this instrument have now provided more detail on parts of the galactic disk longitude distribution of emission. A particular feature of the results is the number of 'point' sources revealed in an incomplete survey with a 2.5° angular resolution. In 160° of longitude, Hermesen *et al.* find 13 sources,

10 of which are COS B discoveries. Two sources, the Crab and Vela, are known pulsars in supernovae remnants, one is possibly the strong X-ray source Cyg X-3, but the other 10 have either a weak or no association with soft X-ray sources or radio pulsars. They may be point-like or emission regions up to 2° wide. One may be associated with a source of hard X-rays seen by Ariel 5 (Coe *et al.* *IAU Circ.* No. 3003, 1976). Hermsen and colleagues stress that the γ -ray sources contribute significantly to the overall γ -ray luminosity of the Galaxy. Indeed, as pointed out by Strong *et al.* (12th *ESLAB Symposium, Frascati*, 1977), removal of point sources leaves very little evidence for a diffuse galactic emission from and beyond the Perseus arm, just outside 10 kpc from the galactic centre. It is tempting to conclude from the much increased fine structure seen in the COS B γ -ray intensity distribution that point source emission dominates and that little information on galactic cosmic ray motion can be gained by study of the disk photon emission.

Attacking the problem from the other end of the spectrum, the

Imperial College group have suggested that at least 8 (maybe >16) galactic hard X-ray sources may plausibly be extrapolated into the γ -ray region to account for a significant amount of the local γ -ray intensity (Plovdiv *C.R. Conf.* 1, 152; 1977). However, conventional accretion models for X-ray sources involving essentially thermal heating processes have difficulty in explaining γ -ray emission and a young pulsar model requiring electromagnetic acceleration would seem to be necessary, as invoked by Lamb *et al.* (*Astrophys. J.* 212, L63; 1977) to explain possible γ -ray production in Cyg X-3.

Until the contribution of point sources to the galactic disk emission is resolved, γ -ray astronomers cannot know whether they are chiefly contributing to knowledge of cosmic ray motion in the Galaxy or studying an important class of high energy photon sources involving non-thermal particle acceleration. Better angular resolution and sensitivity throughout the hard X-ray and γ -ray detector range is required to resolve this particular problem. □

3C 273 has an optical jet, a detached radio source (A) and a central source in which the radio object 3C 273 B has at least three components with milli-arc s diameters. Recent long baseline interferometer measurements (Legg *et al.* *Astrophys. J.* 211, 21; 1977) show that the radio variability is concentrated in one of these three components, on the NE (anti-jet) side of the nucleus. Assuming that the radio emission is synchrotron, the magnetic field in this component appears to be about 10^{-2} gauss (10^{-6} T). This field is very low in comparison with the stellar surface fields usually responsible for circular polarisation and it seems unlikely that this variable radio source can be the origin of circularly polarised optical emission. This must instead be related to the nucleus where much stronger magnetic fields might be encountered. Presumably the linear polarisation which is now observed to vary also originates in the nucleus.

The interesting aspects of the observations now reported are the rapidity of the variations both at radio and optical wavelengths, and the connection they seem to give between the NE radio component and the nucleus. If the results can be confirmed they should give a more coherent picture of the way in which the nucleus is pumping energy into the outlying sources, which is a crucial question in the origin of the extended radio sources frequently associated with quasars and radio galaxies. □

Polarisation in quasars

from F. Graham Smith

THE famous quasar 3C 273, which was the first to be shown to be both a radio source and an optical object with large red-shift, has been studied by every conceivable technique at radio, infrared, optical and X-ray wavelengths. It has spatial structure, a complex spectrum and polarisation, all of which vary with time scales between days and years. The physical processes behind these variations are practically unknown, and observers must sometimes despair of the possibility of making the key observation which will lead to an unravelling of the complex knot of behaviour. The paper from the Crimean Observatory (in this issue of *Nature*, page 493) puts a little order into the great variety of behaviour by showing that some of the radio and optical variations are related. The work has involved measurements of polarisation, both optical and radio, which have been difficult to make and which were for some time in doubt.

Variations in radio flux from 3C 273 were well known in 1966. They are most marked at short wavelengths: at 40 cm not much happens, but at 2 cm the flux density varies by a large factor. The spectra of several quasars

show these variations, which indicate an activity close to the core of the quasar, giving a rapid rise in radio emission first at short wavelengths and later, with less intensity, at longer wavelengths. Aller and Haddock at Michigan found that these variations were accompanied by changes in radio polarisation.

Optical polarisation was naturally searched for at this time, and indeed there was already a positive result by W. Liller in 1963, who reported verbally to the American Astronomical Society. Appenzeller concluded in 1968 that there was 0.26% linear polarisation, probably of interstellar origin. Results from the 2.6 m Crimean telescope then suggested the existence of circular polarisation, but at such a low level that the techniques were questionable. The observations were made at the coudé focus after several reflections, which easily create spurious polarisation. In 1972 these observations were restarted at the Cassegrain focus, with a more symmetrical optical system and checks with stars with well established circular polarisation showed a measuring accuracy of 0.01%. Both radio and optical observations of polarisation are achieving truly remarkable sensitivity.

F. Graham Smith is Director of the Royal Greenwich Observatory.

Electrophysiology of the hypothalamus

from J. L. Barker

A symposium on the Electrophysiology of the Hypothalamus was held at Hemingford Grey House in Cambridge on 24–26 July in conjunction with the 27th International Physiological Congress in Paris. It was organised by R. Dyer (ARC Cambridge) and R. Dyball (ARC Cambridge).

THE hypothalamus is associated with many bodily functions including autonomic reflexes, feeding and sexual behaviour, neuroendocrine control of anterior pituitary activity, thermoregulation and probably, metamor-

J. L. Barker is in the National Institute of Neurological and Communicative Diseases and Stroke, National Institutes of Health, Bethesda, Maryland.

phosis and hibernation. The symposium produced illuminating discussion on the electrophysiological basis of these involuntary activities crucial to the survival of the animal.

The initial aim of those in the field has been to correlate specific circuits with specific functions. However, unlike the cerebellum, cortex and hippocampus, where considerable circuitry has been delineated but where function remains unclear, the hypothalamus is replete with many short-range neurones and complex connectivity, making simple correlation of excitability with specific functions exceedingly difficult. In spite of this many of the contributors to the symposium attempted this correlation.

Those investigations focused on the magnocellular nuclei in the hypothalamus have achieved some measure of success, owing to the apparent homogeneity of magnocellular neurones and their dominant projection to the posterior part of the pituitary. Two populations of neurosecretory cells in these nuclei synthesise, transport and secrete vasopressin and oxytocin into the peripheral circulation to regulate plasma osmolality and various types of smooth muscle contractility. The long projections of the cells have been used to locate the parent cell bodies in the nuclei, since electrical stimulation of the terminal causes 'antidromic' activation of the cell bodies, thus indicating whether the neurone recorded from projects to the posterior pituitary.

Significant contributions to the physiology of these neurosecretory cells have been derived from experiments using natural stimuli. These studies show that one population of neurones is induced to fire in a characteristic 'phasic' manner following circulatory compromise while another group is activated by suckling pups (J. D. Vincent and D. Poulain, University of Bordeaux; Dyer, Dyball and J. B. Wakerley, ARC Cambridge; D. W. Lincoln, University of Bristol; J. J. Dreifuss, Ecole de Médecin, Geneva; H. Yamashita, State University of New York, Brooklyn). The former are assumed to be vasopressin-secreting neurones, while the latter are considered to be oxytocin-secreting cells, because the two different stimuli have been shown to release in a specific and independent manner each of the two neurohormones. Thus, two populations of neurones in the magnocellular nuclei have been tentatively identified by their electrophysiological responses to natural stimuli. The presumed vasopressin cells seem to generate phasic activity independent of each other, while the presumed oxytocin neurones fire high-frequency bursts in a synchronised manner. Not yet established is whether the phasic firing pattern is

a latent, endogenous property of these cells which is activated with appropriate stimuli, as happens in a variety of invertebrate neurosecretory cells, or whether the pattern is synaptically derived. Likewise, the synchronised burst of oxytocin neurones may represent electrical coupling among the elements, as occurs in some invertebrate neurosecretory cells, or it may reflect common synaptic input. Definite relationships between hormone release and electrical activity were demonstrated by Dyball, Lincoln and K. Boer (Netherlands Institute for Brain Research, Amsterdam), all of whom showed that clustering of spike activity into phases or bursts produces considerably more neurohormone output than random, unclustered spike activity. Some clue to membrane mechanisms underlying this facilitated, pulsed release is suggested by studies on invertebrate neurosecretory cells which show that the conductance responsible for spike repolarisation accommodates during bursts (J. L. Barker and T. Smith, National Institutes of Health, Bethesda), thus progressively prolonging individual spikes in a burst and allowing facilitated entry of Ca^{2+} and release of neurotransmitter or neurohormone. Pulses of oxytocin are apparently necessary for lactation, since a constant, low level of hormone is ineffective.

Although considerable effort has been expended on the parvocellular elements in the hypothalamus, most notably by L. P. Renaud (McGill University, Montreal), R. L. Moss (University of Texas, Dallas), B. Dufy (University of Bordeaux), M. C. Harris (University of Birmingham) and K. Yagi (Jichi Medical School, Tochigi-Ken), definite conclusions regarding the roles of these cells in hypothalamic function have yet to be made. This impasse derives mainly from the interconnectivity of the cells, as well as a lack of systematic identification of recorded cells by combined marker stains and immunohistochemistry techniques. The complexity and lack of clear organisation among these hypothalamic elements has caused immense difficulties in specifying the circuits and electrophysiological bases of thermoregulation, sex, autonomic and nutritive behaviour. Some progress has been made by E. T. Rolls (University of Oxford) who has demonstrated hypothalamic units specifically responding to visual stimuli conveying information about food. Mapping of those units conveying information about central and peripheral temperature is being done by those interested in thermoregulation, including R. F. Hellon (National Institute for Medical Research, London), D. Ford (St Bartholomew's Hospital, London),

J. A. Boulant (Ohio State University, Columbus), and J. T. Stitt (Yale University, New Haven). Another line of research, using the push-pull cannula method to apply to, and sample from the hypothalamus various neuroactive substances, has revealed some of the substances released during specific behaviour (R. D. Myers, Purdue University, West Lafayette).

One may conclude from this symposium that the understanding of the cellular basis of assorted hypothalamic functions is fragmentary at best, and that although definite progress has been made, far more research, making use of natural stimuli and multidisciplinary approaches, is required to begin to unravel what is certainly an immensely complex area of the nervous system. □

Identifying carcinogens

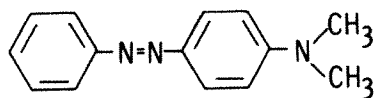
from Alastair Hay

An Ecotoxicology Workshop was held at the University of Surrey from 11 July to 5 August under the auspices of the Eco-Sciences Panel of NATO. The third week of the workshop was taken up by an International Symposium on Industrial Toxicology—the seventh such meeting held at the University of Surrey.

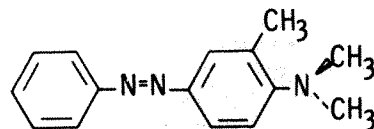
SOME of the problems in identifying possible carcinogens were exemplified during the workshop by work presented on the azo dye butter yellow (I) and the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) released in the Séveso accident. Butter yellow is a known liver carcinogen whereas TCDD is as yet only under suspicion (see *Nature* **258**, 2; 1975) but reports of the behaviour of these chemicals in the tests currently used to identify potential carcinogens well illustrate the problems involved.

Because animal studies are expensive and take a long time much work has been done on developing reliable short-term tests for initial screenings. But in view of the large number of compounds jockeying for attention, pre-selection of chemicals to be submitted to the screen on the basis of structural and physicochemical resemblances to known carcinogens is an attractive proposition. This is the approach advocated by a group of workers at ICI and

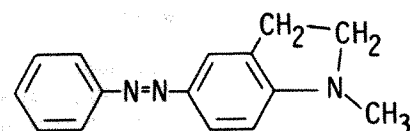
Alastair Hay is a Research Fellow at the Zoological Society of London.



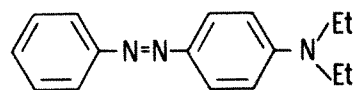
(I)



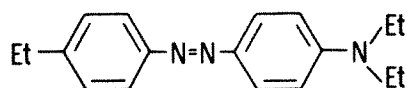
(II)



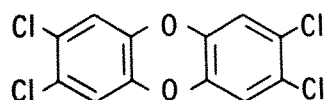
(III)



(IV)



(V)



(VI)

at the workshop John Ashby and his colleagues (ICI, Alderly Park) reported experiments testing the effects of structural modifications of butter yellow on its performance in one of the short-term tests.

Butter yellow gives a predominantly negative response in the Ames test (which tests the ability of a chemical to cause mutations in bacteria). It does, however, give a reproducible positive response in the Styles cell transformation test (*Br. J. Cancer*, in the press). The 3-methyl analogue (II) reported to be non-carcinogenic in animal studies proved negative in this test as well, as might be expected on theoretical grounds. The 3-methyl group forces a change in the angle of the NMe_2 group with respect to the benzene ring. This would reduce or abolish the stability of the nitronium ion thought to be involved in the binding of compounds such as (I) to DNA (Lin *et al. Cancer*

Res. **35**, 844; 1975). To check the importance of the angle change in the carcinogenic deactivation of (II) Ashby *et al.* synthesised and tested the indoline (III). Here the overall substitution pattern is the same, but by joining the 3-methyl group to one of the NMe_2 groups planarity between the nitrogen base pair of electrons and the benzene ring is restored.

In the Styles assay (III) was positive. So far no derivatives of the indoline have been tested *in vivo* for carcinogenicity, but these results suggest that it might be a liver carcinogen. If this turns out to be true it would strengthen the theoretical basis for the difference in activity between (I) and (II).

Curiously the NEt_2 analogue (IV) of butter yellow is reported to be non-carcinogenic, whereas the 4'-ethyl analogue (V) is a carcinogen. The small structural change involved in the addition of an ethyl group to (IV) would hardly be expected to reintroduce carcinogenicity if a fundamental principle were involved in the activity of (IV). Needless to say both (IV) and (V) were positive in the Styles assay. This suggests that either (IV) has been inadequately tested in animals for carcinogenicity, or that perhaps some nonspecific and secondary mechanism such as its solubility, or transport into the cell prevented it from producing tumours *in vivo*. The Styles assay clearly predicts (IV) as a potential carcinogen, and surely represents an efficient short-term test. But the Styles assay does fail to predict β -naphthylamine as a carcinogen whereas this compound produces a positive response with the Ames test. Clearly both tests are necessary for a screening net of finer mesh.

TCDD presents a more complex problem. It is a planar tricyclic aromatic compound (VI) said to be chemically similar to many polycyclic hydrocarbon carcinogens which bind to DNA directly or through the formation of a metabolite (Poland & Kende *Fedn Proc.* **35**, 2404; 1976), or to the acridines which intercalate in DNA (Hussain *et al. Ambio* **1**, 1; 1972). In fact the chemistry of TCDD is not like that of the known carcinogens. Whereas the carbon atoms which bridge the benzene rings in the tricyclic aromatic carcinogens form part of a conjugated bond system, this is not true of the oxygen atoms in TCDD. The oxygens do not conjugate to the benzene rings. From a structural point of view, therefore, TCDD would not be expected to be a carcinogen. The possibility exists, however, that ring epoxidation may occur *in vivo* to convert it into a potential carcinogen. But the metabolism of TCDD, if it does occur, is very slow (Piper *et al. Environ. Health Perspect.* **5**, 241; 1973).

From the evidence available it seems that TCDD is not mutagenic in all bacterial systems. It is reported to be mutagenic in *Escherichia coli* Sd-4 (Hussain *et al., op. cit.*) and *Salmonella typhimurium* TA 1532 (Hussain *et al., op. cit.*, Seiler, *Experientia* **29**, 662; 1975) but not in the *S. typhimurium* strain TA 1530 (Hussain *et al., op. cit.*). Ames and Yamasaki (quoted in Poland & Kende, *op. cit.*) were unable to demonstrate reversion of specific mutant strains of *S. typhimurium* by TCDD treatment.

The only animal study to test the carcinogenic potential of TCDD so far reported is that of Van Miller and Allen (*Fedn Proc.* **36**, 396; 1977). These authors report that in rats TCDD causes carcinomas of the kidney, liver and skin as well as angiosarcomas. This is a disturbing claim which urgently requires confirmation. It is fortunate, therefore, that two other animal studies are nearing completion—those of the Dow Chemical Company and US National Cancer Institute. Their results are awaited eagerly.

It should perhaps come as no surprise that the clinical data on TCDD's potential mutagenic or carcinogenic properties are mixed. G. Reggiani (Hoffmann-La Roche, Basle) reviewed the clinical picture of 800 cases of presumed TCDD exposure in workers—all of whom developed chloracne—exposed to the chemical during the production of 2,4,5-trichlorophenol. The frequency of chromosomal abnormalities in these workers and their families is no greater than the statistical norm. As for the incidence of cancer, the evidence from two factory studies at the Spolana plant (Czechoslovakia) and BASF site (Germany) suggests that the rate is not increased in workers exposed to the dioxin.

Claims to the contrary come from Vietnam. An increase in liver cancer in individuals exposed to the herbicide 2,4,5-T in which TCDD is present as a contaminant may be attributable to the dioxin (Tung reported in *Galston Nature* **258**, 2; 1975). A preliminary study by Tung *et al.* (*Vietnamese Studies* **29**, 53; 1971) also reports an increased incidence of chromosomal abnormalities in the victims of herbicide spraying. The Vietnamese study has been criticised (*Effect of Herbicides in South Vietnam*, National Academy of Sciences, 1974) as being statistically inadequate. It is being repeated, but on a larger scale.

G. Tognoni (Mario Negri Institute, Milan) found the Vietnamese reports disturbing. Although no evidence of chromosomal abnormalities has so far been observed in the Séveso population he was far from optimistic about their long term health prospects.

There is obviously an urgent need

to resolve the question of whether or not TCDD is a potential carcinogen. The Van Miller and Allen study is the only one so far which claims that it is. Their claim needs to be supported by experiments in other animal species. Other short term tests could also be applied; the results from these could perhaps clear up the conflicting evidence. □

Degradative plasmids

from J. R. Saunders

MEMBERS of the bacterial genus *Pseudomonas* are renowned for their capacity to utilise an enormous range of compounds as energy and carbon sources. Over the past few years it has become clear that the ability of these organisms to degrade certain exotic substrates is specified by genes borne on plasmids. A variety of such 'degradative plasmids', some of which are self-transmissible, has been identified (see Chakrabarty *A. Rev. Genet.* **10**, 7; 1976). Thus plasmids can confer on host pseudomonads the ability to break down naphthalene (NAH plasmid), salicylate (SAL plasmid), camphor (CAM plasmid) *n*-octane (OCT plasmid) and toluene and xylenes (TOL plasmid).

TOL plasmids carry the genes encoding the degradation of benzoate and *m* and *p*-toluates by way of the *meta* (α -ketoacid) pathway in *P. putida*. A functional *meta* pathway is also specified by other degradative plasmids. It involves a single set of enzymes allowing growth on a wide range of hydrocarbons, aldehydes, alcohols and carboxylic acids, all of which may present themselves as potential sources of nutrition in the wild. Furthermore, the location of the genetic determinants for the *meta* pathway on plasmids increases versatility by assisting the spread of the degradative trait in the bacterial population. In common with many other saprophytic pseudomonads, *P. putida* possesses an alternative route for dissimilation of benzoate, the *ortho* (β -ketoadipate) pathway. The *ortho* pathway is chromosomally determined and like the *meta* pathway initially involves the oxidation of benzoate to catechol. Subsequently the pathways diverge: the *ortho* pathway requires the conversion of catechol to *cis-cis* muconate by catechol 1,2 oxygenase, the product in this case being the inducer for the enzyme. In contrast benzoate itself probably initiates expression of the *meta* pathway by inducing catechol 2,3 oxygenase which

converts catechol to hydroxymuconic semialdehyde. Consequently when the genes for both pathways are present, as in a Tol⁺ strain of *P. putida*, benzoate is preferentially degraded by the plasmid-coded *meta* pathway and not the chromosomal *ortho* pathway. way.

Ironically Mike Worsey and Peter Williams (*J. Bact.* **130**, 1149; 1977) have found that some strains of *P. putida* grow faster on benzoate when using the *ortho* pathway. Thus growth of such strains on benzoate exerts a selective pressure for loss of Tol function. This may be by elimination (curing) of the entire 78 Mdal TOL plasmid or by deletion from the plasmid of a specific segment of about 27 Mdal which encodes all or part of the TOL pathway (Bayley *et al. Molec. gen. Genet.* **154**, 203; 1977). Growth on benzoate can also select for strains which have an intermediate (B3) phenotype. These can still grow on toluene, *m*-xylene and benzoate but are incapable of using *m*-toluate. Strains of the B3 phenotype apparently possess a regulatory mutation which blocks induction of *meta* pathway enzymes by benzoate and *m*-toluate but enables induction by toluene and *m*-xylene. It is envisaged that early enzymes for utilisation of *m*-toluate and benzoate are positively controlled by two separate regulators, one of which interacts with the inducers toluene and *m*-xylene, whereas the other (lacking in strains of B3 phenotype) interacts with benzoate and *m*-toluate. Further studies on the genetic regulation of this and other degradative pathways promise to be of great interest.

Peter Williams and coworkers at the University of Wales, Bangor, in collaboration with Paul Broda's group at the University of Edinburgh have also characterised the plasmids in thirteen Tol⁺ pseudomonads isolated from soil samples in different geographical locations (Duggleby *et al. J. Bact.* **130**, 1274; 1977). They have assigned the TOL genes to a number of plasmid species in different organisms. Interestingly five of these plasmids are indistinguishable on the basis of their molecular weights (all about 77 Mdal) and gel electrophoresis profiles after endonuclease digestion. One member of this apparently homologous series of plasmids was resident in a strain isolated in Japan whereas the other host strains were found in Wales. This implies widespread distribution of a single TOL plasmid. Although worldwide spread of particular R plasmids is well documented and explicable in terms of international travel and human contact, it is harder to see how a single degradative plasmid could become broadcast amongst the population of soil bacteria. One possibility

would be the spread of carrier strains by large scale import and export of industrial materials such as oil products contaminated with soil bacteria.

Degradative plasmids obviously contribute greatly to the already substantial nutritional versatility of *Pseudomonas* species. Many of the compounds degraded under the control of such plasmids would otherwise be toxic to other forms of life. Such degradation is therefore of some importance in detoxifying localised areas of pollution. Indeed it has been proposed that *Pseudomonas* strains carrying degradative plasmids be used to disperse oil spillages and render them harmless. Since most of the degradative plasmids belong to different incompatibility groups it is possible to construct pseudomonads which carry a battery of different degradative plasmids which together confer enhanced ability to utilise crude oil. Chakrabarty, who has been a prominent advocate of this approach, has pointed out that such strains would also have to harbour mercury-resistance plasmids to cope with the high levels of the heavy metal in oil residues.

The deliberate introduction of such constructed strains into the environment is open to the same kinds of objections as those levelled against recombinant DNA research. Such ventures could rebound if for example they led to the breakdown of petroleum products in storage tanks or the induction of pathogenic characteristics in previously harmless saprophytic species. However initial studies on the 'epidemiology' of TOL plasmids indicate that the population of soil bacteria has itself already responded to the increasing load of organic pollutants that has leaked into the environment. Selective forces, analogous to those that have led to the spread of antibiotic resistance in clinically important bacteria, are probably operating to produce bacteria that are resistant to and able to degrade industrial pollutants. In this case the consequences for mankind should be beneficial. □



A hundred years ago

At the Guy's Hospital *conversazione*, on Monday evening, a new government filter, invented by Major Crease, was shown which reduced strong tea and infusions of logwood to clear tasteless water. The nature of the filtering material is not made known.

From *Nature* **16**, 48; 4 October; 1877.

review article

Thresholds and breakpoints in ecosystems with a multiplicity of stable states

Robert M. May*

Theory and observation indicate that natural multi-species assemblies of plants and animals are likely to possess several different equilibrium points. This review discusses how alternate stable states can arise in simple 1- and 2-species systems, and applies these ideas to grazing systems, to insect pests, and to some human host-parasite systems.

IN all but the most trivial areas of enquiry, there arise questions about the extent to which events are shaped by predictable natural laws as against the accidents of initial conditions and perturbations. Is the human story largely a deterministic tale of civilisations marching to Toynbee's tune, three and a half beats to disintegration, or did the hinge of history turn on the length of Cleopatra's nose? Such questions of the relative roles of chance and necessity¹ are fundamental in modern cosmology^{2,3}, in the foundations of statistical mechanics^{4,5}, and in evolutionary biology¹ and ecology, even though they may arise in less blatant and romantic fashion than the 'what ifs' of history and the social sciences.

Viewing the grand sweep of evolution, we can see many examples where the taxonomic details of the plant or animal that occupies a given niche at a given time and place depend on historical accident, but where the niches themselves, and the broad patterns of community organisation, are remarkably constant⁶⁻⁹.

Taking a much narrower and more local view, it is interesting to consider a particular assembly of species, with specified interactions among them, and to ask questions about the dynamics of the system. Is the dynamical behaviour described by the multi-dimensional generalisation of a single valley (a global attractor)? Or is the dynamical landscape pockmarked with many different valleys, separated by hills and watersheds? If the former, the system has a unique stable state, to which it will tend (like a marble seeking the bottom of a cup) from all initial conditions, and following any disturbance. If the latter, the state into which the system settles depends on the initial conditions; the system may return to this state following small perturbations, but large disturbances are likely to carry it into some new region of the dynamical landscape (so that the system behaves rather like the ball in a pin-ball machine). If there is a unique stable state, historical effects are unimportant; if there are many alternative locally stable states, historical accidents can be of overriding significance. Obviously, questions of this kind are very important in the understanding and management of ecosystems.

A large body of empirical observations shows that many natural communities have a multiplicity of stable states. Sutherland¹⁰ has demonstrated that for the fouling community (a complex assembly of hydroids, tunicates, bryozoans, sponges and associated species) at Beaufort, North Carolina, the order of larval recruitment determines the way the community develops. Reviewing other work on the marine rocky intertidal (see also the review by Levin¹¹), on coral reefs, on freshwater lakes, and on terrestrial plant communities, Sutherland concludes that community structure can often "be explained only by referring to specific historical events" and therefore that "multiple stable points are an undeniable

reality." A similar conclusion emerges from Connell's and Slatyer's¹² survey of mechanisms of succession in natural communities.

The view that complicated ecosystems possess many alternative stable states is also supported by theoretical studies of mathematical models that caricature such systems. From the growing number of possible examples, I mention only two, chosen from opposite ends of the spectrum. Austin and Cook¹³ have made computer studies of a system in which 94 species (embracing plants, herbivores and carnivores) are linked together by interactions that aim to be relatively realistic; the system has many equilibrium points, and is easily transferred from one to another. Case and Gilpin¹⁴ have explored a relatively abstract system, in which the coefficients in the interaction matrix for a n -species Lotka-Volterra model are assigned random values; if n is at all large, the system typically collapses to one or other of a variety of simpler systems with fewer species, and this final steady state depends on the initial population values. The notion of 'resilience' has been introduced by Holling¹⁵ in an attempt to characterise the degree to which a system can endure perturbations without collapsing or being carried into some new and qualitatively different state. Theoretical ideas about resilience, along with interpretive reviews of diverse other meanings that can be attached to 'stability' in an ecological context, are the subject of many recent papers¹⁶⁻²².

It is thus clear that real ecosystems possess multiple stable states, as do plausible mathematical models. Unfortunately, the complications inherent in multi-species systems almost invariably preclude any quantitative confrontation between theory and data. For multi-species communities, the empirical observations remain largely anecdotal, and the theory remains largely metaphorical.

For simple 1- and 2-species situations it is, however, beginning to become possible to put theory and observation together, to gain insight into the workings of systems with more than one stable state. This review is a synthesis of several examples of this kind. I begin with grazing ecosystems (and then, more generally, any harvested crop or animal population), go on to insect pest systems (particularly the spruce budworm), and conclude with some human host-parasite systems.

Grazing ecosystems

Consider^{23,24} a population of herbivores, maintained at a constant density H , and sustained by vegetation whose biomass is V .

Our interest is centred on the dynamics of the vegetation biomass. Following Noy-Meir²³, suppose that in the absence of grazing the growth rate of the vegetation as a function of V is $G(V)$, and that the herbivores consume the vegetation at a net rate $C(V)$ (corresponding to a per capita consumption at a rate $c(V)$: $C(V) = Hc(V)$). Then the rate at which V changes is given by

*Biology Department, Princeton University, Princeton, New Jersey 08540

$$dV/dt = G(V) - C(V) = G(V) - Hc(V) \quad (1)$$

The vegetation biomass will thus tend to settle to an equilibrium level where the natural growth rate exactly balances the loss rate due to grazing; that is, to a value of V such that $G(V) = C(V)$. Noy-Meir shows how these equilibrium V -values can be found graphically, for various assumptions about the way $G(V)$ and $C(V)$ depend on V .

Fig. 1 illustrates one plausible situation. Here, as shown by the solid curve, $G(V)$ is positive even when $V = 0$ (corresponding to some constant background contribution to the vegetation growth rate, for example from the sprouting of seeds blown in from other areas). At first $G(V)$ increases as V increases, but as V continues to increase $G(V)$ decreases as shading and competition for nutrients becomes important, until $G(V) = 0$ at $V = K$; K is the maximum stable biomass of ungrazed vegetation. The general shape of the per capita consumption function $c(V)$ is a saturation curve: at low V , the herbivore intake is limited by forage availability, so that $c(V)$ increases with increasing V ; at high V , $c(V)$ saturates to some constant determined by the animal's intake capacity or digestion rate. It is useful to christen V_0 as the characteristic value of V at which the consumption function saturates. The dashed curves for total consumption rate $C(V) = Hc(V)$ in Fig. 1 are for a $c(V)$ that increases linearly with V at small V , corresponding to a herbivore that searches randomly (Holling's "Type II" or "invertebrate" predator search pattern²⁵).

We see from Fig. 1 that for relatively small values of H there is a single equilibrium value for V , given by the point A where $G(V)$ and $C(V)$ curves intersect; this value is slightly less than K . For relatively large values of H , there is again a unique equilibrium value for V (corresponding to the point E), now at a low value of V . For intermediate values of H , the $G(V)$ and $C(V)$ curves intersect at three points. The points B and D correspond to locally stable V -values, whose domains of attraction are divided by the unstable equilibrium point corresponding to C; B and D are the bottoms of adjacent valleys, and C marks the watershed between them.

The essential feature that leads to two alternative stable states for intermediate H values in Fig. 1 is the assumption that $c(V)$ saturates to a constant for values of V significantly below the ungrazed equilibrium. That is, defining

$$\alpha = V_0/K \quad (2)$$

Fig. 1 is for α significantly less than unity. If α exceeds, or is of the

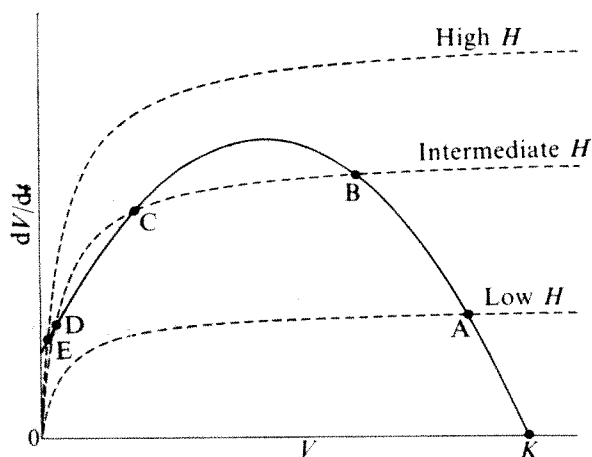


Fig. 1 The rate of change of vegetation biomass, dV/dt , is shown as a function of V . The solid curve is the natural, ungrazed vegetation growth rate (which here is finite even for $V = 0$). The dashed curves are loss rates due to grazing (of Type II pattern) at high, intermediate and low herbivore densities, H . Where the solid curve lies above the dashed one, the net growth rate is positive; where the solid curve lies below the dashed one, the net growth rate is negative; the points of intersection of the curves correspond to possible equilibrium points. For further discussion, see the text.

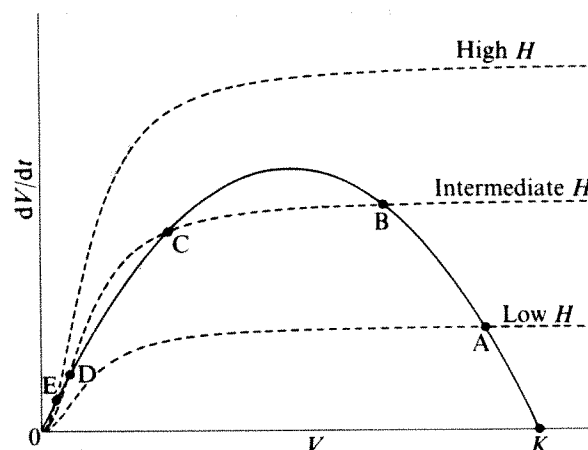


Fig. 2 As for Fig. 1, except that here the natural growth rate (solid curve) is linearly proportional to V at small V , and the loss rate due to grazing (dashed curves) is of Type III. Specifically, this figure corresponds to equations (3) and (4); the α of equation (2) is here $\alpha = 0.1$, and 'high, intermediate and low H ' are represented by $\gamma = 0.35, 0.22$ and 0.10 , respectively. The basic features shown by the figure are, however, generic.

order of, unity, there is always only one stable state in the figure corresponding to Fig. 1.

Fig. 2 illustrates another plausible circumstance, which differs from Fig. 1 in details but not in essentials. Here, as V increases, $G(V)$ increases from zero to some maximum value and then decreases back to zero at $V = K$. The per capita consumption function $c(V)$ is now for herbivores whose foraging efficiency increases faster than linearly with V at low V values, but which again saturates to a constant for V above some characteristic value V_0 (Holling's "Type III" or "vertebrate" predator consumption function²⁵, which is also typical of many invertebrates²⁶⁻²⁸). Provided that V_0 is significantly less than K (that is, α is small), we can again distinguish three domains of dynamical behaviour for the vegetation biomass: for small H there is a unique equilibrium value of V , slightly below K (the point A); for large H there is a unique equilibrium at a low V value (the point E); for intermediate H there are two alternative stable states (the points B and D), divided by an unstable point (C).

Specifically, Fig. 2 illustrates the situation where $G(V)$ is given by the familiar logistic equation, $G(V) = rV(1 - V/K)$, and where $C(V)$ is the "Type III" consumption function $C(V) = \beta HV^2/(V_0^2 + V^2)$. Then equation (1) has the particular form

$$\frac{dV}{dt} = rV \left(1 - \frac{V}{K}\right) - \frac{\beta HV^2}{V_0^2 + V^2} \quad (3)$$

This equation may equivalently be written in dimensionless form, by using the earlier definition of α , and introducing the rescaled variables $X = V/K$, $\tau = rt$ and $\gamma = \beta H/rK$

$$dX/d\tau = X(1 - X) - \gamma X^2/(\alpha^2 + X^2) \quad (4)$$

This equation can exhibit (D. Ludwig, D. Jones and C. S. Holling, to be published) two alternative stable states if, and only if, $\alpha < 1/\sqrt{3}$.

Figure 3 is derived from the situation depicted in Fig. 2, and shows the stable equilibrium value(s) of the vegetation V , as a function of the herbivore density H . For low H , the vegetation biomass tends to settle to a unique steady value, slightly below K . As H increases beyond a threshold value (T_1), a second stable state for V appears, in a discontinuous fashion. As H continues to increase, there occurs a second threshold (T_2), beyond which there is again only one stable equilibrium for V (the original low- H state having disappeared, discontinuously). For stocking densities in the intermediate region, $T_1 < H < T_2$, the vegetation will tend

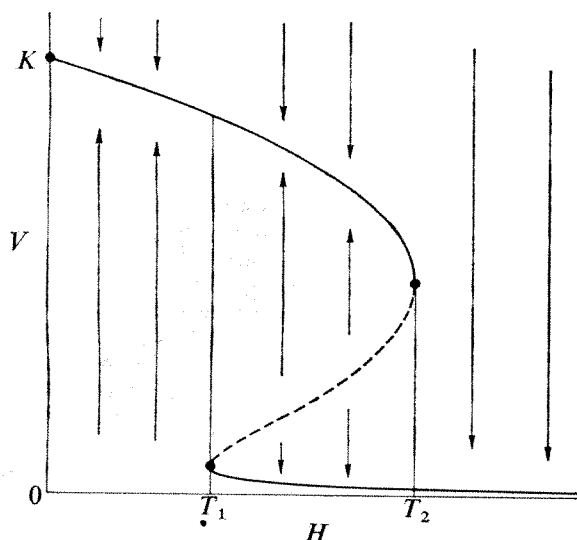


Fig. 3 The equilibrium values of the vegetation biomass, V , are shown as a function of the stocking density, H . For a fixed value of H below the lower threshold at T_1 , or above the upper threshold at T_2 , there is a unique equilibrium value of V ; any initial V value will move to this equilibrium, as indicated by the arrows. For H between T_1 and T_2 , there are two alternative equilibria for V : as shown by the arrows, the system will move to the upper or lower equilibrium, depending on whether the initial value of V lies above or below the dashed 'breakpoint' curve. (This curve is constructed from Fig. 2 and equations (3) and (4), with $\alpha = 0.1$.)

toward either the upper or the lower equilibrium value, depending on the initial value of V : for initial values lying above the dashed line in Fig. 3, V will move toward the upper equilibrium; initial values below the dashed line will move to the lower equilibrium. Borrowing an epidemiological term²⁹, we may call the dashed line the locus of the "breakpoint" values of V .

Although Fig. 3 was constructed from the specific equation (3), its important features are generic to any grazing ecosystem where the dynamics of the vegetation biomass is described by something like Figs 1 or 2. Even in the specially simple case where the $G(V)$ of Fig. 2, with $G(0) = 0$, is combined with the $C(V)$ of Fig. 1, with a linear dependence on V for small V , we still obtain Fig. 3, except that now the lower equilibrium value (occurring for $H > T_1$) is $V = 0$.

If it is assumed that gross animal production, P_G , is linearly proportional to total consumption, $C(V)$, then the equilibrium level of P_G as a function of stocking density H can be read off from Fig. 3. This is done in Fig. 4. Fig. 4 has all the properties just adumbrated for Fig. 3, and is similarly generic.

Noy-Meir first points out some management morals implicit in all this, and then discusses the extent to which such theoretical insights accord with known facts.

If one has a grazing system capable of manifesting the discontinuities illustrated in Figs 3 and 4, then the vegetation will tend always to recover to a high level following an environmental disturbance only if H is kept below some threshold density, T_1 . But, as can be seen from Fig. 4, this usually implies unacceptably low gross productivity. Conversely, for H above T_1 , there is the danger that an environmental fluctuation will carry V below the breakpoint value (the dashed line), whereupon the system will move into the alternative steady state, with dramatically lower values of V and P_G . The closer the system is pushed toward the point of maximum productivity (at $H = T_2$), the more likely is this discontinuous collapse. And of course if the stocking rate for maximum productivity is misjudged, so that H is pushed beyond T_2 , then the system must collapse to the lower equilibrium state. As Noy-Meir²³ sums it up, "a discontinuously stable system is highly labile at the stocking rate which allows the highest productivity. Such a grazing system can be maintained at or near this maximum production rate only by very frequent, almost constant, adjust-

ments of stock density in response to fluctuations in vegetation. A somewhat lower stocking rate will ensure more stable, though on the average somewhat lower, production."

Noy-Meir reviews two classes of empirical evidence.

For intensive, or pasture, systems he pulls together data on the growth curves as a function of green biomass, $G(V)$, for ryegrass-clover in New Zealand^{30,31} and for clover in Australia³², and on the consumption curves for sheep, $C(V)$, for *Phalaris*-clover³³, for *Phalaris*-annuals-clover³⁴, and for ryegrass-clover³⁵, all in Australia. He concludes that the sheep tested by Arnold and by Willoughby would be capable of discontinuous stability properties in October (down under) in pastures of ryegrass, ryegrass-clover, and possibly in clover. There is also direct evidence for two alternate stable states in an experiment done by Morley³⁶, which involves sheep at three different stocking rates in two grazing systems (continuous and with rotation), with three replicates of each.

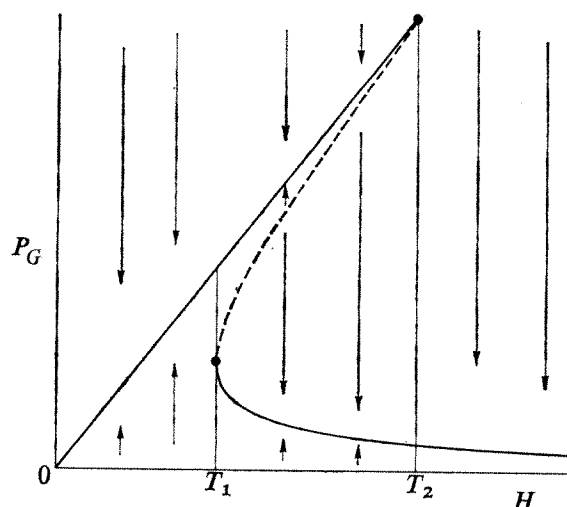
For extensive, or range, systems, indirect evidence can be culled from traditional management practices. Noy-Meir lists the conventional distinction between 'safe' and 'maximum' stocking rates, the notion of range readiness (allowing the vegetation to build to some pre-determined level before introducing animals), and the observation that productivity and animal condition may remain high even though the range is on the verge of collapse. This 'conventional wisdom' is readily justified by Figs 3 and 4.

Throughout this discussion, the herbivore density H has been treated as a constant. In natural (as opposed to managed) situations, H itself will be a dynamical variable, whose rate of change depends on V and H . The consequent pair of differential equations for $H(t)$ and $V(t)$ have been discussed by many people: see the review by Caughley²⁴. The system may have a unique stable point, or two (or even more) alternate stable points, or the populations may oscillate in a stable limit cycle. This subject will be pursued further, when we come to the budworm model, below.

Harvesting animal populations

In grazing ecosystems, we considered the dynamical behaviour of a population (the 'vegetation', $V(t)$) which was being harvested by herbivores. Much of the discussion can be translated to apply to other systems where a plant or animal population is harvested, either directly or indirectly, by man. In particular, fisheries provide an example where one is interested in the dynamics of a fish population, and where the net population growth rate involves a natural growth term, $G(V)$, and a loss rate due to harvesting, $C(V)$, which in general depends both on the fishing effort and on the fish

Fig. 4 Gross animal productivity, P_G , is shown as a function of H . This curve is derived from Fig. 3, under the assumption that P_G is proportional to the total consumption, $C(V)$, of equation (1). The main features are as in Fig. 3; for further discussion, see the text.



population density. The same is true of whaling industries, and of commercial forestries.

Brauer and Sanchez³⁷ have considered a fish or other population that is harvested to give a constant yield. Their analysis corresponds to Fig. 2 in the limit $\alpha \rightarrow 0$; that is, they have the $G(V)$ curve of Fig. 2, but their consumption or harvesting curves are purely horizontal lines, already saturated at $V = 0$. The resulting equilibrium fish population (V) as a function of yield (H) is a limiting version of Fig. 3, with T_1 at the origin, and the lower equilibrium curve collapsed to the H axis ($V = 0$).

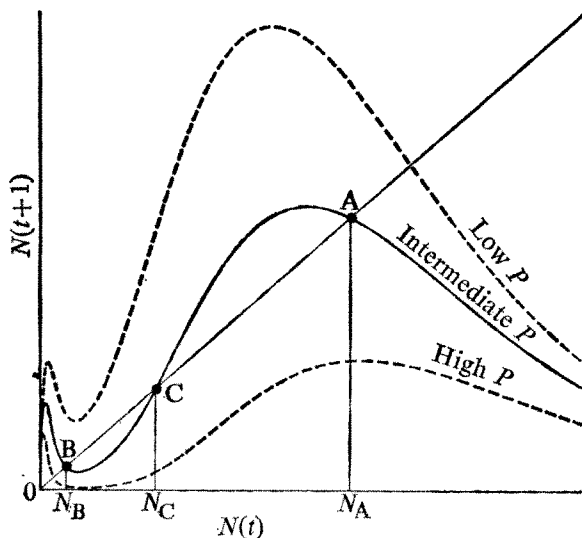


Fig. 5 This figure is a difference equation version of Fig. 3, for animals with non-overlapping generations. The curves relating the population magnitudes in successive generations are shown for various levels of predation, P . Possible equilibrium points occur where the curves intersect the 45 line. (Specifically, the difference equation used here is $N(t+1) = N(t) \exp[r\{1 - N - PN(x^2 + N^2)\}]$, with $\alpha = 0.1$, $r = 4$, and $P = 0.35, 0.22, 0.15$.)

A more realistic harvesting curve will acknowledge that, at low fish population values, the effort needed to keep the yield constant is prohibitively high. Consequently, a realistic curve for attempted 'constant yield' harvesting will be more like those in Fig. 1 and 2, and much of the discussion of the system's dynamics (ref. 38 and J. R. Beddington, to be published) parallels that by Noy-Meir. Beddington and May's³⁸ study is focused on the response of the system to environmental fluctuations, and to this end employs differential equations with stochastic coefficients. Nevertheless, the qualitative conclusions of this relatively sophisticated study (especially the caution against trying to maximise sustained yield under a strategy of constant quotas, which is roughly equivalent to operating around T_2 in Fig. 4) are laid bare by the simple and deterministic graphical analysis outlined above.

Other recent studies of harvested systems which can have alternate stable states are due to Goh³⁹ and to Huberman⁴⁰ (who uses equation (3)). Clark⁴¹ has given a fine review.

Insect pests: general remarks

We now alter our viewpoint, to think of $V(t)$ more generally as a 'prey' population. Its dynamical behaviour depends on the trophic level above it (the 'predators', which were the herbivores H in the grazing systems) and on the trophic level below it (the resources, which were the light and nutrients determining K in the grazing systems). At the risk of confusing the reader, I shall symbolise this change of viewpoint by using $N(t)$, to replace $V(t)$, for the prey population, and by using P , to replace H , for the predators.

In dealing with insect pests, our attention has shifted up one rung in the trophic ladder. Instead of the vegetation $V(t)$ which

depends on a variable H and a constant K , we have a population $N(t)$ of herbivorous insects which depends on predators P and vegetational resources K , either of which may vary.

For a given value of the environmental carrying capacity (K) and for a specified density of predators (P , formerly H), the considerations that determine the dynamics of the prey population (N , formerly V) are likely to be as in Fig. 2. That is, the growth rate of the prey population may be typified as logistic, and the predators' attack rate will have the general form shown in Fig. 2 both for vertebrate²⁵ and for many invertebrate²⁶⁻²⁸ predators.

One final complication must be disposed of. For the majority of temperate zone insects, population growth is a seasonal (often annual), rather than a continuous, affair. Thus the continuous rate of population change, dN/dt , should usually be replaced by discrete changes, $N(t+1) - N(t)$, at time intervals one unit (often one year) apart. The relation between the population values in successive generations will still typically be determined by Fig. 2, but with $N(t+1) - N(t)$ replacing dN/dt on the y -axis. It is more traditional, in this case, to plot the curve that relates $N(t+1)$ to $N(t)$. This is done in Fig. 5. The 45 line represents population values that are unchanging from one generation to the next, and therefore the points where the curves intersect this line are possible equilibrium states. Where the solid curve lies above the dashed curve in Fig. 2, the net growth rate is positive, and the curve lies above the 45 line in Fig. 5. Conversely, where the solid curve lies below the dashed one in Fig. 2, the curve lies below the 45 line in Fig. 5.

When predation is relatively unimportant (low H in Fig. 2, low P in Fig. 5), there is a unique equilibrium point, at a population value slightly below K ; the population level is set primarily by resources. When predation is relatively important (high H in Fig. 2, high P in Fig. 5), there is again a unique equilibrium point, this time at a low population level; the population is predator-controlled. But for intermediate levels of predation there are, as before, two attracting equilibrium points (A and B in Fig. 5), divided by a repelling point (C in Fig. 5).

The consequences of this intermediate level of predation, with its two alternative equilibria for the pest population, have been noted by Takahashi⁴² and Watt⁴³. They observe that such pests may usually fluctuate at low numbers, around the lower equilibrium point at N_B . But if fluctuations in the number of predators, or of pests, or of the carrying capacity, happen to carry pest numbers above N_C in any one year, then the population will explode towards the upper equilibrium point at N_A . A subsequent crash is likely (the complications that make the dynamics of difference equations more unsteady than those of differential equations are discussed in the conclusion), with the population returning to the neighbourhood of N_B . The pest population may thus exhibit a periodic or episodic pattern of "outbreaks", followed by relatively long intervals at low densities.

Southwood⁴⁴⁻⁴⁶ has recently given a more quantitative discussion, and he and others have incorporated the practical conclusions into a morphology of strategies appropriate for the control of various kinds of insect pests⁴⁷⁻⁵⁰.

For example, Southwood and Comins⁴⁴ have interpreted Clark's thorough studies of the eucalyptus psyllid (a plant louse) *Cardiaspina albiteretura* in the light of Fig. 5. Using Clark's data for egg-to-adult survival for 29 generations, along with information about the depression of natality by direct crowding and by host plant deterioration, they estimate the shape of the $N(t+1)$ -versus- $N(t)$ curve. They find the curve to be of 'intermediate P ' type, and assign to N_B , N_C and N_A values corresponding to 2.8, 22 and 107 eggs per shoot, respectively. Not only does this give a qualitative explanation of the observed episodic outbreaks, but it is in remarkable quantitative agreement: Clark's field data give an average of 3.0 eggs per shoot at low densities, suggest a "rapid increase to outbreak level" beyond 10-15 e/s, and have a mean for 12 high density populations of 110 e/s (with a range 23-280).

Sufficient data have also been published on the European spruce sawfly, *Diprion hercyniae*, in New Brunswick for a tentative analysis of this kind. Southwood⁴⁷ suggests the present situation is one of 'intermediate P ', with the sawfly controlled by a com-

bination of virus disease and parasitoids: life-table estimates and field observations agree on a value of N_c around $0.2 \text{ larvae m}^{-2}$; fluctuations to higher densities result in runaway to around $N_A \sim 1 \text{ larvae m}^{-2}$, which is economically acceptable. Before the advent of the virus disease, the sawfly in New Brunswick seems to have been^{4,7} in a "low P " situation, with outbreaks to densities around 20 larvae m^{-2} followed by crashes.

Insect pests: the spruce budworm

One of the best studied of all insect pests is the spruce budworm, *Choristoneura fumiferana*, in Canada. This forest defoliator erupts at approximately 40-year intervals, causing much damage in northern coniferous forests, and economic stress in the lumber industry. The massive amount of data gathered by Morris⁵¹ and his associates has been subjected to much analysis by Holling and his collaborators^{15,52} (and D. Ludwig, D. Jones and C. S. Holling, to be published) at UBC and at the International Institute for Applied Systems Analysis (IIASA). One of the most pleasing features is the way the models have become progressively simpler as the basic mechanisms have become better understood. Thus the earlier 'systems models' have given way to Ludwig *et al.*'s⁵³ 3-component model (budworms; average leaf area per tree; energy reserve determining the condition of trees and foliage); the present review gives a crude 2-component model (budworms; leaf area) that retains the essentials.

Consider first the dynamics of the budworm population, $N(t)$. Yet again, this is plausibly described by Fig. 2 (with N replacing V), with the net population growth rate counterpoised between a natural growth term and losses due to predators. The general features described by Fig. 2 may be typified by the specific equation (3), which now reads

$$\frac{dN}{dt} = rN \left(1 - \frac{N}{K}\right) - \frac{\beta P N^2}{N_0^2 + N^2} \quad (5)$$

Here r is the intrinsic growth rate for budworms, and K is the carrying capacity, which depends on the average leaf area per tree, S ; we write $K = \kappa S$. N_0 is the characteristic budworm population at which the predator attack rate saturates (to the constant level β per predator). This characteristic value for predator switching will usually depend on budworm density per unit leaf area rather than on absolute budworm numbers, so that we may write $N_0 = \eta S$. Finally, for a given value of average leaf area S , it is convenient to use the rescaled variables $X = N/\kappa S$, $\tau = rt$ and $\gamma = \beta P/\kappa S$ to rewrite equation (5) in dimensionless form as

$$dX/d\tau = X(1-X) - \gamma X^2/(x^2 + X^2) \quad (6)$$

Here x is formally defined as $x = \eta/\kappa$, but biologically it retains the meaning it had in equations (2) and (4): for any given value of S , x is the ratio between the budworm density that saturates the predator attack capacity and the maximum budworm density that the vegetation can sustain. Equations (4) and (6) are identical.

As before, it is clear, either from the general Fig. 2 (which is drawn for small x) or from the specific equation (6) (provided $x < 1/3$, 3), that the budworm system may have two alternative stable states. If we fix the resource level S , and plot the stable budworm density N as a function of the number of predators P , we get exactly Fig. 3 (with N replacing V , and P replacing H).

It is more interesting, however, to fix the predator level P and consider how the equilibrium value(s) of N varies with S . This can be done graphically from Fig. 2, or algebraically from equation (5). The result is Fig. 6. A simpler way to arrive at Fig. 6 from Fig. 3 is to notice, from equation (6), that the equilibrium value(s) of N/S depends only on γ , once x is fixed. But $\gamma = \beta P/\kappa S$, so that S plays a part exactly like $1/P$: small P is equivalent to large S , and vice versa, whence Fig. 6 (with S for the x axis) follows from Fig. 3 (with P or H for the x axis). Moreover this makes biological sense. For budworms, the good life is few predators or lots of food; low P or high S . The bad life is the converse.

The main features of Fig. 6 need no elaboration. At low values of

S , there is a unique, and low, budworm density: the system is under predator control. At T_2 a second stable state appears discontinuously, and, for $T_2 < S < T_1$, N will tend to one or other of the two stable states, depending on which side of the 'breakpoint' locus it starts from. For S above T_1 there is again a unique stable state, in which the predators play little part.

This discussion of the budworm dynamics is not sufficient for a full elucidation of the way the interactive system of budworms and forest foliage behaves. We need also to consider how the average leaf area per tree, S , depends on N .

The essentials of such a discussion can be carried out⁵³, independent of any further details, by noting that the characteristic time scales for changes in N and in S are very different. The time scale for budworm population growth is months, or even weeks. The basic time scale for change in the average leaf area per tree depends on average branch area, and thence on the time scale for tree growth, which is typically measured in decades. Thus N changes on a relatively fast time scale, S on a slow one.

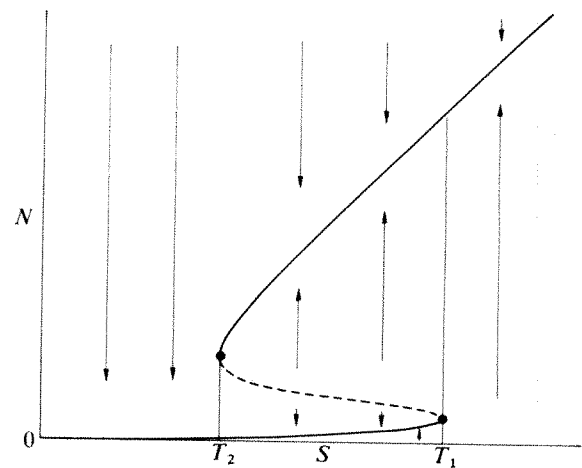


Fig. 6 The equilibrium budworm population, N , shown as a function of the average leaf area per tree, S . (This figure is based on equation (6), with $x = 0.06$.) The threshold and breakpoint features are similar to those in Fig. 3, and are discussed more fully in the text.

Returning to Fig. 6, we further suppose that budworm populations along the lower, 'predator-controlled' equilibrium curve do not have a significant impact on the foliage, so that S undergoes slow natural growth in this regime. Conversely, we assume that the large budworm populations along the upper equilibrium curve have a significant adverse effect on S , decreasing it. The qualitative nature of the system's dynamical behaviour now follows. Starting from any low values of N and S , the system will move rapidly (on the fast time scale) on to the lower budworm equilibrium curve in Fig. 6. S will now slowly increase, with N always quickly adjusted to the current S value, and the system will move (on the slow time scale) to the right along the lower equilibrium curve. When the system arrives at the point T_1 , no further continuous change is possible, and N jumps (on the fast time scale) to the upper equilibrium branch. Now S decreases because of the uncontrolled depredations of the budworms, and the system moves (fairly fast) to the left on the upper equilibrium curve. Finally, when the point T_2 is reached, N must fall (again on the fast time scale) back to the lower equilibrium branch, and the cycle begins again.

The upshot is a cyclic pattern of budworm explosions and crashes, as shown in Fig. 7. The system spends most of the cycle at low budworm densities, traversing the lower equilibrium curve from T_2 to T_1 . The cycle length is thus set by the slow time scale, and is of the order of decades.

A more explicitly mathematical treatment can be given crudely by assuming that the rate of change of S is given by a natural logistic growth term (with an intrinsic growth rate ρ), offset by

losses linearly proportional to the budworm density

$$dS/dt = \rho S(1 - S/S_{\max}) - \epsilon N. \quad (7)$$

Under the assumptions discussed above, the locus of equilibrium values of S (the points where $dS/dt = 0$) are as indicated by the dashed line in Fig. 7. The pair of equations (5) and (7) then lead to a stable limit cycle, as shown in Fig. 7. The limit $\rho \ll r$ corresponds to the foregoing distinction between slow and fast time scales, and leads to a limit cycle with the almost discontinuous character that was discussed above.

The work of Ludwig *et al.*⁵² is more detailed (involving three

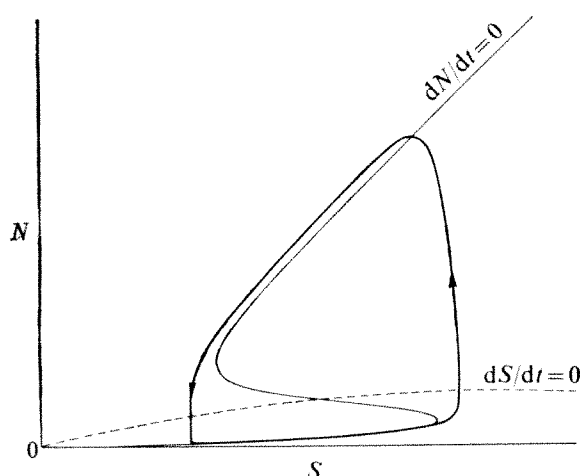


Fig. 7 The (solid, $dN/dt = 0$) equilibrium curve for budworms, N , as a function of foliage, S , is as in Fig. 6. The (dashed, $dS/dt = 0$) equilibrium curve for S as a function of N has the form of equation (7). Under these circumstances, the system will settle to oscillate in a stable limit cycle, as indicated. The figure is for $\rho = 0.01r$, so that the budworm growth time scale is fast compared with the time scale for tree growth: as explained more fully in the text, this explains the main features of the cycle.

differential equations), and is compared with field data. They obtain numerical agreement with the observed 40-year cycle.

This model has implications for the management of budworm populations. Notice first that a program aimed at stopping budworm outbreaks has a propensity to hold the population perpetually poised on the brink of explosion, around the threshold point T_1 on the lower equilibrium curve in Fig. 6. More quantitatively, the effects of using insecticide against budworms can be mimicked by adding an extra mortality term, $-sN$, to the right hand side of equation (5). If we could maintain $s > r$ for many years, the budworms could in principle be eradicated. In the more likely event that $r > s$, the new version of equation (5) can again be brought into the dimensionless form of equation (6), with α replaced by $\alpha' = (r/r-s)\alpha$. We recall that the kinkiness in Figs 3, 4 and 6, and the ensuing alternative stable states, depends on α being small. Thus use of insecticides can increase the effective value of α to such an extent as to straighten out the kink in Fig. 6 (that is, can increase α' to a value $\alpha' > 1/3 \sqrt{3}$), leading to a unique stable state. The price, however, is daunting: not only are we committed to endless insecticide use, but the new equilibrium is necessarily established at a budworm density somewhere intermediate between the original upper and lower equilibrium levels. Such a budworm density represents a perpetual low-level outbreak.

It should be added that this discussion has ignored climatic fluctuations. One argument in favour of trying to 'hold the line' with insecticides is that one may thereby buy time, waiting for a year when unfavourable weather will carry the population back down to very low values.

Human host-parasite systems

Similar threshold and breakpoint phenomena occur in the transmission dynamics of many infections of humans and other

animals, particularly when the transmission cycle involves intermediate vectors.

Malaria is probably the best-known example exhibiting a threshold^{54,55}. Suppose that a single malarious mosquito is introduced into a closed and previously malaria-free community of people and mosquitoes. Each person bitten by this mosquito may, with a probability that can be estimated, become victim to the disease. These infected people may in turn be bitten by uninfected mosquitoes, which thereby (again with some probability factor, and after the elapse of a latent period of around 10 days) are recruited as malaria vectors. The key question is whether the original infectious mosquito produces, on the average, more than one subsequently infectious mosquito, or not. If it does, the system is 'above threshold', and the fraction of the human and of the mosquito populations that have malaria at any one time will grow until an equilibrium is reached, at which new infections are balanced against recoveries and deaths. Conversely, if it does not, the system is 'below threshold', and the introduced infection cannot be maintained.

This discussion makes clear the underlying nature of the threshold relation for malaria. The relation can be written explicitly in terms of the number of mosquitoes, biting rates, and recovery and death rates of mosquitoes and people; as reviewed by Macdonald^{54,55} and Conway⁵⁶, this threshold formula has many implications for management.

The main fact, however, is that for simple models of the malaria transmission process there is always a unique stable state: for mosquito densities above threshold, there is some endemic level of infection; below threshold the level is zero. For many helminthic infections the situation is made more complicated by the parasites' having a sexual stage in the human host. Thus, for example, an adult female schistosome will produce eggs only if she has a mate, which may be unlikely if the mean worm load per person is less than, or of the order of, unity.

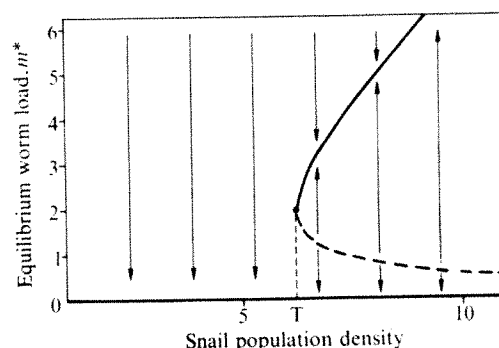


Fig. 8 For a simple model of the transmission dynamics of schistosomiasis, the equilibrium mean worm load per person, m^* , is shown as a function of the snail population density (or other relevant transmission factor). The threshold and breakpoint phenomena are as discussed in the text. (From Bradley & May⁵⁹.)

As first stressed by Macdonald^{29,55}, the result is that for schistosomiasis and other parasitic infections with a sexual stage in the primary host there is, as before, a threshold transmission value which depends on the population density of the snails or other intermediate vector, on the rate of egg production in the primary host, and on other transmission parameters. Below threshold, there is only one equilibrium state, namely no infection. Above threshold, there are two alternative equilibrium states: if the initial mean worm load per person, m , is sufficiently high, the infection cycle is maintained; but if m is initially too small, the number of mated females (which will scale as m^2) may be insufficient for the system to 'take off' and attain its endemic equilibrium level. This situation is illustrated in Fig. 8, which corresponds to simple models for schistosomiasis^{29,56-58}. If the snail density (or other relevant transmission factor) is below the threshold value T , the

equilibrium level of adult schistosomes is zero. Above threshold, the mean worm load per person moves either to the upper equilibrium curve, or to zero, depending on whether its initial value lies above or below the "breakpoint" line.

Figure 8 is for a closed system, with no immigration of infectious snails or people from outside. If a small amount of such immigration is included (I. Nasell, to be published) the lower equilibrium curve in Fig. 8 must shift up to some small, but finite, level of infection, and a figure similar to Fig. 6 is obtained (with m replacing N , and snail density or the like replacing S).

The existence of a breakpoint holds important implications for control of the system^{29,55-59}. For diseases such as malaria, where there is always a unique equilibrium state, the only control strategy is to reduce the mosquito numbers and/or the biting rate and other parameters in such a combination as to take the system below threshold. The system must, moreover, subsequently be kept below threshold. But infections whose dynamics are as described by Fig. 8 can in principle be eradicated by temporarily displacing the mean parasite load below the breakpoint, thus taking advantage of the two alternative equilibrium states. This control strategy has the advantage that the basic transmission parameters do not have to be modified to, and maintained at, sub-threshold values. (If the infection dynamics of snails operates on a fast time scale, and of humans on a slow time scale, the locus of the breakpoint depends only on the level of infection among humans: more generally, the breakpoint will depend on both snail and human infection levels⁵⁶⁻⁵⁸. It also depends on whether the adult parasites are distributed among humans in random or clumped fashion^{58,59}.)

Current estimates are that about 200 million people have schistosomiasis, and about 300 million suffer from the various filarial infections (which include 'river blindness' and elephantiasis). But empirical information on the overall transmission dynamics of schistosomiasis and filariasis is scant, so that theoretical notions about threshold and breakpoints remain untested in this context.

Conclusion

This review has drawn together a variety of mathematical models for ecological systems, each of which is dealt with individually in the scattered literature. The models are united by the common theme of possessing a regime of dynamical behaviour in which there are two alternative stable states, so that continuous variation in a control variable can produce discontinuous effects. Thus smooth changes in stocking rates can cause discontinuous changes in the grazed vegetation; continuous changes in harvesting rates can cause discontinuous collapse in fisheries; continuous changes in environmental parameters or foliage growth or predation rates can lead to discontinuous outbreaks of insect pests; continuous changes in snail or dipteran population densities can cause discontinuous appearance or disappearance of helminthic infections.

These models can often be tied to empirical data, whence they yield specific insights into the management of the system. (Some of these insights can be recast in the language of catastrophe theory, but this is usually *post hoc* window-dressing. It is too often forgotten that catastrophe theory is, strictly speaking, a local theory; we want global descriptions of the dynamics.)

The review has been largely confined to the dynamical behaviour of a single population, albeit as a function of other populations of resources and predators. As the number of interacting dynamical variables, and of equations, increases things get more complicated. Consider, for example, a predator-prey system where the predator's dynamics depends on the prey population according to the backward-bending curve of Fig. 6 (N versus S), and where the prey's dynamics in turn depends on the predator population according to Fig. 3 (V versus H). These two curves—one for the predator equilibrium, the other for the prey equilibrium—are in principle capable of intersecting at 9 points, corresponding to no fewer than four alternative stable states (separated by four saddle points and one central hilltop), each with its own domain of attraction. As the dimensionality of the system increases to encompass more and more species, the dynamical

landscape can begin to look like the surface of the moon, and any detailed comparison with field or laboratory data becomes very difficult.

A different and additional complication is that systems with discrete generations are described by difference equations, which can exhibit kaleidoscopic dynamics. For such systems, of which Fig. 5 is an example, increasingly severe nonlinearities can make the dynamical behaviour range from a stable point, through a bifurcating hierarchy of stable cycles, into a regime which is in many ways indistinguishable from random noise⁶⁰⁻⁶². Similarly, patterns of stable points or cycles giving way to chaos can be found in systems described by differential equations with time lags, or even in simple first-order ordinary differential equations once 3 or more species are interacting^{60,62,63}.

These manifold complications can lead one to take a gloomy view of the possibility of making predictions about multi-species systems^{60,62}. The one kindly light amidst this encircling gloom is that many complex communities are, arguably, made up mainly of loosely coupled lower-order systems^{17,64,65}, to which the simple models reviewed here are pertinent.

I thank Henry S. Horn for helpful conversations, and the US NSF for support (grant DEB 75-10464).

- ¹ Monod, J. *Chance and Necessity: an Essay on the Natural Philosophy of Modern Biology* (Knopf, New York, 1971).
- ² Weinberg, S. *The First Three Minutes: a Modern View of the Origin of the Universe* (Basic Books, New York, 1977).
- ³ Hawking, S. W. *Sci. Am.* **236**, 34-40 (1977).
- ⁴ Layzer, D. *Sci. Am.* **233**, 56-69 (1975).
- ⁵ Blatt, J. M. *Proc. theor. Phys.* **22**, 745-756 (1959).
- ⁶ Simpson, G. G. *The Major Features of Evolution* (Columbia University Press, New York, 1953).
- ⁷ Raup, D. M. *Am. Sci.* **65**, 50-57 (1977).
- ⁸ MacArthur, R. H. *Geographical Ecology* (Harper and Row, New York, 1972).
- ⁹ May, R. M. in *Progress in Theoretical Biology* 3 (eds Rosen, R. & Snell, F.) 1-50 (Academic, New York, 1974).
- ¹⁰ Sutherland, J. P. *Am. Nat.* **108**, 859-873 (1974).
- ¹¹ Levin, S. A. *A. Rev. ecol. Syst.* **7**, 287-310 (1976).
- ¹² Connell, J. H. & Slatyer, R. O. *Am. Nat.* (in the press).
- ¹³ Austin, M. P. & Cook, B. G. *J. theor. Biol.* **45**, 435-458 (1974).
- ¹⁴ Case, T. J. & Gilpin, M. E. *Nature* **261**, 40-42 (1976).
- ¹⁵ Holling, C. S. *A. Rev. ecol. Syst.* **4**, 1-23 (1973).
- ¹⁶ Lewontin, R. C. in *Diversity and Stability in Ecological Systems* 13-24 (US National Bureau of Standards, Springfield, Virginia, 1969).
- ¹⁷ May, R. M. *Stability and Complexity in Model Ecosystems* 2nd ed. (Princeton University Press, Princeton, 1974).
- ¹⁸ Orians, G. H. in *Unifying Concepts in Ecology* (eds van Dobben, W. H. & Lowe-McConnell, R. H.) 139-150 (W. Junk, The Hague, 1975).
- ¹⁹ Margalef, R. in *Unifying Concepts in Ecology* (op. cit.) 151-160.
- ²⁰ May, R. M. in *Unifying Concepts in Ecology* (op. cit.) 161-168.
- ²¹ Whittaker, R. H. in *Unifying Concepts in Ecology* (op. cit.) 169-181.
- ²² Grimm, H. R. *IASA Publication RR-76-005* (2361 Laxenburg, Austria, 1976).
- ²³ Noy-Meir, I. *J. Ecol.* **63**, 459-481 (1975).
- ²⁴ Caughley, G. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 94-113 (Blackwell, Oxford, 1976).
- ²⁵ Holling, C. S. *Mem. ent. Soc. Can.* **45**, 3-60 (1965).
- ²⁶ Hassell, M. P., Lawton, J. H. & Beddington, J. R. *J. Anim. Ecol.* **46**, 249-262 (1977).
- ²⁷ Murdoch, W. W. & Oaten, A. *Adv. ecol. Res.* **9**, 1-131 (1975).
- ²⁸ Hassell, M. P. *Arthropod Predator-Prey Systems* (Princeton University Press, Princeton, in the press).
- ²⁹ Macdonald, G. *Trans. R. Soc. trop. Med. Hyg.* **59**, 489-506 (1965).
- ³⁰ Brougham, R. W. *Aust. J. agric. Res.* **6**, 804-812 (1955).
- ³¹ Brougham, R. W. *N. Z. J. Sci. Technol.* **A38**, 78-87 (1956).
- ³² Davidson, J. L. & Donald, C. M. *Aust. J. agric. Res.* **9**, 53-72 (1958).
- ³³ Willoughby, W. M. *Aust. J. agric. Res.* **10**, 248-268 (1959).
- ³⁴ Arnold, G. W. in *Grazing in Terrestrial and Marine Environments* (ed. Crisp, D. J.) 133-154 (Blackwell, Oxford, 1963).
- ³⁵ Alden, W. G. *Proc. Aust. Soc. Anim. Prod.* **4**, 163-166 (1962).
- ³⁶ Morley, F. H. W. *Proc. N. Z. Soc. Anim. Prod.* **26**, 8-21 (1966).
- ³⁷ Brauer, F. & Sanchez, D. A. *Theor. Pop. Biol.* **8**, 12-31 (1975).
- ³⁸ Beddington, J. R. & May, R. M. *Science* **197**, 463 (1977).
- ³⁹ Goh, B. S. *Math. Biosci.* **33**, 359 (1977).
- ⁴⁰ Huberman, G. *Theor. Pop. Biol.* (in the press).
- ⁴¹ Clark, C. W. *Mathematical Bioeconomics* (Wiley, New York, 1976).
- ⁴² Takahashi, F. *Res. Pop. Ecol.* **6**, 28-36 (1964).
- ⁴³ Watt, K. E. F. *Ecology and Resource Management* (McGraw-Hill, New York, 1968).
- ⁴⁴ Southwood, T. R. E. & Comins, H. N. *J. Anim. Ecol.* **45**, 949-966 (1976).
- ⁴⁵ Southwood, T. R. E. in *Proceedings of XI International Congress of Entomology* (ed. Packer, J. S.) 36-51 (Entomological Society of America, College Park, Maryland, 1977).
- ⁴⁶ Southwood, T. R. E. *Am. Sci.* **65**, 30-39 (1977).
- ⁴⁷ Southwood, T. R. E. in *The Origins of Pest, Parasite, Disease and Weed Problems* (eds Cherrett, J. M. & Sagar, G. R.) (Blackwell, Oxford, 1977).
- ⁴⁸ May, R. M. *Nature* **264**, 211-212 (1976).
- ⁴⁹ Conway, G. R. in *Theoretical Ecology: Principles and Applications* (op. cit.) 257-281.
- ⁵⁰ Conway, G. R. *Nature* (in the press).
- ⁵¹ Morris, R. *Mem. ent. Soc. Can.* **31**, 1-332 (1963).
- ⁵² Holling, C. S., Dantzig, G., Baskerville, C., Jones, D. & Clark, W. *Proc. Int. (Canadian) Conference on Applied Systems Analysis*, 1975 (in the press).
- ⁵³ Levandowsky, M. & White, B. S. *Evol. Biol.* (in the press).
- ⁵⁴ Macdonald, G. *The Epidemiology and Control of Malaria* (Oxford University Press, London, 1957).
- ⁵⁵ Macdonald, G. *Dynamics of Tropical Disease* (Oxford University Press, London, 1973).
- ⁵⁶ Nasell, I. & Hirsch, W. M. *Comm. pure appl. Math.* **26**, 395-453 (1973).
- ⁵⁷ Nasell, I. *Theor. Pop. Biol.* **10**, 133-144 (1976).
- ⁵⁸ May, R. M. *Math. Biosci.* (in the press).
- ⁵⁹ Bradley, D. J. & May, R. M. *Trans. R. Soc. trop. Med. Hyg.* (in the press).
- ⁶⁰ May, R. M. & Oster, G. F. *Am. Nat.* **110**, 573-599 (1976).
- ⁶¹ May, R. M. *Nature* **261**, 459-467 (1976).
- ⁶² Guckenheimer, J., Oster, G. F. & Ipakchi, A. *Theor. Pop. Biol.* (in the press).
- ⁶³ Rossler, O. E. *Z. Naturforsch.* **31a**, 1664-1670 (1976).
- ⁶⁴ Gilbert, L. E. in *Coevolution of Animals and Plants* (eds Gilbert, L. E. & Raven, P. H.) (Texas University Press, Austin, 1975).
- ⁶⁵ Lawton, J. H. *Nature* **264**, 138-139 (1976).

articles

Hypervelocity cratering and impact magnetisation of basalt

G. Martelli & G. Newton

Plasma and Space Physics Group, School of Mathematical and Physical Sciences, The University of Sussex, Brighton, Sussex, UK

Suitably modified explosive shaped charges have been used to produce small hypervelocity projectiles ($v \sim 15 \text{ km s}^{-1}$) to study e.m. effects associated with high-velocity cratering of basalts, thus simulating in a terrestrial environment some features of meteoritic impact on the lunar surface. The experiments have shown that plasma is formed during impact and that an external field can be impressed in the basalt.

IN an experiment to test whether meteoritic impact can magnetise lunar rocks (either by creating and impressing a transient magnetic field or by impressing and implanting a pre-existing field pattern), we made observations of magnetic phenomena associated with the cratering due to the impact of macroscopic ($m = 0.1\text{--}1 \text{ g}$) hypervelocity projectiles ($v \sim 15 \text{ km s}^{-1}$) on basalt blocks. For these experiments the 'meteoroid' consisted of the tip of the jet from a shaped charge and the target of a basalt block similar in composition to some lunar basalts. These tests were conducted in evacuated vessels, both with and without external magnetic fields either parallel or perpendicular to the basalt surface, and observations show that (1) a transient magnetic field followed by a short burst of electromagnetic oscillations is created near the impact region, and (2) it is possible to impress an externally applied magnetic field into the basalt.

Measurements of the magnetic field of lunar rocks have given values ranging between 10^{-2} and 1.2 Oe (refs 1, 2) for inducing fields necessary to account for the observed natural remanent magnetisation. Recent indirect observations from the Apollo 17 sub-satellite³ have confirmed the wide range of magnetic field intensities and orientations recorded by magnetometers flown on board previous Apollo missions.

Theoretical models based on lunar dynamo⁴ and fossil field theories^{5,6} have been invoked to explain the observations. Suggestions have also been made that there is evidence concerning the existence of a magnetic core⁷. Theories which can account, however, for both magnetic field amplification and/or local magnetisation mechanisms can explain more readily the wide range of observed values and are more amenable to direct experimental verification. Of these theoretical models, some assume the magnetisation to be associated with meteoritic impact⁸⁻¹⁰, while cometary impact has also been considered as a mechanism for impressing and magnifying an external magnetic field¹¹. Finally, during discussions with Professor K. Anderson, Space Science Laboratory, University of California, Berkeley in 1975 we considered the possibility that the plasma created during a hypervelocity impact could support transient electric

fields and associated electric currents, as observed in laser 'impact' experiments¹²⁻¹⁴. For hypervelocity impact, the residual magnetic field would be the remanent of an induced field, due to both the shock remanent magnetisation (SRM, or piezoremanence) and to the thermal remanent magnetisation (TRM) associated with the heating effects of the impact. The basic system of currents and inducing magnetic fields which can arise in laser 'impact' experiments, and can probably be present in hypervelocity impact experiments, are discussed in refs 15, 16 and illustrated in ref. 17 (see also for bibliography).

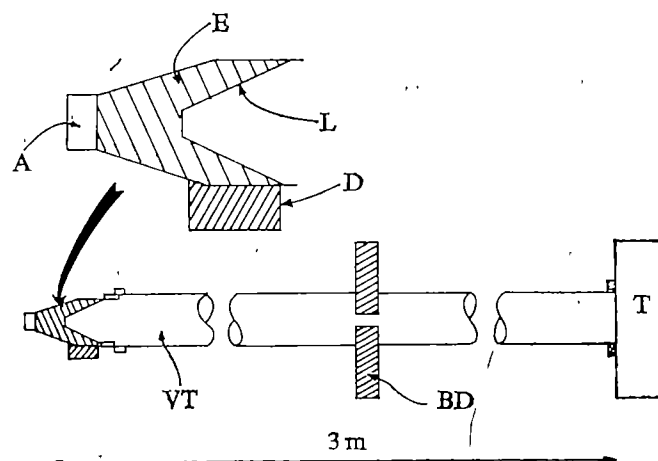
The only experimental technique we know of which has been used so far for the purpose of simulating shock compression associated with high-velocity impact is that of the explosively driven flying plate technique^{18,19} and interesting results have been achieved by this method²⁰. It differs, however, from the technique used here on a major point, since it does not actually simulate very closely the impact of a relatively small object on a large surface.

We describe here results from some experiments designed to simulate meteoritic impact, the 'meteoroid' consisting of the tip of the jet from a shaped charge, the target being a block of basalt with composition reasonably similar to that of some lunar basalt.

Experimental technique

Central to the experiments described below is the use of shaped charges to produce a high velocity jet of material, of which the

Fig. 1 Experimental set-up. L, Aluminium liner; D, deflector (TNT); E, explosive shaped charge (Octol); A, detonator; VT, vacuum tube; BD, blast deflector; T, target (basalt block).



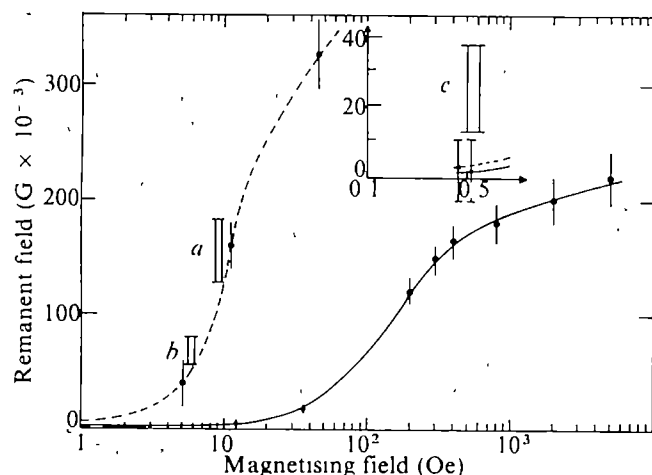


Fig. 2. IRM field (continuous curve) and TRM field (broken curve) for basalt samples used in the experiment, *a*, SARM for a 10 G field perpendicular to the target surface; *b*, SARM for a 6 G field parallel to the target surface; *c*, SARM for the geomagnetic field parallel to the target surface.

tip only is allowed to impact on the basalt target; the remaining, and slower, portion of the jet is deflected by means of a subsequent explosion. As is well known, a metal lined shaped charge consists of a cylinder of explosive having a high detonation velocity (v_d), the cylinder having a metal lined conical cavity at one end. When detonated at the other end, the (plane) detonation wave implodes the cone and parts of it are ejected with a velocity larger than the detonation velocity (Monroe effect). The maximum velocity v_m depends on the opening angle α of the cone and the ratio of the mass of material that lags behind (the 'slug') is also a function of α . This ratio can be improved by truncating the cone at, say, about one tenth of its height, measured from the apex. The velocity gradient along the jet causes it to stretch and break up into individual pellets. We are interested in the fastest fragments of the jet, hereafter referred to as the 'tip'. If the density ρ_l of the liner is close to that of the explosive ρ_e , the shock impedance of the liner is well matched to the implosion wave, thus minimising surface reflection and maximising the amount of liner material in the tip. Provided the experiments are conducted in an evacuated system, the temperature of the jet is not expected to exceed a few hundred degrees Celsius, because of the cooling associated with the adiabatic expansion which it undergoes during flight.

In our experiments the liner was made of HE30 aluminium

alloy, which has a relatively low density ($\rho_e = 2.73 \text{ g cm}^{-3}$), is easily machinable and non-toxic. The explosive was Octol ($v_d \sim 8.4 \text{ km s}^{-1}$, $\rho_e \sim 1.8 \text{ g cm}^{-3}$). The angle α was 45° .

The experiment

The experimental set-up is illustrated schematically in Fig. 1. The system was evacuated by a rotary pump to $\sim 10^{-2}$ torr. On ignition of the explosive at A, the propagation of the detonation causes: (1) the explosive ejection of the tip of the jet, and (2) the shock ignition of the TNT deflector.

If the TNT deflector is properly placed, only the tip proceeds unperturbed while the remaining solid and gaseous debris is swept sideways. The best positioning of the deflector was obtained by trials against plates which recorded the impact. It was found that the debris could be deflected through $\sim 30^\circ$, and that only a small tip would reach the target.

The velocity of the tip was deduced by timing the flight over a set distance (1.5 m), the passage being recorded by the rupture of current carrying foils. Maximum values of v_m of $\sim 15 \text{ km s}^{-1}$ were recorded.

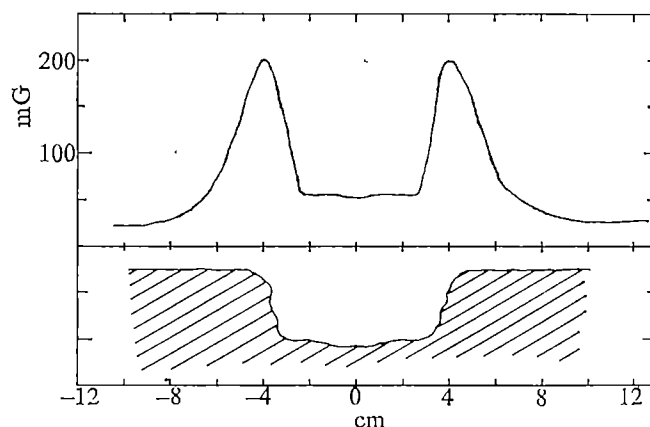


Fig. 3. A crater profile in a basalt block (shaded area). The field due to the SARM was measured 1.5 cm above the target surface, the magnetising field was $\sim 10 \text{ G}$ perpendicular to the target

We believe that the differences in magnetic structure between lunar and terrestrial basalts do not invalidate the aims of our experiments. We have, however, attempted to select basalt which minimises these differences. This was found at a quarry at Rowley Regis near Birmingham, which supplied us with approximately 30 t of selected blocks. The composition of the samples is given in Table 1.

Experimental results

Magnetic mapping of the surface of the basalt blocks before and after impact was carried out using both a Hall probe and a flux-gate magnetometer.

Before impact the field at the surface of the basalt blocks was found to be of the order of $10 \pm 10 \text{ mG}$.

The magnetic properties of the basalt have been measured using a large number of cores, up to 2.5 cm diameter, extracted from various blocks. Figure 2 shows the contribution to the measured field due to the isothermal remanent magnetisation at room temperature ('IRM', continuous curve) and to the thermal remanent magnetisation ('TRM', broken curve) obtained after heating the samples to about 800°C and letting them cool inside a solenoid. The largest contribution to the error comes from the spread of values obtained from different samples. The size of the cores was chosen so as to make the

Table 1 Composition of some Basalt samples from the quarry at Rowley Regis

	%
SiO ₂	50.17
Fe ₂ O ₃	4.61
Al ₂ O ₃	18.75
CaO	7.33
MnO	0.16
MgO	5.41
K, Na oxide	4.93
FeO	5.79
Others	2.86

The blocks were cut, faced and inspected for homogeneity and absence of microfractures. The selected blocks were individually encased in alumina cement inside a reinforced dural frame, $45 \text{ cm} \times 45 \text{ cm} \times 25 \text{ cm}$, to prevent rupturing during test. The experiments were carried out in a reinforced concrete bunker at one of the proving grounds of the Raufoss Ammunition Factory, near Oslo.

field measurements practically independent of the core size, thus enabling the measurements to be compared directly with those at the surface of the basalt blocks.

We emphasise that, because of the short duration of the impact process, it is not possible to differentiate reliably between thermal remanent magnetisation and piezoremanent effects, so, hereafter, we refer to the magnetisation associated with the impact as 'shock associated remanent magnetisation' (SARM).

Three main types of hypervelocity impact tests were performed, and we present data from one of each. (1) with a 10 Oe external magnetic field at right angles to the basalt surface; (2) with a 6 Oe field parallel to the basalt surface;

conducted successfully in an evacuated vessel without an externally applied magnetic field, revealed magnetic perturbations at the basalt surface reaching up to ~ 40 mG near the crater (see inset in Fig. 2 marked 'c'). This value is no more than three to four times larger than the fluctuations recorded across the surface before impact. It may be that since the impact created a crater larger than was the case in the other tests, any more strongly magnetised material in the neighbourhood of the impact area may have been lost with the ejecta.

In some of the experiments mentioned above we recorded the electromagnetic noise generated on impact, using pickup coils surrounding the glass tube near the basalt surface and a 20-kHz bandwidth instrumentation recorder. In all cases we observed a rapid (probably bandwidth limited) transient magnetic field during impact followed by a number of oscillations (Fig. 4a). This suggests the creation of plasma capable of sustaining azimuthal currents, and possibly, transient flute instabilities. If this interpretation is correct, the current can be estimated from the amplitude of the recorded signals, to be of the order of 10^{-1} A. A similar experiment performed using the same geometry but at atmospheric pressure showed a faster transient magnetic field but much less pronounced oscillations (Fig. 4b). The question of how much plasma is formed on impact, at what temperature, and the type of transient instabilities generated therein needs further investigation.

Conclusions

Our experiments indicate that: (1) plasma is created by hypervelocity impact of small projectiles on a basalt surface; (2) an external magnetic field is impressed during impact, the SARM value being of the order of, or slightly exceeding that, of the TRM of samples of the same basalt. However, when the external field is as low as the geomagnetic field, the observed SARM is substantially larger than the corresponding TRM. (3) By extrapolating this result to impacts occurring in a field free region, it would seem that a magnetic perturbation can be created by the impact and impressed in the basalt. This point, however, requires further detailed measurements in a field-free environment.

We thank the Raufoss Ammunisjonsfabriker AS for hospitality and support and, in particular, the help of the Chief Engineer E. Myrvold, and Dr E. Trøen. We thank Professor R. Hide, of the Meteorological Office, Bracknell, UK, Dr L. J. Srnka, of the Lunar Science Institute, Houston, Texas, USA, and Mr J. Dangerfield, Institute of Geological Sciences, London, UK for discussions. The Turners Hill quarry at Rowley Regis provided the basalt blocks free of charge. We also thank Mr F. Schofield of Sussex University for building most of the experimental apparatus and Mr O. P. Solberg, Mr B. Blackman for assisting during the tests.

Received 16 May; accepted 3 August 1977.

- Strangeway *et al.* *Lunar Sci.* **IV**, 697-743 (1973).
- Collinson, D. W. *et al.* *Proc. 6th Lunar Sci. Conf.* 3049-3062 (1975).
- Anderson, K. *et al.* *IUGG Conference*, (Grenoble, 1975).
- Runcorn, S. K. *Proc. R. Soc. A* **325**, 157-174 (1971).
- Urey, H. & Runcorn, S. K. *Science* **180**, 636-638 (1973).
- Goswami, J. N. *Nature* **261**, 675-677 (1976).
- Goldstein, B. E., Phillips, R. J. & Russell, C. T. *Proc. 7th Lunar Sci. Conf.* (1976).
- Wasilewski, P. J. *The Moon* **6**, 264-291 (1974).
- Fuller *et al.* *The Moon* **9**, 57-86 (1971).
- Pohl, J. & Stoffel, H. *Z. Geophys.* **37**, 857 (1971).
- Hide R. *The Moon* **4**, 39 (1972).
- Gold, T. & Soter, S. *Planet. Space Sci.* **24**, 45-54 (1976).
- Stamper, J. A. *et al.* *Phys. Rev. Lett.* **26**, 1012-1015 (1971).
- Bird, R. S. *et al.* *Phys. Rev. A* **7**, 1328-1331 (1973).
- Stamper, J. A. & Ripin, B. H. *Phys. Rev. Lett.* **34**, 138-141 (1975).
- Widner, M. M. *Phys. Fluids* **16**, 1778-1780 (1973).
- Braynski, S. I. *Transport Processes in a Plasma*, in *Reviews of Plasma Physics*, p. 272. Consultant Bureau, New York, (1965).
- Srnka, L. *Spontaneous magnetic field generation in hypervelocity impact. Proc. 8th Lunar Conf., Houston, Texas* (1977).
- Cable, A. J. *High velocity impact phenomena* (ed. Kinslow) p. 11. (Academic, New York, 1970).
- Rose, M. F. & Grace, F. I. *Brit. J. appl. Phys.* **18**, 671-674 (1967).
- Cikowski, S., Fuller, M. *et al.* *Proc. IV Lunar Conference*, in *Geochim. Cosmochim. Acta* **3**, 3003-3017 (1974).

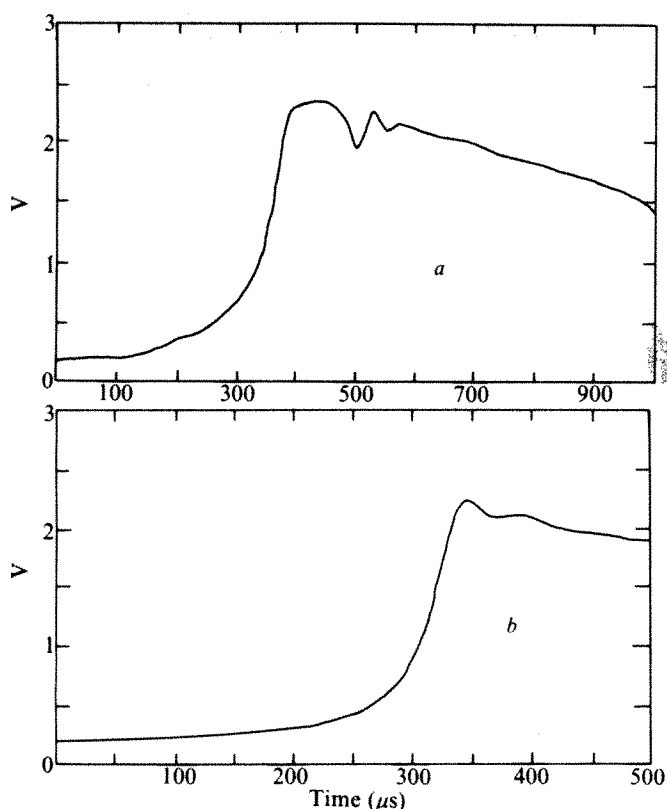


Fig. 4 Pickup signals from a coil surrounding the impact area. *a*, The vessel evacuated to 10^{-2} torr; *b*, in air. Note the different time scales.

(3) in the ambient magnetic field which, inside the bunker, was 0.5 G in the vertical direction.

The results of each of these tests are as follows:

Test type (a). Magnetic field measurements of the impact area yielded profiles such as that shown in Fig. 3. It can be seen that the largest SARM values are found in the neighbourhood of the field after impact is parallel to that of the externally applied field. The extreme values of the measured field due to SARM effects in the neighbourhood of the crater are shown in Fig. 2, as the error box marked 'a'.

Test type (b). In the region neighbouring the crater, relatively large magnetic fields were detected (approximately 100 mG). The maximum perturbations seem to be concentrated near the crater rim, along the fracture lines, that is, where the shock reached probably its highest value. The extreme values of the measured magnetic field for this test is also shown in Fig. 2, and is marked 'b'.

Test type (c). The results of the only impact experiment

Thermodynamic theory of size dependence of melting temperature in metals

P. R. Couchman* & W. A. Jesser

School of Engineering and Applied Science, Department of Materials Science, University of Virginia, Charlottesville, Virginia 22901

The way that small particles melt is a crucial element in the construction of a thermodynamic treatment of the relation between particle size and melting temperature. There are indications that melting is initiated at the surface and that the solid-liquid interface sweeps rapidly through the solid at the melting temperature. The formal and physical elements of the indicated nucleation and growth criterion for melting are discussed and the existence of upper and lower limits on the melting temperature is outlined. Theoretical predictions show satisfactory agreement with experimental observations.

THE effect of particle size on melting temperature has promoted explicit theoretical discussions since the early 1900s and extensive experimental investigations have been reported. The qualitative experimental features are fairly simple—a monotonic decrease in melting temperature with decreasing particle size^{2–16}, this variation seems to be more substantial in the lower end of the size spectrum than in the 'large' size regime^{5–13, 15, 16}.

Previous theories have considered the solid-liquid-vapour mutual equilibrium¹⁷ (actually a size-dependent triple point^{18–20}) as the chemical equilibrium condition for a solid sphere embedded in bulk liquid¹⁸ (this, however, is related to the classical supercooling problem rather than to the melting of small particles), chemical equilibrium between solid and liquid spheres of identical radius¹⁹. In a later theory the 'thermodynamic melting temperature' was defined as that of Pawlow¹⁷ (although this is not necessarily the actual transformation temperature)²¹. Several of these and other works^{4–10, 19} have considered solid-liquid contact to be a central point: the favoured liquid skin theory considers chemical equilibrium between a finite liquid layer and the surrounded solid at the melting temperature^{4, 5, 7, 9}. Unfortunately, no physical criterion has emerged by which to determine the liquid thickness; this quantity (assumed constant) and the solid-liquid interfacial tension are determined by fitting the theoretical expression to experimental results.

In view of these problems associated with previous treatments of the size-dependence of melting temperature we have reconsidered the problem, and we present a thermodynamic theory of the relation between particle size and melting temperature.

Size dependence of melting temperature

Unlike in bulk systems, in small particles the mode of melting is a crucial element for the construction of a thermodynamic theory of melting. The size-dependence of intensive parameters in these systems requires that the geometry and extent of both phases be determined at the melting temperature. Consequently, it is imperative to consider the question of the mechanism of melting in general, and of small particles in particular.

First, note the essential asymmetry between melting and freezing—substantial liquid supercoolings are normal, whereas there is no significant superheating in solids. There is evidence that this lack of superheating is a surface-related effect^{22–24}; additional support for the important role of the surface in initiating melting is provided by kinetic studies of superheating^{25, 26}, low-energy

electron diffraction (LEED) studies of surface melting and freezing of (Pb, Bi and Sn) single crystal surfaces²⁷ and neutron scattering experiments indicating the absence of any bulk phenomena associated with melting in Al to within 10^{-1} K of the melting temperature²⁸. Moreover, molecular dynamics simulations of melting in small clusters of (rare gas) atoms indicate quite unambiguously that the transition is initiated at the surface^{29–31}. Finally, the fact that liquid metals wet the parent solid, energetically favours the initiation of melting at the surface²³.

Evidently, at the melting temperature a liquid layer is formed on the surface and moves at a substantial rate into the solid. It is these nucleation and growth characteristics of the melting phenomenon that may be used to construct a thermodynamic theory of the size-dependence of melting temperature.

The (closed, constant total volume) system considered is an isolated one-component collection of non-contiguous metal spheres at a uniform temperature and individually in mechanical equilibrium, resting on a non-interacting substrate. There are no dissociated species, no evaporation occurs during melting and gravitational effects are assumed negligible. Additionally, the difference between surface stress and surface free energy per unit area³² is neglected.

It is useful to recognise that a size-dependent absolute lower limit on the melting temperature exists. Neglecting the small vapour free-energy change this temperature, T_{lb} , is defined by the condition that the Helmholtz free energy difference between the final, entirely liquid, and initial, entirely solid, particles vanishes. The Helmholtz potential is appropriate as there is a pressure discontinuity associated with the melting of small particles. In equation (1) the subscripts s and l refer to solid and liquid; σ , areal surface work; r , particle radius; v , atomic volume; T_0 , bulk melting temperature; and l_0 latent heat, whence neglecting all but first order terms

$$T_{lb} = T_0 \left\{ 1 - \frac{3}{l_0} \left[\frac{r_s \sigma_s}{r_s} - \frac{r_l \sigma_l}{r_l} \right] \right\} \quad (1)$$

Although T_{lb} has been discussed as the two-state melting temperature^{7, 9, 34} it cannot be the actual melting temperature unless the mechanism by which the final state is attained is irrelevant (that is, in the limit of vanishing particle size).

The melting mode, and consequently the actual melting temperature, involves the formation of a liquid layer and a criterion to determine its spontaneous growth to consume the solid. The derivation and formal analysis of the Helmholtz free energy difference ΔF_i between a core of solid surrounded by a concentric liquid layer and an entirely solid particle is thus complemented by the development of a melting criterion; their combined use leads directly to a quantitative estimate of the relation between particle size and melting temperature and, finally, to a comparison with experimental results. For brevity and simplicity we reproduce the principal features of the results of this energetic analysis by considering (where appropriate) only first-order terms. The superscript prime denotes quantities of the combined (solid-liquid) system. Those of the entirely solid system have no superscript. Pressure, temperature, chemical potential, number of atoms, volume and area are respectively, P , T , μ , N , V , A . The double subscript sl denotes quantities of the solid-liquid interface. Matter conservation is assumed between the solid and liquid states. Formally, ΔF_i is given by

*Present address: Materials Research Laboratory and Polymer Science and Engineering Department, University of Massachusetts, Amherst, Massachusetts 01002.

$$\Delta F_i = -P_s'V_s' + P_l'V_l' - P_i'V_i' + (\mu_s' - \mu_s)N_s' + (\mu_s' - \mu_s)N_l' + \sigma_{sl}A_{sl} + \sigma_l A_l - \sigma_s A_s \quad (2)$$

Explicit integration of the relevant Gibbs–Duhem relations provides a lengthy and analytically intractable expression for ΔF_i (ref. 1). Fortunately there are two simplifying approximations which retain all essential physical insight of the problem and provide an expression amenable to formal qualitative investigation: the liquid and solid atomic volumes are taken to be identical ($v_s = v_l \equiv v$) and both of these phases are assumed incompressible. These approximations yield the following expression

$$\Delta F_i = \frac{l_0(T_0 - T)}{T_0} \times \frac{4\pi}{3} \frac{r^3 - (r-t)^3}{v} + \sigma_{sl}4\pi(r-t)^2 + (\sigma_l - \sigma_s)4\pi r^2 \quad (3)$$

where r denotes the initial solid (and here, final liquid) radius and t the liquid thickness. This function may be shown to have a stationary value (σ_{sl} , σ_l and σ_s are here approximated as size-independent) with respect to t (or, here, N_l) at constant temperature, when

$$\frac{l_0(T_0 - T)}{T_0} = \frac{2\sigma_{sl}v}{(r-t)} \quad (4)$$

This is simply the condition that the solid core has the same chemical potential as the surrounding liquid layer. For $\sigma_{sl} < 0$, physical solutions of this relation demand that $t > r$ and $T > T_0$. The extremum of equation (4) is then a maximum

$$\frac{1}{8\pi} \left(\frac{\partial^2 \Delta F_i}{\partial t^2} \right)_T = -\sigma_{sl} \quad (5)$$

and thus, equation (4) defines an unstable chemical equilibrium. An approximate upper limit, T_{ub} , on the melting temperature may be inferred from the condition $\delta \Delta F_i / \delta N_l|_T > 0$ for vanishing t

$$T_{ub} = T_0 \left(1 + \frac{2\sigma_{sl}v}{l_0 r} \right) \quad (6)$$

Below T_0 the transformation of solid to liquid is favoured by the surface term (due to the wetting condition) and retarded due to the volume (latent heat) term. This is the converse of the classical nucleation problem and is consistent with the surface-induced nature of melting³⁵.

If the non-wetting condition $\sigma_s > \sigma_l + \sigma_{sl}$ is obeyed, the possibility of superheating exists; the condition $T < T_0$ restores the latent heat as the driving force for melting. It seems that a theory of the size-dependence of solid superheating analogous to the melting theory is possible. This explanation and such a theory relate to Peppiatt's experiments²².

The Helmholtz free energy difference, ΔF_i , required to form a liquid layer on and from the completely solid particle is a maximum at the critical liquid thickness, t_c , defined by equation (4). In nucleation theory the probability of attaining this critical fluctuation will determine the speed of movement of the solid–liquid interface into the solid. A formal relation between temperature, the critical Helmholtz energy fluctuation ΔF_c and a measure, J , of this rate of movement is given by

$$J = C \exp[-\Delta F/kT] \quad (7)$$

where the pre-exponential factor C is only mildly temperature dependent and is taken as constant³⁵ and equal to that calculated by Stowell³⁶. The critical melting fluctuation ΔF_c is formally given here by equation (3) evaluated at the chemical potential equality condition of equation (4). The various dependences (calculated, estimated or measured) of quantities needed to determine correction terms to the first-order expression quoted here are discussed in detail elsewhere¹.

Comparison of theory with experiment

A comparison of the predictions of this theory with experimental results is given in Figs 1–3. Sn, In and Au were chosen because these

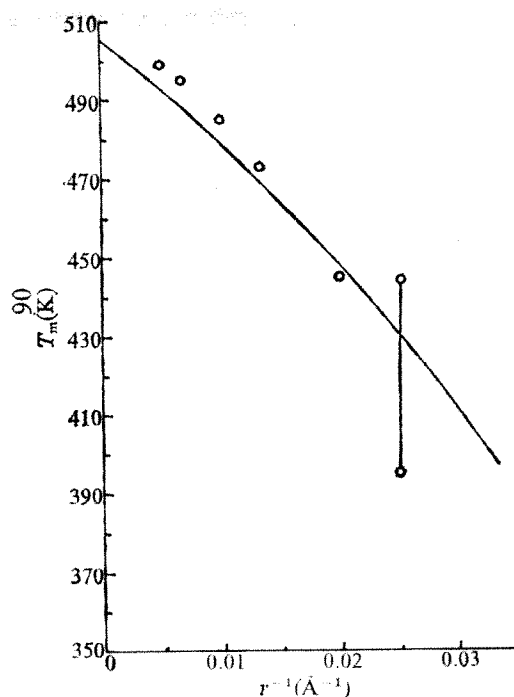


Fig. 1 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for tin. —, Calculated; O, experimental. The latter were obtained directly or by interpolation from Wronski⁵ and are (except at $r^{-1} = 0.025 \text{ Å}^{-1}$) midway between the experimental scatter. The scatter increases as particle size decreases and consequently is largest (and mostly unavoidable) at smallest r . At $r^{-1} = 0.025 \text{ Å}^{-1}$ the apparent upper and lower values of T_m are illustrated. The solid curve represents the results of the calculation using equations (3, 4 and 7).

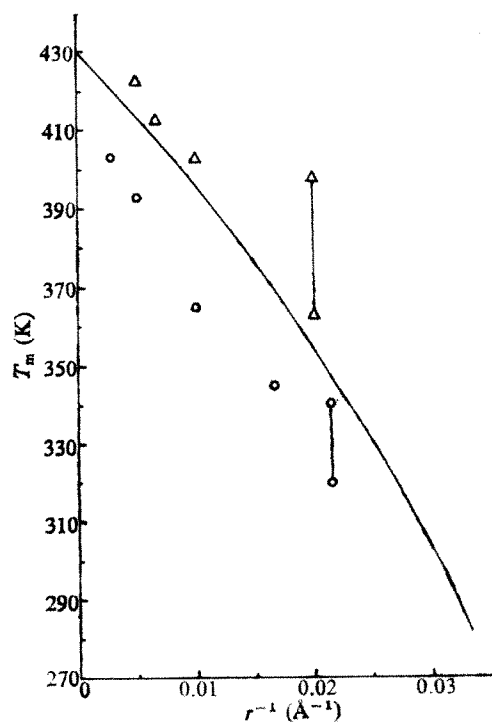


Fig. 2 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for indium. —, Calculated; O, experimental (Pöcza, Barna and Barna¹³); Δ, (Berman and Curzon¹¹). The points are taken directly from Pöcza *et al.* and directly or by interpolation from Berman and Curzon. The scatter in the small size regime is illustrated for Berman and Curzon's data; the error bars reported by Pöcza *et al.* for small r are likewise given. The solid curve represents the results of the calculation using equations (3, 4 and 7).

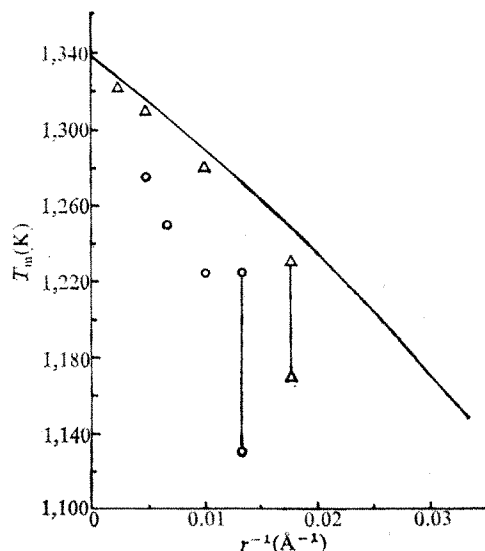


Fig. 3 Relation between melting temperature (T_m) and reciprocal particle radius (r^{-1}) for gold. —, Calculated; Δ , experimental (Sambles¹⁰); \circ , (Buffat and Borel¹⁶). Data obtained and treated as for Fig. 1.

were the only metals with both a sufficient number of surface parameters available (latent heats and heat capacities are relatively well documented) to allow theoretical calculations and a sufficient number of experimental results to allow a significant comparison between theory and experiment. The qualitative agreement is good. Further, the quantitative agreement is satisfactory in view of the approximations involved in the theory, the known lack of precision (and accuracy) in some of the quantities and their variation and the difficulty in obtaining accurate melting temperature and size measurements over such a large range. Actually, the only surface parameter we used which had any substantial experimental disagreement is σ_{sl} . We have used values consistent with the strong evidence that its direct determination from grain boundary grooving measurements³⁷ yields a significantly larger value than that inferred from supercooling measurements—the latter give a lower limit to σ_{sl} .

These calculations indicate that the critical liquid thickness (t_c) is not constant for each metal^{4,5,7,9} but decreases monotonically with decreasing particle size.

Conclusion

The thermodynamic theory of the size-dependence of melting temperature outlined here seems to provide a satisfactory and physically acceptable explanation of the phenomenon and is in reasonable agreement with experimental observations. Although other treatments have implied that solid-liquid interface movement is important in the transition^{4-9,15,19} (Tammann³⁸ was the first to suggest that melting is a surface phenomenon) none has sought to explore the surface nucleation and liquid layer growth characteristics of melting in order to provide a formal treatment of the size-dependence of melting temperature.

One expected and important feature of melting in small systems that this treatment predicts is the 'smearing out' of the transition as particle size decreases. This effect stems from the basic (nucleation and growth) characteristics of the melting phenomenon although its predicted extent is minimal; and the transition is predicted as extremely sharp down to very small particle size. (< 1 K even at the small end of the size spectrum.)

Received 3 May; accepted 28 July 1977.

1. Couchman, P. R. thesis, Univ. Virginia (1975/1976).
2. Takagi, M. *J. phys. Soc. Japan* **9**, 359–363 (1954).
3. Blackman, M. & Curzon, A. E. in *Structure and Properties of Thin Films* (eds Neugebauer, C. A., Newkirk, J. B. & Vermilyea, D. A.) 217–222 (Wiley, New York, 1959).
4. Curzon, A. E. thesis, Imperial College, Univ. London (1959).
5. Wronski, C. R. M. thesis, Imperial College, Univ. London (1963).
6. Wronski, C. R. M. *Br. J. appl. Phys.* **18**, 1731–1737 (1967).
7. Coombes, C. J. thesis, Imperial College, Univ. London (1970).
8. Coombes, C. J. *J. Phys. F* **2**, 441–448 (1972).
9. Sambles, J. R. thesis, Imperial College, Univ. London (1970).
10. Sambles, J. R. *Proc. R. Soc. Lond. A* **324**, 339–351 (1971).
11. Berman, R. P. & Curzon, A. E. *Can. J. Phys.* **52**, 923–929 (1974).
12. Gladkikh, N. D., Niedermayer, R. & Spiegel, K. *Phys. stat. Solid.* **15**, 181–192 (1966).
13. Pocza, J. F., Barna, A. & Barna, P. B. *J. Vac. Sci. Tech.* **6**, 472–473 (1969).
14. Stowell, M. J., Law, T. J. & Smart, J. *Proc. R. Soc. Lond. A* **318**, 231–241 (1970).
15. Peppiatt, S. J. & Sambles, J. R. *Proc. R. Soc. Lond. A* **345**, 387–399 (1975).
16. Buffat, Ph. & Borel, J.-P. *Phys. Rev. A* **13**, 2287–2298 (1976).
17. Pawlow, P. *Z. phys. Chem.* **65**, 1–35; 545–548 (1909).
18. Rie, E. *Z. phys. Chem.* **104**, 354–362 (1923).
19. Reiss, H. & Wilson, I. B. *J. Colloid Sci.* **3**, 551–561 (1948).
20. Defay, R., Prigogine, I., Bellemans, A. & Everett, D. H. *Surface Tension and Adsorption* 244–251 (Longmans Green, 1966).
21. Hanszen, K.-J. *Z. Phys.* **157**, 523–553 (1960).
22. Peppiatt, S. J. *Proc. R. Soc. Lond. A* **345**, 401–412 (1975).
23. Christian, J. W. *The Theory of Transformations in Metals and Alloys* (Pergamon, New York, 1965).
24. Domb, C. *Il Nuovo Cimento, Suppl. Series* **10**, 9–26 (1958).
25. McKinney, J. T., Jones, E. R. & Webb, M. B. *Phys. Rev.* **160**, 523–530 (1967).
26. Lander, J. J. *Progress in Solid State Chemistry* **2** (ed Reiss, H.) (Pergamon, New York, 1965).
27. Goodman, R. M. & Somorjai, G. A. *J. chem. Phys.* **52**, 6325–6335 (1970).
28. Klastrup-Kristensen, J. & Cotterill, R. M. J. *Physics of Non-Equilibrium Systems: Fluctuations, Instabilities and Phase Transitions* (ed Riste, T.) (Leiden, Noordhoff, 1975).
29. Kristensen, W. D., Jensen, E. J. & Cotterill, R. M. J. *J. de Physique, Colloque C2, suppl. no. 4*, **36**, C2–C21 (1975).
30. Briant, C. L. & Burton, J. J. *J. chem. Phys.* **63**, 2045–2058 (1975).
31. Cotterill, R. M. J. *Phil. Mag.* **32**, 1283–1288 (1975).
32. Linford, R. G. *Solid State Surface Science* **2** (ed Green, M.) (Dekker, New York, 1973).
33. Couchman, P. R. & Everett, D. H. *J. electroanal. Chem.* **67**, 382–386 (1967).
34. Hill, T. L. *Thermodynamics of Small Systems*, Part 1, 114–132 (Benjamin, New York, 1963).
35. Christian, J. W. *The Theory of Transformations in Metals and Alloys* 2nd edn, Part I (Pergamon, New York, 1975).
36. Stowell, M. J. *Phil. Mag.* **22**, 1–6 (1970).
37. Nash, G. E. & Glucksmann, M. E. *Phil. Mag.* **24**, 577–592 (1971).
38. Tammann, G. *Zeit. anorg. allg. Chem.* **10**, 166 (1920).

Corrected age of the Pliocene/Pleistocene boundary

Bilal U. Haq & W. A. Berggren

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

John A. Van Couvering

University of Colorado Museum, Boulder, Colorado 80302

Calcareous plankton datum-events are referred to the Pliocene/Pleistocene boundary at Le Castella, the stratotype Calabrian at Santa Maria di Catanzaro, and to deep-sea sediments in six piston cores. The boundary is correlated by multiple overlapping criteria to a level equivalent to, or slightly younger than, the top of the Olduvai Event, giving a revised estimate of about 1.6 Myr for the age of the boundary. The Pliocene/Pleistocene boundary is thus coeval with the earliest of four major climatic deteriorations in the Pleistocene, reconciling palaeoclimatic concepts with the chronostratigraphical definition of the epoch.

ESTIMATES of the age of the Pliocene/Pleistocene boundary have varied from about 0.6 Myr¹ to > 4 Myr^{2,3}. The absence of reliable palaeomagnetic measurements on the stratotype of the Calabrian at Santa Maria di Catanzaro⁴ and the boundary-stratotype section at Le Castella^{5,6} in southern Italy has prevented direct correlation with the palaeomagnetic polarity 'scale'. Over the last decade biostratigraphical studies on the critical sections in southern Italy and also on palaeomagnetically-dated deep-sea cores have resulted in essentially two estimates for the age of the boundary: 1.6–1.8 Myr, by association with the Olduvai Event^{7–11} and 2.8 Myr, by association with the Kaena Event^{4,12–14}. Those who agree with the younger date have generally believed that the base of the Calabrian, as defined at Santa Maria di Catanzaro¹⁵, was stratigraphically equivalent to the 'marker-bed' at Le Castella¹⁶; these workers have

referred to the Le Castella 'marker-bed' section as the reference section for dating the age of the boundary. On the other hand, those who agree with the older date have consistently referred the boundary to a level near the physical base of the Catanzaro section. This level, the approximate transition from Pliocene clays to sandier beds above that indicate a different environment—the so-called Sandy Calabrian—is more than 75 m below the level of the prominent sandy calcarenite, Bed G-G' of Gignoux's defining study, which was the designated base of the Calabrian Stage originally and which is now placed at the base of the Calabrian stratotype^{17,18}.

We present here the results of a study of the calcareous plankton

(first appearance datum) of the members of the coccolithophore genus *Gephyrocapsa* in four of the palaeomagnetically-dated deep-sea cores (Fig. 1) and at Le Castella (Fig. 2). The minute size of the specimens (commonly 1.5–7 µm) and the subtlety of the criteria that distinguish various *gephyrocapsid* taxa, makes species differentiation (except the end members of the lineage, *G. aperta* and *G. oceanica*) under light microscope at best unreliable. Scanning electron microscopy, as used in this study, is essential (Fig. 4).

In core V12-18 *G. aperta*, the earliest (Fig. 1) and morphologically simplest form (Fig. 4a), appears at about 2.25 Myr. More evolved forms, such as *G. protohuxleyi* and *G. omega*, appear within the Olduvai Event in cores CH61-171 and V12-18,

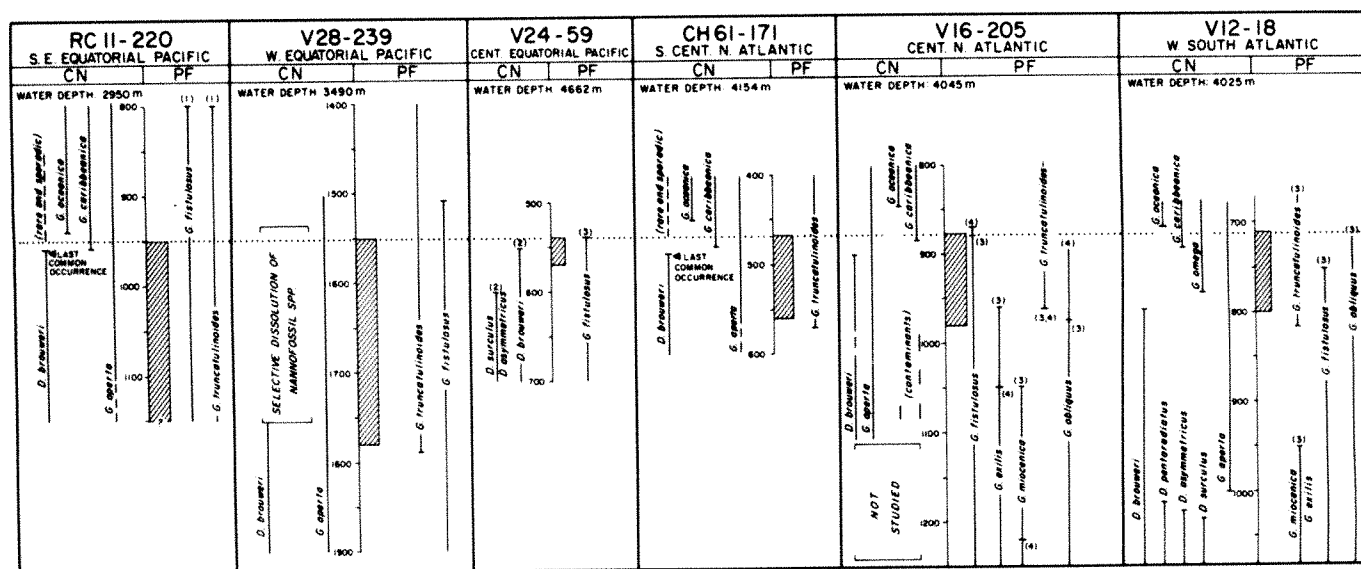


Fig. 1 Biostratigraphy of piston-core samples, showing ranges of taxa that are critical to the correlation of the Pliocene/Pleistocene boundary as related to normal-polarity Olduvai Event (hatched interval). Ranges are shown according to observations made in this study; previously published observations of range-limits in these cores are indicated by numbered points, as follows. (1), Bielak and Briskin⁴⁰; (2), Gartner⁴¹; (3), Saito *et al.*¹¹; (4), Briskin and Berggren³⁷. Location of the various cores is: RC11-220: 14° 49'S, 139° 58'W; V28-239: 03° 15'N, 159° 11'E; V24-59: 02° 34'N, 145° 32'W; CH61-171: 26° 41.5'N, 39° 23'W; V16-205: 15° 24'N, 43° 24'W; V12-18: 28° 41.7'S, 34° 29.6'W.

of six palaeomagnetically-dated deep-sea cores, and of the Calabrian sections of southern Italy. We have established a calcareous plankton biochronology in the deep sea cores that is applicable to these sections and which provides a more accurate means of estimating the age of the Pliocene/Pleistocene boundary than has previously been possible. We use the geomagnetic polarity scale of Cox¹⁹, consider the Gilsa and Olduvai events to be age equivalent²⁰, and assign ages of 1.61–1.79 Myr to the upper and lower limits of the Olduvai Event, respectively.

Material

We have examined, or reviewed literature on, calcareous plankton microfossils from six palaeomagnetically-dated deep-sea cores (Fig. 1). Planktonic foraminifera from core V16-205 were examined by W.A.B. for another study so that two sets of observations are shown for that core. Calcareous nannoplankton are selectively dissolved and fragmented over the interval of the Olduvai in V28-239, but the remaining biostratigraphical data on the cores is shown in Fig. 1. By way of comparison, calcareous nannoplankton and selected foraminifera have been examined in 60 samples from the stratigraphical section at Le Castella (Fig. 2) and samples from Santa Maria di Catanzaro (Fig. 3) which were collected by F. Barbieri in 1971.

Calcareous nannoplankton

The basic biochronological framework for correlating the Pliocene/Pleistocene boundary is provided by the sequential FAD

respectively. In four cores (CH61-171 and V16-205 from the South Central North Atlantic; V12-18 from the western South Atlantic; and RC11-220 from the South-East Equatorial Pacific) the upper boundary of the Olduvai is straddled by the FAD of *G. caribbeana* (below) and *G. oceanica* (above). Mean ages of 1.62 Myr and 1.57 Myr respectively are assigned to these FAD events.

In the relatively long South Atlantic core V12-18, it has also been possible to assign ages to the LAD (last appearance datum) of the last four remaining discoasters of late Pliocene age. *Discoaster surculus*, *D. asymmetricus* and *D. pentaladialis* disappear sequentially around 2.3 Myr, just before the FAD of *G. aperta* (Fig. 1). The terminal discoaster, *D. brouweri*, disappears within the Olduvai Event, but apparently slightly earlier (that is, near the base of the Olduvai, ~1.8 Myr) in the South Atlantic core V12-18 than in the Central North Atlantic (CH61-171, V16-205), or Equatorial Pacific (RC11-220, V24-59) where it disappears close to the top of the Olduvai Event, ~1.64 Myr. Specimens occur rarely and sporadically above this level in some cores. Considering the ease with which calcareous nannoplankton can be reworked into younger strata, the *D. brouweri* LAD as shown in cores CH61-171, V16-205, and RC11-220 may be slightly younger than the actual extinction level of this species. A three-rayed variety of this species becomes more numerous near the LAD in many cores.

The sequence of evolutionary events in the *Gephyrocapsa* lineage seen in the deep sea cores also occurs in the Le Castella section (Fig. 2). We have examined in detail 60 nannoplankton samples from this section under both light and scanning electron

microscopes. *Gephyrocapsa aperta* is found in sample CAT 3, some 105 m below the boundary marker bed. *G. protohuxleyi* is found in sample CAT 14, about 40 m below the marker-bed and morphologically typical *G. caribbeana* (that is, comparable with the holotype) and *G. oceanica* are found in samples CAT 34 and CAT 36, ~30 and 25 m below the marker-bed, respectively. Throughout the Le Castella section, reworked discoasters of early and late Tertiary age are found. But, some 50 m below the marker-bed, *D. brouweri*, the terminal representative of the genus, decreases abruptly in abundance; approaching this level from below, it occurs relatively numerous and continuously and the three-rayed variety grows relatively more numerous. Above this level, however, it occurs relatively rarely and sporadically and about 15 m below the marker-bed it virtually disappears. This distribution pattern of *D. brouweri* has been observed previously²¹, and we consider the true LAD of *D. brouweri* to be ~50 m below the marker-bed.

Planktonic foraminifera

In the same samples, a series of planktonic foraminiferal datum levels are also associated with the early Matuyama Epoch, and the Olduvai Event in particular⁹⁻¹¹.

In the subtropical South (V12-18) and North (V16-205) Atlantic the LAD's of *Globorotalia miocenica* and *Globorotalia exilis* fall within the interval of 2.2 to 2.0 Myr, slightly below the Olduvai Event. The FAD of *Globorotalia truncatulinoides* is associated with the base of the Olduvai Event, occurring some 10-20 cm below it in Equatorial Pacific core V28-239, and

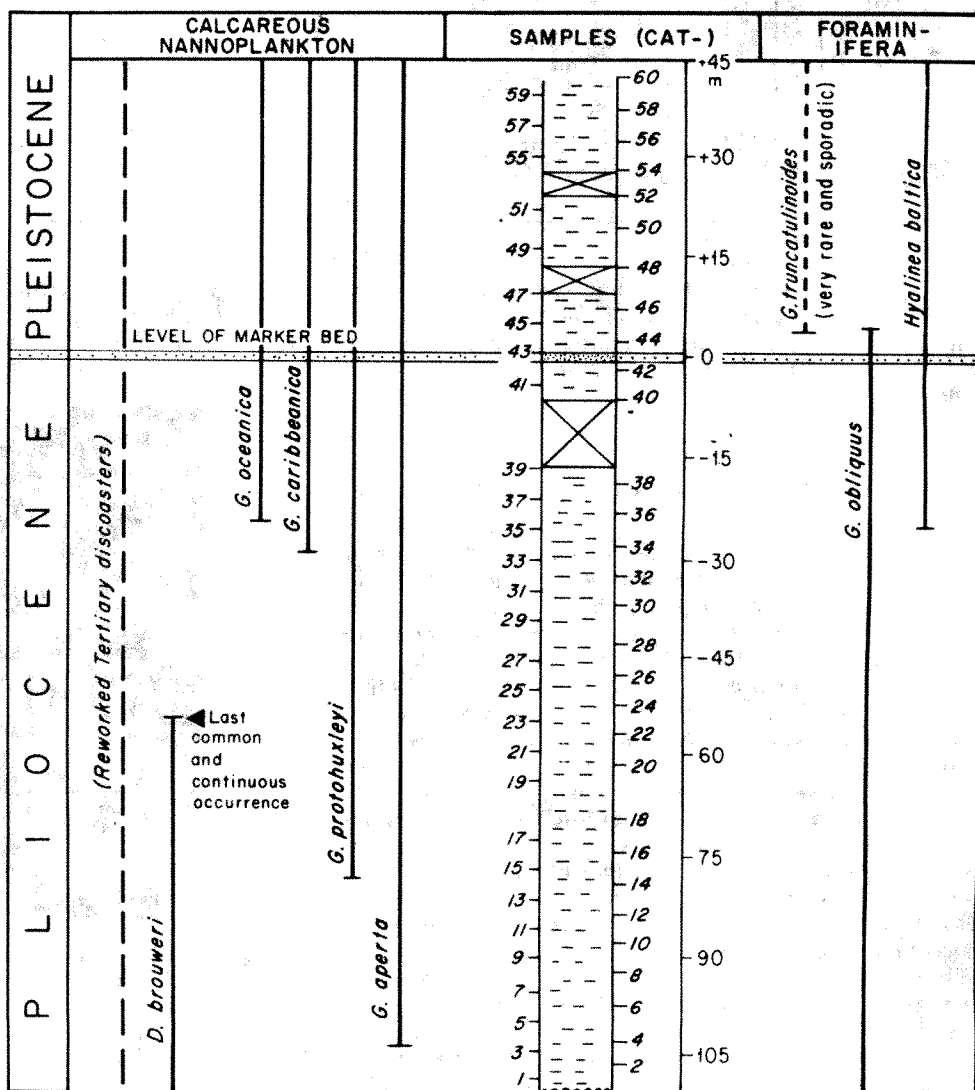
10-20 cm above it in subtropical North Atlantic cores V16-205 and CH61-171. The LAD of *Globigerinoides obliquus* is virtually coincidental with the top of the Olduvai Event in V16-205 and V12-18 (Fig. 1) and with the marker bed at Le Castella (Fig. 2). The LAD of *Globigerinoides fistulosus* also seems to be close to the top of the Olduvai Event (mid-part of Olduvai in V12-18; coincident with the top in V24-59 and slightly above it in V28-239). An anomalously young (~1.3 Myr) LAD of this species is noted in RC11-220.

The lowest recorded occurrence of *Globorotalia truncatulinoides* at Le Castella is ~2 m above the Pliocene/Pleistocene boundary (Fig. 2) and at Santa Maria di Catanzaro ~30-40 m below Bed G-G' (Fig. 3). Various other levels for this first occurrence have been cited^{1,6,16,22,23} but this unreliability seems to be due primarily to the extreme rarity of specimens.

Benthonic foraminifera

The calcareous benthonic foraminifer *Hyalinea baltica* is generally regarded as one of the primary biostratigraphical criteria for the base of the Pleistocene in the Mediterranean²². At Le Castella the FAD of this taxon is generally regarded as coincident with the lithostratigraphical marker-bed (between samples CAT 50 and 51) separating Pliocene and Pleistocene¹⁶. But, the range of *H. baltica* has been found²⁴ to extend at least 25 m below the marker-bed, at which level it constitutes 17% of the total benthonic fauna. At Santa Maria di Catanzaro *H. baltica* occurs near the base of the exposed section, at least 65 m below Bed G-G' (Fig. 4). If the FAD of this taxon was approximately simultaneous within the basin in

Fig. 2 Stratigraphical occurrence of calcareous plankton taxa and *Hyalinea baltica* at Le Castella (Calabria, Italy). Samples (CAT), collected by F. Barbieri in 1971, are the same as those studied by Rio²⁵.



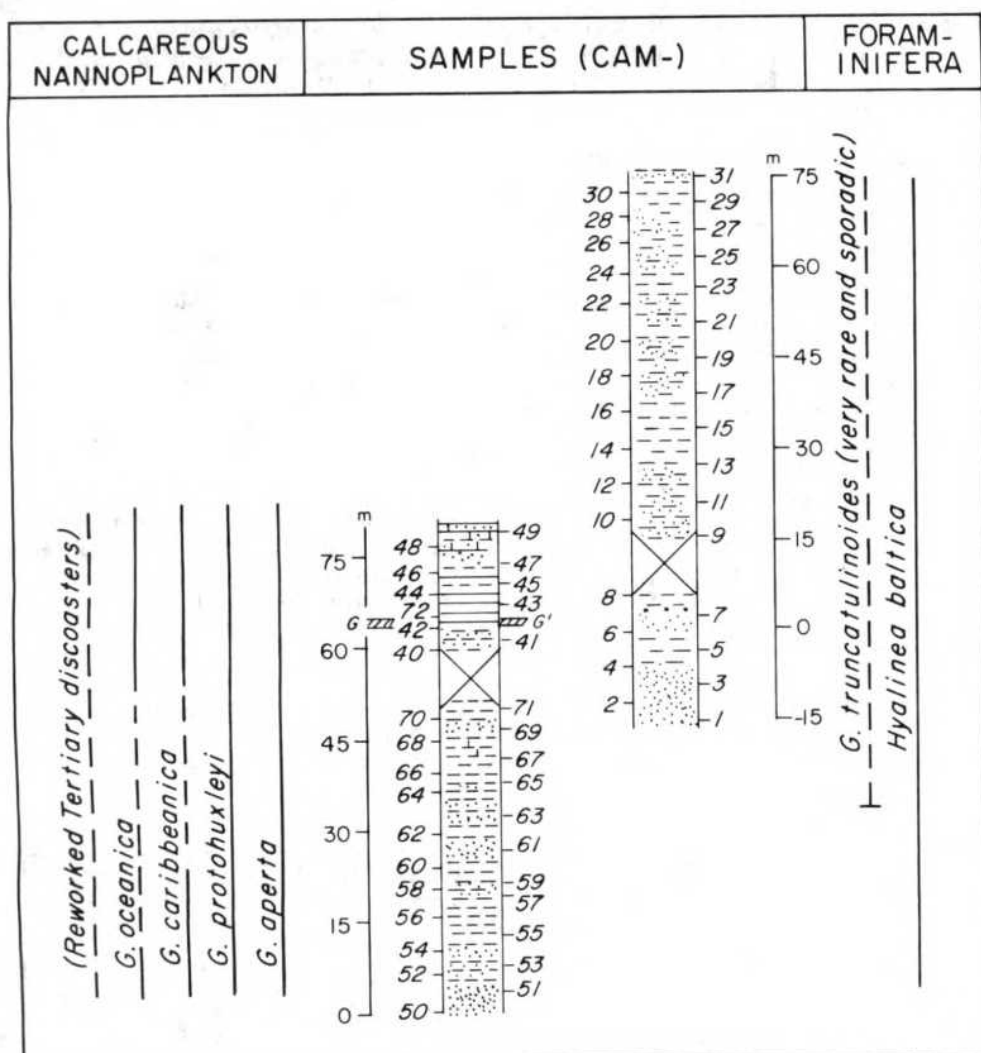


Fig. 3 Stratigraphical occurrence of calcareous plankton and *Hyalinea baltica* at Santa Maria di Catanzaro (Calabria, Italy). Samples (CAM), collected by F. Barbieri in 1971, are the same as those studied by Rio²⁵.

which these two sections are located, it would suggest that the marker bed at Le Castella is significantly older than Bed G-G' at Santa Maria di Catanzaro. This is, in fact, the conclusion that we have reached from the comparison of calcareous plankton datum events.

Age of Pliocene/Pleistocene boundary

If we apply the calcareous nannoplankton biochronological framework established from deep-sea cores (LAD of discoasters at 1.65 Myr and FAD of *Gephyrocapsa caribbeanica* and *Gephyrocapsa oceanica* at 1.60 Myr; Fig. 1) to the Le Castella section and assume a relatively constant rate of sedimentation, a minimum age of ~1.55 Myr is derived for the level of the marker bed denoting the Pliocene/Pleistocene boundary. A similar conclusion with a 1.4-Myr estimate for this boundary was published by D. Rio²⁵. But, the association of the *Globigerinoides obliquus* LAD with the top of the Olduvai Event in deep-sea cores and with the marker-bed at Le Castella suggests an age closer to 1.6 Myr for the boundary level. The upper bathyal clays of the Le Castella section are known to include (at least minor amounts of) clastic turbidites that contain displaced shallow-water benthonic foraminifera. A uniform sedimentation rate therefore cannot be assumed, and in view of these uncertainties we suggest a mean age of 1.6 Myr for the Pliocene/Pleistocene boundary at Le Castella.

There are two lithostratigraphical levels which are cited as definitive for the conceptual basis of the Pliocene/Pleistocene boundary: (1) Bed G-G', at Santa Maria di Catanzaro, a prominent calcarenitic lens (of turbidite origin) which contains abundant fossils of the shallow-water mollusc *Arctica islandica*

and is generally considered to define the lithostratigraphical base of the Calabrian Stage^{17,18}; (2) the indurated (turbiditic) sand layer at Le Castella (between samples CAT 50 and 51) and which has been generally considered as the boundary-stratotype of the

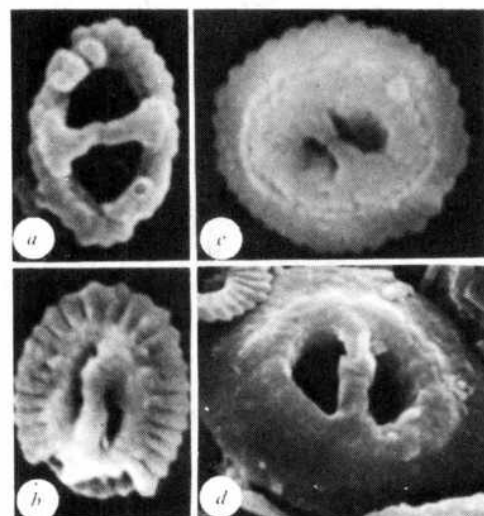


Fig. 4 Scanning electron micrographs of important gephyrocapsid species used in the biochronology of the Pliocene/Pleistocene boundary interval: a, *Gephyrocapsa aperta* Kamptner, Core CH61-171, 431 cm; b, *G. protohuxleyi* McIntyre, Santa Maria di Catanzaro Section, CAM-1; c, *G. caribbeanica* Boudreaux and Hay, Core V12-18, 709 cm; d, *G. oceanica* Kamptner, Core CH61-171, 431 cm. $\times 10,000$.

Pliocene/Pleistocene^{16,26}. Correlation between the stratigraphic sections and the age of the boundary markers at Le Castella and Santa Maria di Catanzaro has caused much controversy. Orthodox opinion has been that the two marker-beds are stratigraphically equivalent^{16,22} and that the boundary is about 1.8 Myr^{10,11,27}. One strongly dissenting opinion^{12-14,28} has been that the true base of the Calabrian, and the Pliocene/Pleistocene boundary, is represented by the base of sandy Calabrian beds that extend more than 75 m stratigraphically below Bed G-G' at Santa Maria di Catanzaro, and that this level should be dated at about 2.8 Myr. In this view, the marker bed at Le Castella is about 1 Myr younger, and represents the base of the Emilian Stage. In contrast, another dissenting opinion²⁹⁻³¹ holds that the lowermost Calabrian—equivalent to the Le Castella section—is missing at Santa Maria di Catanzaro because the sandy Calabrian is transgressively unconformable on the clayey Pliocene beds, and as a consequence all of the Calabrian (*s.l.*) above this contact is younger than the Le Castella marker-bed. The contact has also been described as a fault¹⁸, but even so, Bed G-G' clearly seems to be younger than the marker-bed at Le Castella. This is because the entire 65 m of sandy Calabrian underlying Bed G-G' contains *Hyalinea baltica*²⁴ together with the various Pliocene/Pleistocene species of *Gephyrocapsa* noted above (Fig. 3; see also ref. 14) in an association that first appears at Le Castella only a short distance below the Pliocene/Pleistocene boundary (Fig. 2). The fact that *G. obliquus* has not been observed in the sandy Calabrian tends to confirm the correlation of this section to a level above the Le Castella marker-bed (see Fig. 5).

The presence of discoasters in the Catanzaro and Le Castella sections to levels well above the marker-beds has been cited^{14,16} as evidence that the Calabrian (and the Pliocene/Pleistocene boundary) is older than the Olduvai Event, but last occurrences of nannofossils are less reliable, due to reworking, than first occurrences as biochronological datums; in this particular case the argument that the sudden reduction in numbers of discoasters about 50 m below the Le Castella marker-bed represents the actual

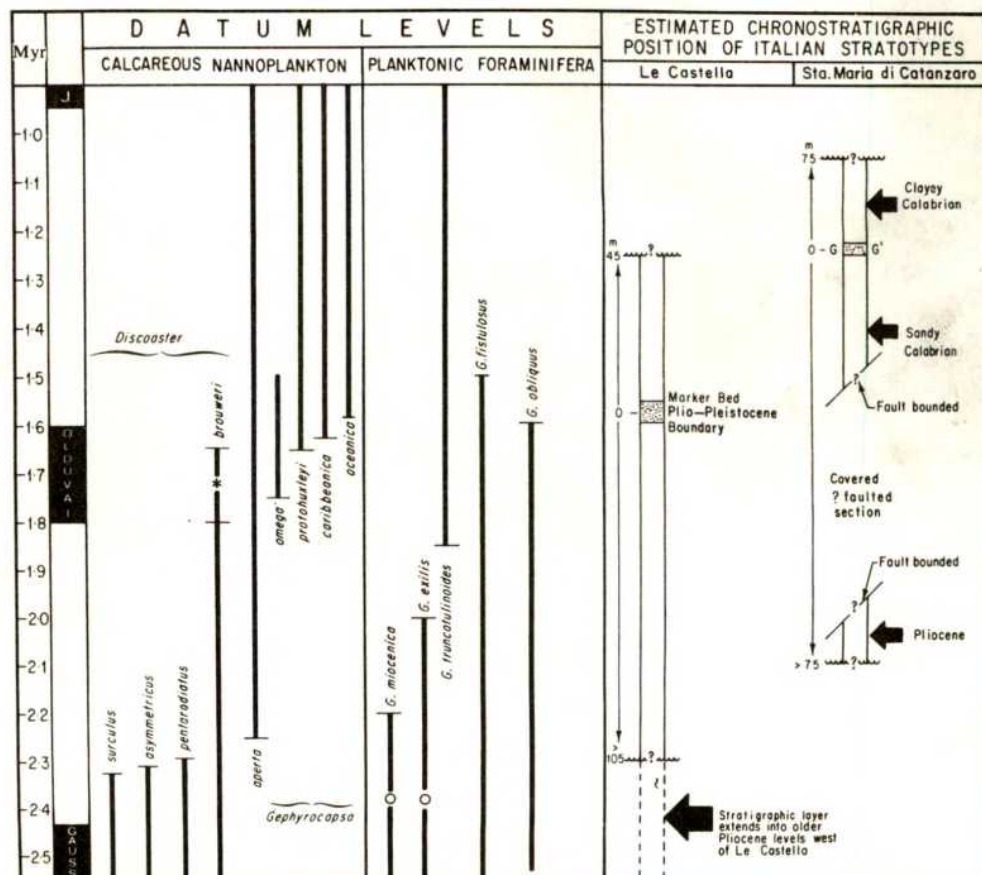
LAD agrees with the nanoplankton biostratigraphy in the deep-sea cores (Fig. 1). Thus, all of the discoasters collected in the Santa Maria di Catanzaro Calabrian (*s.l.*), both below and above Bed G-G' are probably reworked because they are stratigraphically above the FADs of *G. caribbeana* and *G. oceanica* and the LAD of *G. obliquus*.

We seem now to be faced with a paradox. According to the criteria already cited, Bed G-G' at Santa Maria di Catanzaro (which denotes the base of the stratotype of the Calabrian Stage) is younger than the marker-bed at Le Castella (which denotes the Pliocene/Pleistocene boundary). If this is so, then the section of sandy Calabrian below Bed G-G' is both Pliocene because it is pre-Calabrian, and Lower Pleistocene because it is younger than the Pliocene/Pleistocene boundary. By accepting the view that there is only one definition of the Pliocene/Pleistocene boundary, that of Le Castella, we resolve the paradox and simultaneously demonstrate the value of the 'golden spike' in picking apart such conundrums.

Calabrian, Emilian and Sicilian

The stage-subdivisions of the Italian Lower Pleistocene are based partly on geomorphological and continental evidence, and partly on marine invertebrate biostratigraphy, because of the environmental and stratigraphic effects of glacio-eustatic changes³. Radiometric dating^{3,32,33} of strata assigned to Pleistocene stages, therefore, is not clearly applicable to the marine sequence, nor is the marine sequence well-correlated. To cope with this, Ruggieri and his colleagues³¹ proposed the use of invertebrate datum events—the FADs of *Arctica islandica*, *Hyalinea baltica*, and *Globorotalia truncatulinoides*—to identify the beginning (base) of the successively younger Calabrian, Emilian, and Sicilian Stages, respectively. In this proposed arrangement, the section at Santa Maria di Catanzaro from Bed G-G' upward is specifically correlated to the Sicilian Stage of Palermo³¹ because the occurrence of *A. islandica* in Bed G-G' is (rightly) held to be accidental,

Fig. 5 Pliocene/Pleistocene calcareous plankton biochronology in deep-sea cores and estimated chronostratigraphic position of about 1.8 Myr (*) in one of the cores studied (V12-18). The upper limit of this species, as shown here, may thus be somewhat younger than the actual extinction datum, due to reworking at the depositional interface. ○, Atlantic only.



but the occurrence of *Globorotalia truncatulinoides* close to this level is held to represent the true FAD of this species and thus the base of the Sicilian.

Aside from violating the principle that stratotypes, not fossils, define a stage, the proposal³¹ is untenable on the basis of biostratigraphy cited here. First, *H. baltica* ranges down through the sandy Calabrian at Catanzaro²³, and down to 25 m below the Pliocene/Pleistocene marker-bed at Le Castella²⁴, which would indicate that these strata belong to the Emilian, not the Calabrian Stage, and that there is, apparently, no Calabrian in Calabria at all. Second, the occurrences of *Globorotalia truncatulinoides* in the Pliocene/Pleistocene of Calabria are rare and unreliable, and the evidence from areas where it is more abundant indicate that its FAD precedes that of *G. caribbeana* and *G. oceanica* (Fig. 5) and thus, one may presume, the FAD of *H. baltica* (see Fig. 2). In summary, to follow the proposal of Ruggieri and others³¹ in this region would first eliminate the Calabrian in its present form by invalidating its stratotype and burying it in the Sicilian Stage, and then would extend the base of the Sicilian (in theory) below that of the Emilian as well. These assumptions as to the relationships of the lower age limits of the proposed defining taxa are incorrect, or (in the case of the shallow-water mollusc *A. islandica*) not demonstrable, at least in the type Calabrian area.

Correlation of discoaster extinction level

We have observed that the extinction of discoasters at Le Castella, marked by a sudden drop in relative abundance of *D. brouweri* that signifies the transition from autochthonous to recycled status, is in close association with the sequential first appearance of various species of *Gephyrocapsa* (Fig. 2). The same relationship in the biostratigraphy of deep-sea cores that overlap the top of the Olduvai Event (Fig. 1) allows us to correlate the Pliocene/Pleistocene of Calabria to the deep-sea record (Fig. 5). Kaneps³⁴ has likewise observed that in equatorial-Pacific core RC12-66, the uppermost autochthonous occurrence of discoasters is within carbonate-minimum 17 near the top of the Olduvai Event, at about 1.65 Myr. But, at DSDP Site 132 in the Mediterranean this datum was found to be below Mediterranean carbonate-minimum M21, within the upper Gauss polarity-stage at about 2.6 Myr, and so it was suggested that the extinction datum of discoasters was correspondingly earlier in the Mediterranean basin than in the open ocean due to differential cooling of the Mediterranean. Our data suggest, on the other hand, that *D. brouweri*, the last of the discoasters, became extinct in the Mediterranean and in the tropical Pacific and Atlantic oceans almost simultaneously. The apparent diachroneity cited in DSDP Site 132³⁴ may be due to local effects, or to miscorrelation.

Palaeoclimatic history and the beginning of the Pleistocene

When the Pliocene/Pleistocene boundary was correlated to the beginning of the Olduvai Event according to the best data of a few years ago⁷⁻¹¹, oceanographers found that there were no identifiable major climate changes in the deep-sea record (the land record was simply too vague) that could be correlated to such a boundary. This seemed to justify a new approach to the boundary, implicit in the recommendation of the 1948 International Geological Congress³⁵ that the boundary be defined by the base of the Calabrian Stage in Italy—the beginning of the golden spike concept. Abandoning any reference to palaeoclimatology seemed healthy iconoclasm at the time, because of irritating and unreconcilable arguments that were beginning to arise out of radiometric dating on 'the earliest glacial event' ranging from 0.5 Myr to 3 Myr^{2,14} or more—evidently palaeoclimatic models had different attributes in different places.

The *Globorotalia truncatulinoides* datum has now proved to be a weak reed, and with the correlations described here we are on firmer, slightly different ground. It has long been supposed that the

change from Piacenzian clayey sediments to the markedly different lithology and colder-water fauna of the Calabrian was a reflection of Pleistocene climate replacing pre-glacial Pliocene^{1,6,27,28}, and the 1948 Commission referred to this in its recommendation³⁵. (For those unfamiliar with golden spikes, the reasons for establishing a physical reference point as a boundary definition become immaterial once the definition, or spike, is pounded in.) But, oceanographers, accustomed to the *truncatulinoides* connection, have habitually ignored or discounted the palaeoclimatic criterion as wishful thinking by workers bemused by over-dramatic concepts of the Ice Ages.

With the revised estimate of 1.6 Myr for the boundary, these conflicting concepts of the beginning of the Pleistocene seem to be reconciled. Synthesis of marine and nonmarine data for the Late Neogene had already shown that the first major glacial advance in North America, the Nebraskan, and correlative climate-induced effects elsewhere, should be dated close to 1.5 Myr³⁰, and that three others begin at approximately 0.9, 0.6, and 0.3 Myr respectively. More recently, quantitative factor-analysis and oxygen-isotope studies of microfossils in cores from the equatorial Pacific and tropical North Atlantic^{37,38} have been used to derive Pleistocene-palaeoclimatic curves. These show clearly-developed periodicities of 92,000 yr—as predicted long ago by Milankovitch according to computations of orbital precession and recently used, with varying success, in Late Pleistocene climatostratigraphy^{1,16,39}—superimposed on much broader and stronger climate cycles with a periodicity of about 500,000 yr. The earliest of four of these major cold-water, high-salinity, high-seasonality peaks seen in the North Atlantic core³⁷ centres at 1.5 Myr, just above the top of the Olduvai Event. This palaeo-oceanographic feature is clearly to be equated in age with the Nebraskan glaciation³⁶. From the data presented here, it can also be clearly equated with the Pliocene/Pleistocene boundary as defined by the marker-bed at Le Castella.

We thank the Lamont-Doherty Geological Observatory, F. Barbieri, Gulf Oil Corporation and M. Briskin for core and outcrop material studied here. D. Rio (Parma) has already published some conclusions about the relationship of the Calabrian marker-beds²³ that anticipate ours, with fewer data. D. Bayliss, C. Denham, D. Johnson, and T. Saito reviewed the manuscript. This research was supported by USNSF grant OCE 76-21274 (to B.U.H. and W.A.B.).

Received 21 June; accepted 20 July 1977.

- ¹ Emiliani, C. *J. Geol.* **63**, 562 (1955); *Ann. N.Y. Acad. Sci.* **95**, 534 (1961).
- ² Savage, D. E. & Curtis, G. H. *Am. Ass. Petrol. geol. Bull.* **51**, 480 (1967).
- ³ Ambrosetti, P. *et al. Boll. Soc. Geol. Ital.* **87**, 333 (1968).
- ⁴ Watkins, N. D., Kester, D. A. & Kennett, J. P. *Earth planet. Sci. Lett.* **24**, 113 (1974).
- ⁵ Nitsuma, N. *Mar. Geol.* **6**, 105 (1970).
- ⁶ Lamb, J. L. *Gulf Coast Ass. geol. Soc. Trans.* **21**, 415 (1971).
- ⁷ Berggren, W. A., Phillips, J. D., Bertels, A. & Wall, D. *Nature* **216**, 253 (1967).
- ⁸ Glass, B., Ericson, D. B., Heezen, B. C., Opdyke, N. D. & Glass, J. A. *Nature* **216**, 437 (1967).
- ⁹ Hays, J. D., Saito, T., Opdyke, N. D. & Burckle, L. H. *Geol. Soc. Am. Bull.* **80**, 1500 (1969).
- ¹⁰ Hays, J. D. & Berggren, W. A. in *Micropaleontology of the Oceans* (eds Funnell, B. M. & Riedel, W. R.), 680 (Cambridge University Press, London, 1971).
- ¹¹ Saito, T., Burckle, L. H. & Hays, J. D. in *Late Neogene Epoch Boundaries* (eds Saito, T. & Burckle, L. H.), 235 (Micropaleontology, New York 1975).
- ¹² Lamb, J. L. *Geol. Soc. Am. Abstr. Progr.* Part 7, 280 (1969).
- ¹³ Beard, J. H. *Geol. Soc. Am. Abstr. Progr.* Part 7, 256 (1969).
- ¹⁴ Lamb, J. L. & Beard, J. H. *Univ. Kansas Paleontol. Contrib. Art.* 57 (Protozoa 8), 27 (1972).
- ¹⁵ Gignoux, M. *Ann. Univ. Lyon N.S.* **36**, 22 (1973).
- ¹⁶ Emiliani, C., Mayeda, T. & Selli, R. *Geol. Soc. Am. Bull.* **72**, 682 (1961).
- ¹⁷ Selli, R. *Geol. Soc. Am. Bull.* **37**, 58 (1971).
- ¹⁸ Selli, R. *Geol. Soc. Am. Bull.* (in the press).
- ¹⁹ Cox, A. *Science* **163**, 237 (1969).
- ²⁰ Gromme, C. S. & Hay, R. L. *Earth planet. Sci. Lett.* **10**, 179 (1971).
- ²¹ Takayama, T. *J. Mar. Geol.* **6**, 72-73 (1970).
- ²² Bayliss, D. D. *Lethaia* **2**, 133 (1969).
- ²³ Bandy, O. L. & Wilcoxon, J. A. *Geol. Soc. Am. Bull.* **81**, 2939 (1970).
- ²⁴ Bremer, M., Briskin, M. & Berggren, W. A. *10th INQUA Congr., Abstr.* (in the press).
- ²⁵ Rio, D. *Ateneo Parmense. Acta Nat.* **10**, 416, 421 (1974).
- ²⁶ Vanzo, S. *Ateneo Parmense. Acta Nat.* **11**, 423 (1975).
- ²⁷ Selli, R. *Prog. Oceanogr.* **4**, 67 (1967).
- ²⁸ Smith, L. *Gulf Coast Ass. geol. Soc. Trans.* **19**, 583 (1979).
- ²⁹ Ruggieri, G. *Boll. Soc. Geol. Ital.* **91**, 639 (1972).
- ³⁰ Sprovieri, R., d'Agostino, S. & di Stefano, E. *Riv. Ital. Paleont.* **79**, 127 (1973).
- ³¹ Ruggieri, G., Buccheri, G., Greco, A. & Sprovieri, R. *Boll. Soc. Geol. Ital.* **94**, 889 (1976).
- ³² Ambrosetti, P. *Quaternaria* **9**, 267 (1967).
- ³³ Bonadonna, F. B. *Mem. Soc. Geol. Ital.* **7**, 261 (1968).
- ³⁴ Kaneps, A. G. in van Andel, T. H., *et al. Initial Reports of the Deep Sea Drilling Project* **16**, 876 (1973).
- ³⁵ Committee for the Study of the Plio-Pleistocene Boundary, *Int. Geol. Congr. Rep. 18th Session, Great Britain* **9**, 6 (1948).
- ³⁶ Berggren, W. A. & Van Couvering, J. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **16**, 1 (1974).
- ³⁷ Briskin, M. & Berggren, W. A. in *Late Neogene Epoch Boundaries* (eds Saito, T. & Burckle, L. H.), 167 (Micropaleontology, New York 1975).
- ³⁸ Shackleton, N. J., and Opdyke, N. D. *Geol. Soc. Am. Mem.* **143**, 449 (1976).
- ³⁹ CLIMAP Project Members *Science* **191**, 1131 (1976).
- ⁴⁰ Bielik, L. E. & Briskin, M. *Mar. Micropaleont.* **2** (in the press).
- ⁴¹ Gartner, S. *Geol. Soc. Am. Bull.* **84**, 2021 (1973).

Cultured epithelial cells of cornea, conjunctiva and skin: absence of marked intrinsic divergence of their differentiated states

Tung-Tien Sun & Howard Green

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Keratinocytes of three different epithelia grown in cell culture express a large number of differentiation markers with either no differences or relatively small differences, depending on the species. Much of the distinctive phenotype of these epithelia in vivo must be due to external modulation and relatively little, at least in the case of the human, to permanent intrinsic divergence during development.

THE corneal and conjunctival epithelia and the epidermis are stratified squamous epithelia. Cells in the basal layer of all three seem similar, but differentiation in the superficial layers is obviously different. The corneal and conjunctival epithelia do not possess the granular cell layer and anucleate stratum corneum typical of epidermis. Since all three epithelia rise embryologically from closely related precursors, it is not surprising that they should share some common properties; but as these epithelia later become very different, they might be thought to have diverged markedly and irreversibly during embryogenesis.

With proper fibroblast support, human epidermal cells (keratinocytes) can be grown serially in cell culture^{1,2}, where they show many of the differentiation markers characteristic of this cell type *in vivo*. Single cells give rise to colonies, each forming a stratified squamous epithelium containing multiplying and differentiating cells. The cells synthesise keratins (T.-T.S. and H.G. unpublished) and grow in size, eventually forming cornified (cross-linked) envelopes^{3,4}. The final stages of epidermal differentiation are promoted by placing the cells in suspension—a condition which does not permit the cells to grow; instead they become permeable to Trypan blue, their keratin filaments become detergent insoluble, they form cross-linked envelopes, and with the aid of serum plasminogen, their nuclei are digested⁵.

We describe here the behaviour of corneal and conjunctival epithelial cells when grown in the same conditions. They can multiply and carry out the same programme of differentiation as the epidermal cells, including some aspects not observed *in vivo*. All three cell types are clearly keratinocytes and, at least in the case of the human, their behaviour in common culture conditions is so nearly the same that we are not able to distinguish them. In the case of the rabbit, there are some persistent, though small, differences between the keratinocytes of different origin.

Fibroblast dependence and effect of EGF

In earlier studies of cultured corneal epithelial cells, fibroblast support was not provided⁶⁻⁸, but epidermal keratinocytes have since been found to require the support of fibroblasts in order to form colonies¹. This support was usually provided in the form of lethally-irradiated 3T3 cells at a density of about $20,000\text{ cm}^{-2}$ inoculated together with or before the keratinocytes. In these conditions, the keratinocytes form expanding colonies, displacing the 3T3 cells from the vessel surface. The epidermal cells are serially cultivable and grow through many cell generations. In the human, epidermal cells do not transform into established lines and retain the diploid chromosome number¹.

This culture system was applied to epithelial cells of cornea and

conjunctiva. Human corneas were obtained from local eye banks and after the endothelial layer and Descemet's membrane were removed with jeweller's forceps, the remaining tissue (epithelium plus a small part of the stroma) was minced to approximately $1-2\text{ mm}^3$ and digested with 0.25% trypsin and 0.002% EDTA at 37°C for 30–45 min. The disaggregated single cells were then plated with irradiated 3T3 cells. Multiplication of human fibroblasts was inhibited by the 3T3 cells, but if necessary, human fibroblasts were selectively removed with 0.02% EDTA after the epithelial colonies grew to appreciable size^{1,3}.

Figure 1 shows subcultures containing corneal epithelial and epidermal colonies 11 d after inoculation of 5×10^4 cells together with irradiated 3T3 cells. The colonies stained red with Rhodanile Blue and were of similar size and appearance. In the absence of 3T3 cells, neither epithelial cell type formed colonies; as there were no fibroblast colonies either, the cultures did not contain appreciable numbers of living human fibroblasts.

In the presence of epidermal growth factor⁹ (EGF), the cells of large colonies of epidermal keratinocytes sustain their growth rate much better than in its absence². Corneal epithelial cells were similarly responsive, judging from the size of the colonies (Fig. 1). Such an effect of EGF is to be expected from its action on intact corneal epithelium¹⁰. Conjunctival epithelial cells were similar to corneal epithelial cells in their colony-forming properties and EGF responsiveness.

When examined under the phase microscope, the basal cells of colonies of human corneal epithelial cells (Fig. 2a) seemed similar to those of epidermal cells (Fig. 2b) in their shape, their mosaic-like arrangement and their ability to displace the 3T3 cells from the vessel surface. The stratified structure of the colonies, and the silver-staining properties of the superficial cells³ were also very similar.

Four strains of corneal epithelial cells, originating from donors aged between 25 and 60, and growing in the presence of EGF, were subcultured twice and grew through a total of about 30 cell generations. Although epidermal cells of newborn donors can grow through many more generations², the culture lifespan of corneal epithelial cells in the present experiments seems similar to that of epidermal cells of adult donors¹.

Synthesis of keratins and other cellular proteins

The keratins of bovine epidermal stratum corneum have been characterised electrophoretically^{11,12}. Those of cultured human epidermal cells and human stratum corneum have recently been found to be similar in solubility, electrophoretic behaviour, immunological reactivity and ability to assemble into filaments *in vitro* (T.T.S. and H.G., unpublished).

The proteins of cultured epidermal, corneal and conjunctival epithelial cells were compared by electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate (SDS). Figure 3a (track 1) shows the total protein of human corneal epithelial cells extracted with 2% SDS and 1% β -mercaptoethanol. Identical patterns were obtained from epidermal and conjunctival cells. In addition to the large number of protein bands unrelated to keratins, there were present several intense bands of keratins with a molecular weight range from slightly greater than that of actin (42,000) to slightly greater than that of tubulin (55,000). The non-

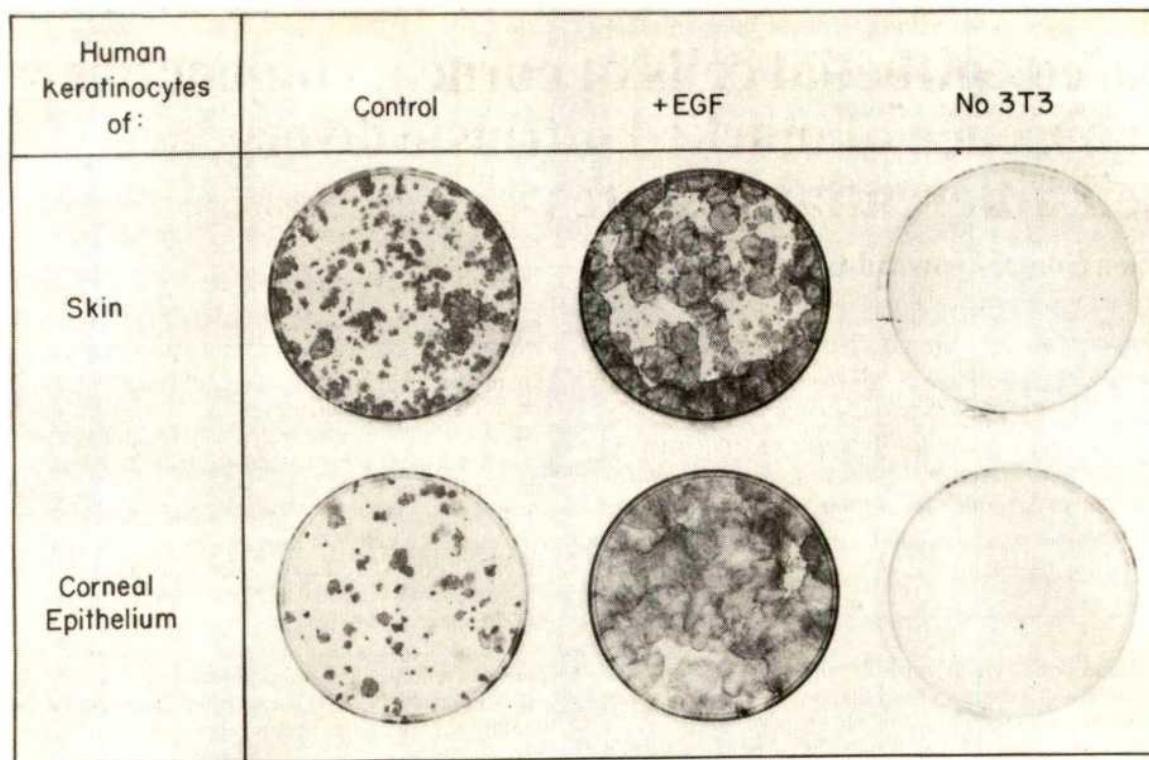
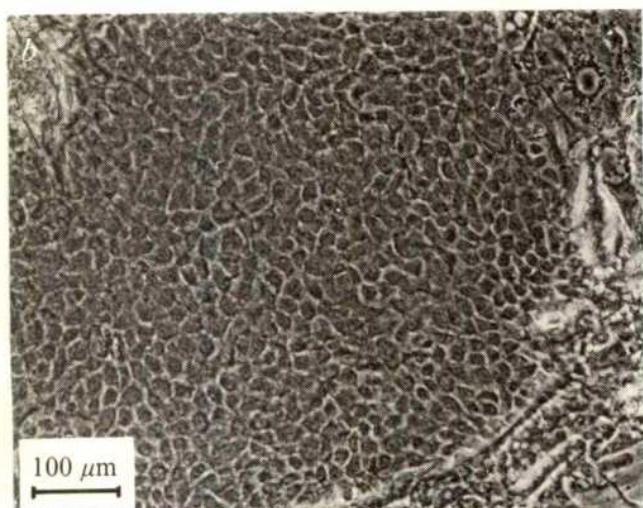
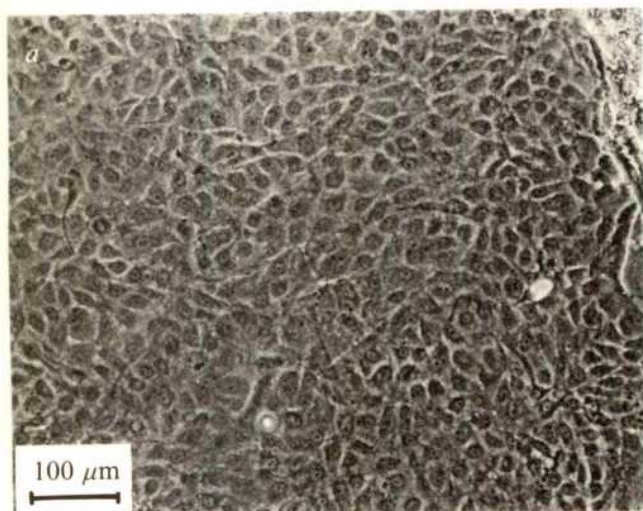


Fig. 1 Colony formation, fibroblast dependence and the effect of EGF. Human corneal epithelial cells (5×10^4 , strain B, donor aged 25 yr, secondary culture) and epidermal cells (5×10^4 , strain M, newborn donor culture) were plated in 60-mm dishes with or without 4×10^5 lethally-irradiated 3T3 cells. To some dishes, epidermal growth factor (EGF) was added to a final concentration of 15 ng ml^{-1} , starting on the third day after plating². After a total of 11 d, the cultures were fixed with 10% formalin and stained with Rhodanile Blue¹⁹.



keratin proteins can be removed selectively by extraction in dilute buffer, leaving substantially only the keratins to be extracted with SDS and reducing agent (Fig. 3a, tracks 2–5). Tracks 2, 3 and 4 show no consistent differences in the position or relative intensity of the keratin bands of corneal and conjunctival epithelial cells and of epidermal cells. No keratins were detected in fibroblast extracts (track 5).

In order to compare the keratins of the different cell types further, antiserum specific for epidermal keratins of human stratum corneum was prepared and tested in double diffusion experiments against an extract of the total protein of each cell type. It was found that the proteins of cultured epithelial cells of epidermis, cornea and conjunctiva reacted strongly with the antiserum and showed a precipitin band in common with one of the bands produced by the keratins of stratum corneum (Fig. 4). A second precipitin band produced by the keratins of stratum corneum was not present in any of the cultured cell types and may have been due to a 63,000-molecular weight protein known to be present in stratum corneum, but not in corneal epithelium or any cultured keratinocytes (unpublished). No visible precipitin band was produced by the proteins extracted from human fibroblasts.

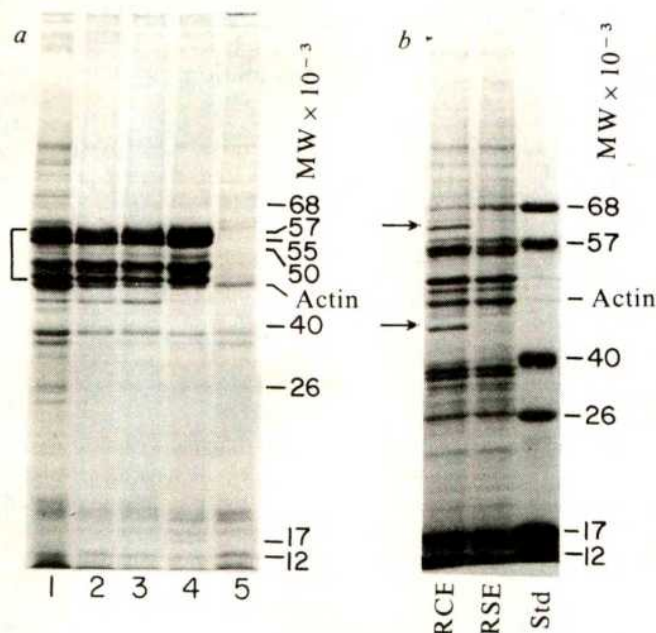
Increase in cell size and formation of cross-linked envelope

During differentiation in culture, human epidermal cells increase their cell size and protein content³ as they do *in vivo*¹³. Figure 5a shows that, like epidermal cells, trypsin-disaggregated corneal epithelial cells adopted an approximately spherical shape and could be seen to vary greatly in size. The smallest cells, which are probably the basal cells³, had a diameter of about $12 \mu\text{m}$. The

Single colonies of corneal epithelial and epidermal cells. Primary colony of corneal epithelial cells of strain B (a) and secondary colony of epidermal cells of strain M (b) 11 d after inoculation. The cultures were fixed with buffered 10% formalin and photographed directly without staining. One margin of each colony appears in the right hand side of the pictures. Phase-contrast microscopy.

largest cells, probably the superficial cells of the epithelium³, exceeded 35 μm in diameter. Some of the large cells possessed cross-linked envelopes similar to those made by terminally differentiated epidermal keratinocytes. The presence of these envelopes was demonstrated by heating a trypsinised cell suspension in the presence of 5% SDS and 1% β -mercaptoethanol, a procedure which dissolves entirely cells without such envelopes but only the intracellular contents of cells with envelopes³ (Fig. 5b). The insolubility of the envelopes is due to proteins cross-linked by ϵ -(γ -glutamyl)-lysine bonds⁴. As in the case of epidermal cells, usually 5–10% of the cells in surface cultures of corneal epithelial cells possessed cross-linked envelopes.

Cross-linked envelopes were also found in human corneal epithelium *in vivo*. Samples were scraped from the surface of excised corneas with a knife, and treated directly with detergent and reducing agent. Microscopic examination showed that envelopes were abundant. These envelopes preserved the flattened shape of the cells *in vivo*, as they do even in cultures of epidermal



The proteins of cultured epithelial cells of cornea, conjunctiva and epidermis. *a*, Human, 3T3 cells were removed from nearly confluent epithelial cultures with isotonic buffer containing EDTA (refs 1, 3). The epithelial cells were collected in a small volume of the same buffer with the aid of a rubber policeman. Half of the cells were extracted directly with a solution of 2% SDS and 1% β -mercaptoethanol at 100 °C for 5 min. The extract was analysed by 12.5% disc polyacrylamide gel electrophoresis in the presence of SDS. The other half of the cells were first extracted with several changes of 100 vol 20 mM Tris-HCl (pH 7.4) and the insoluble proteins were then dissolved in detergent and β -mercaptoethanol as before and subjected to electrophoresis; protein (50–100 μg) was applied to each slot. The direction of electrophoresis was from top to bottom. Numbers on the right hand side of the gel denote molecular weights of standard proteins including human serum albumin (68,000), tubulin (57,000 and 55,000), the heavy chain of human gammaglobulin (50,000), and actin (42,000). The bracket at the left hand side of the gel indicates the mobility range of the keratin proteins. Samples in different tracks are: 1, total proteins of cultured human corneal epithelial cells; 2, insoluble proteins of the same cultured corneal epithelial cells; 3, insoluble proteins of cultured conjunctival epithelial cells; 4, insoluble proteins of cultured epidermal keratinocytes; 5, insoluble proteins of cultured human foreskin fibroblasts. Any apparent difference in band intensity between the extracts of the three epithelial cell types was not reproducible. *b*, Rabbit, 12-d-old primary cultures of corneal epithelial cells and epidermal cells derived from the same 1-yr-old animal were collected. Total cellular protein was extracted and analysed as for the human. Arrows show two non-keratin proteins present only in the corneal epithelial cells. The keratin bands, which lie between actin and molecular weight 57,000, differ slightly in mobility from those of the human and may be less abundant. RCE, Rabbit corneal epithelial cells; RSE, rabbit epidermal cells; Std, molecular weight standards.

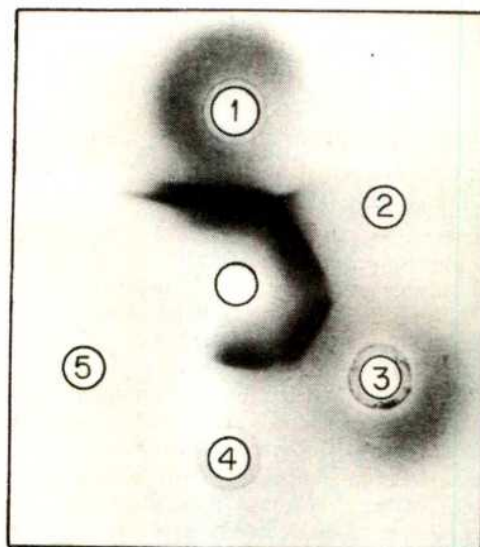


Fig. 4 Immunological cross-reactivity between keratins of human stratum corneum and proteins of cultured human epithelial cells. Stratum corneum was minced and pre-extracted with 8 M urea to remove non-keratin proteins. The keratins were then dissolved in 8 M urea containing 10 mM dithiothreitol. Rabbits were immunised at multiple sites with a total of 20 mg protein over a period of 2 months. Antiserum thus obtained was placed in the centre well of an Ouchterlony plate and tested against 80–120 μg of total cell protein of different cell types extracted with a solution containing 1% SDS, 10 mM dithiothreitol and 10 mM Tris-HCl (pH 7.4). 1, Keratins of stratum corneum (30 μg); 2, extract of cultured epidermal keratinocytes; 3, extract of cultured corneal epithelial cells; 4, extract of cultured conjunctival epithelial cells; 5, extract of cultured human dermal fibroblasts. The double diffusion test was performed in an agar plate containing 0.1% SDS, 0.5% Triton X-100, 0.01% Thimerosal, in addition to phosphate buffer and 1% Agarose, basically according to Yen *et al.*²⁰.

keratinocytes when the cells are treated with the reducing agent and detergent without previous trypsinisation³.

Terminal differentiation of suspended cells

When human epidermal cells are suspended as single cells in medium stabilised with methylcellulose (methocel), they develop disulphide-stabilised keratin filaments and become insoluble in SDS solutions. These conditions also greatly favour the formation of cross-linked envelopes. Eventually, the cell nuclei are destroyed⁵.

By all three criteria, human corneal epithelial cells were found to behave similarly. Within a few days in suspension, all the cells became insoluble in detergent, and about 50% developed cross-linked envelopes (Fig. 6). About 80% of the cell nuclei were destroyed within 9 d.

Properties of rabbit keratinocytes in culture

In order to compare the same cell types from another species, experiments were carried out on epidermal and corneal epithelial cells of the rabbit (newborn to 1 yr old). Both formed stratified squamous epithelial colonies in surface culture and developed detergent-insoluble keratin filaments and cross-linked envelopes in suspension. Both cultured cell types were shown to possess desmosomes and tonofilaments on electron microscopic examination, and both contained keratins demonstrable by electrophoresis (Fig. 3b). It is clear that, as in the human, both cell types are keratinocytes; the two cell types of the rabbit could, however, be distinguished in culture. For example, although the corneal epithelial cells formed cross-linked envelopes in methocel suspension, they did so with lower frequency than epidermal cells and did not form envelopes at all in surface culture. This is perhaps related to the fact that, unlike that of the human, the corneal epithelium of the rabbit *in vivo* does not possess cells with cross-linked envelopes. Rabbit corneal epithelial cells grew poorly on subculture in comparison with the epidermal cells. Finally, the

cultured rabbit corneal epithelial cells contained two water-soluble non-keratin proteins not present in epidermal keratinocytes (Fig. 3b).

External modulation or intrinsic divergence of differentiated state

By the criteria already described, the cultured cells of all three epithelia are keratinocytes (to be distinguished from fibroblasts of corneal stroma, sometimes referred to as keratocytes). In the human cell cultures we have been unable to distinguish the three cell types by any criterion. The same differentiation markers can be expressed in culture by epidermal and corneal epithelial cells of the rabbit, although the culture phenotypes of the two cell types are not identical. These results suggest that an important part of the differences between these epithelia *in vivo* results from differences in the local environment in which the cell type is found.

One obvious local difference is related to the adhesiveness of the superficial cells of the epithelium. A dry-surfaced epithelium (skin) invariably possesses an anucleate stratum corneum, whereas wet-surfaced epithelia frequently do not. Whether an anucleate cell layer is present or not may simply be a matter of the adhesiveness of the superficial cells, which in turn could be affected by the degree of hydration. If adhesiveness is low, detachment may occur before differentiation is complete. This occurs in surface cultures of epidermal keratinocytes; nuclear destruction is completed, for the most part, after detachment of the squames⁵, rather than before, as in intact skin. Similarly, we have shown here that nuclear destruction takes place in corneal keratinocytes suspended in methocel much as it does in epidermal keratinocytes, although corneal epithelium does not normally possess a stratum corneum.

A second and perhaps more important difference is related to the nature of the cells located beneath the epithelium. Instructive effects of the underlying connective tissue are known to specify keratinocyte behaviour in adults¹⁴ as well as in embryonic life¹⁵. In our cultures of the various epithelia, the fibroblast population consists mainly of (irradiated) 3T3 cells. For the reasons mentioned, fibroblasts of the type associated with the epithelium *in vivo* were virtually absent from the cultures studied. The very similar phenotype exhibited by the cultured keratinocytes might be explained by either absence of specific fibroblast instruction or the presence of common instruction provided by the 3T3 cells. The possibility exists that any one of these keratinocyte types, especially in the human, placed in the *in vivo* site of one of the other, even after birth, would conform in phenotype to the other keratinocyte type and generate the site-specific epithelium^{14,16}.

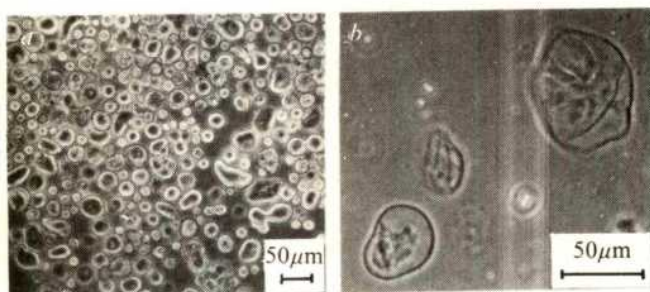


Fig. 5 Cell enlargement and formation of cross-linked envelopes by cultured human corneal epithelial cells. *a*, Heterogeneity of cell size in the growing colonies. 3T3 cells and any contaminating living fibroblasts were removed selectively from a nearly confluent 17-d primary culture with EDTA. The remaining corneal epithelial cells were trypsinised and an aliquot of the single-cell suspension examined in a haemocytometer chamber under the phase microscope. Note the presence of cells of variable diameter from 12 μm to > 35 μm . *b*, Cross-linked envelopes formed in surface culture. To a trypsinised single-cell suspension of cultured human corneal epithelial cells, SDS and β -mercaptoethanol were added to final concentrations of 5% and 1% respectively and the solution was heated to 100 $^{\circ}\text{C}$ for 3 min. The photograph taken under phase microscopy shows several of the large envelopes remaining. All small cells are dissolved.

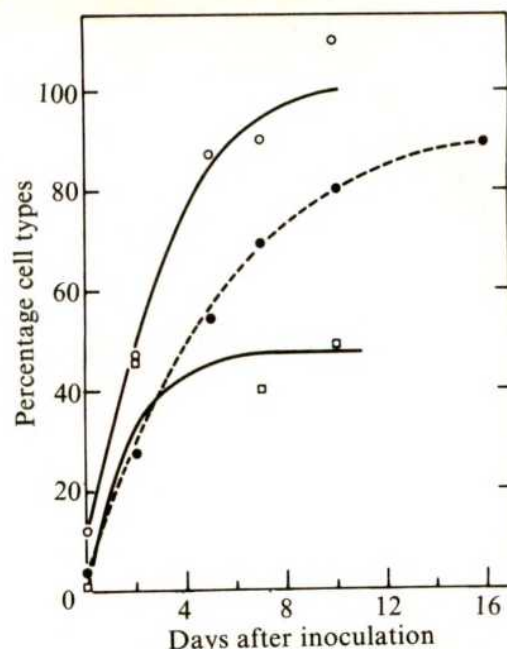


Fig. 6 Terminal differentiation of human corneal epithelial cells in methocel-stabilised suspension. Thirteen-day tertiary cultures of human corneal epithelial cells grown in the presence of EGF were trypsinised and resuspended at 2×10^5 cells ml^{-1} in medium containing 20% foetal calf serum and 1.2% methylcellulose. Aliquots were removed at intervals, diluted in isotonic buffer and the cells centrifuged. Cells insoluble in detergent (○) alone possess disulphide-stabilised keratin filaments. Cross-linked envelopes (□) were scored by insolubility in ionic detergent plus reducing agent. Cells were also deposited on filters, fixed and stained with haematoxylin and eosin, and the proportion of cells possessing nuclei was determined⁵. ●, Anucleate cells.

3T3 cells are known to be fibroblasts¹⁷, but it is not known from what organ they originated, as the line was evolved from mixed mouse embryo cell cultures¹⁸. This may not be important insofar as ability to support proliferation is concerned, since human diploid fibroblasts of non-dermal as well as of dermal origin support the growth of epidermal keratinocytes^{1,19}. The experiments described here also show that the ability of 3T3 cells to support keratinocyte multiplication is not specific for the type of keratinocyte. Although the fibroblast products necessary for the support of keratinocyte growth have not yet been identified, they may be different from those involved in instructive effects.

Whatever the special conditions that may modulate their differentiated state *in vivo*, the three cell types can, in the human, show a common phenotype in culture in which all the properties examined, including the differentiated ones, are expressed equally. In the case of the rabbit, although all the differentiated properties can also be expressed in culture by corneal and epidermal cells, the differences between the two cell types grown in identical culture conditions show that conversion to a common phenotype is not complete. Thus in this species, the intrinsic differences may make a significant contribution to the phenotypic differences between the two epithelia *in vivo*.

These investigations were aided by grants from the National Cancer Institute. Human corneas were provided from local eye banks by Drs Beatrice Y. J. T. Yue, and Jules L. Baum and Miss Rasma Niedra.

Received 20 June; accepted 4 August 1977.

- Rheinwald, J. G. & Green, H. *Cell* **6**, 331-344 (1975).
- Rheinwald, J. G. & Green, H. *Nature* **265**, 421-424 (1977).
- Sun, T.-T. & Green, H. *Cell* **9**, 511-521 (1976).
- Rice, R. H. & Green, H. *Cell* **11**, 417-422 (1977).
- Green, H. *Cell* **11**, 405-415 (1977).
- Allman, M. I. et al. *Invest. Ophthalmol.* **15**, 666-668 (1976).
- Newsome, D. A., Takasugi, M., Kenyon, K. R., Start, W. F. & Opelz, G. *Invest. Ophthalmol.* **13**, 23-32 (1974).
- Yanoff, M. *Documenta ophthalmol.* **41**, 157-204 (1976).
- Cohen, S., Carpenter, G. & Lemback, K. J. in *Advances in Metabolic Disorders* **8**, (ed. Lust, R. & Hall, K.) 265-284 (Academic, New York, 1975).

¹⁰ Savage, C. F., Jr & Cohen, S. *Expl Eye Res.* **15**, 361-366 (1973).¹¹ Steinert, P. M. *Biochem. J.* **149**, 39-48 (1975).¹² Baden, H. P., Lee, L. D. & Kubilus, J. J. *Invest. Derm.* **67**, 573-576 (1976).¹³ Yardley, H. J. & Goldstein, D. F. *Br. J. Derm.* **95**, 621-626 (1976).¹⁴ Billingham, R. E. & Silvers, W. K. *J. exp. Med.* **125**, 429-446 (1967).¹⁵ Sengel, P. *Morphogenesis of Skin* (Cambridge University Press, Cambridge, 1976).¹⁶ Van Scott, E. J. & Reinertson, R. P. *J. invest. Derm.* **36**, 109-131 (1961).¹⁷ Goldberg, B. *Cell* **11**, 169-172 (1977).¹⁸ Todaro, G. J. & Green, H. *J. Cell Biol.* **17**, 299-313 (1963).¹⁹ Rheinwald, J. G. & Green, H. *Cell* **6**, 317-330 (1975).²⁰ Yen, S.-H., Dahl, D., Schachner, M. & Shelanski, M. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 529-533 (1976).

letters to nature

Rapid fluctuations of radio flux and polarisation in quasar 3C273

QUASAR 3C273, one of the brightest and most studied quasars, is variable over a wide wavelength range. The changes of its luminosity may occur on a timescale of a few years or of several days in both the radio¹ and optical^{2,3} range. There are variations of linear and circular polarisation in the radio domain⁴ and sometimes the optical emission is circularly polarised⁵. The data from our simultaneous radio and optical observations show rapid variations of radio flux and circular polarisation at $\lambda = 1.35$ cm, and of optical linear polarisation of 3C273 within one day or several hours. These variations are possibly more rapid than those previously reported.

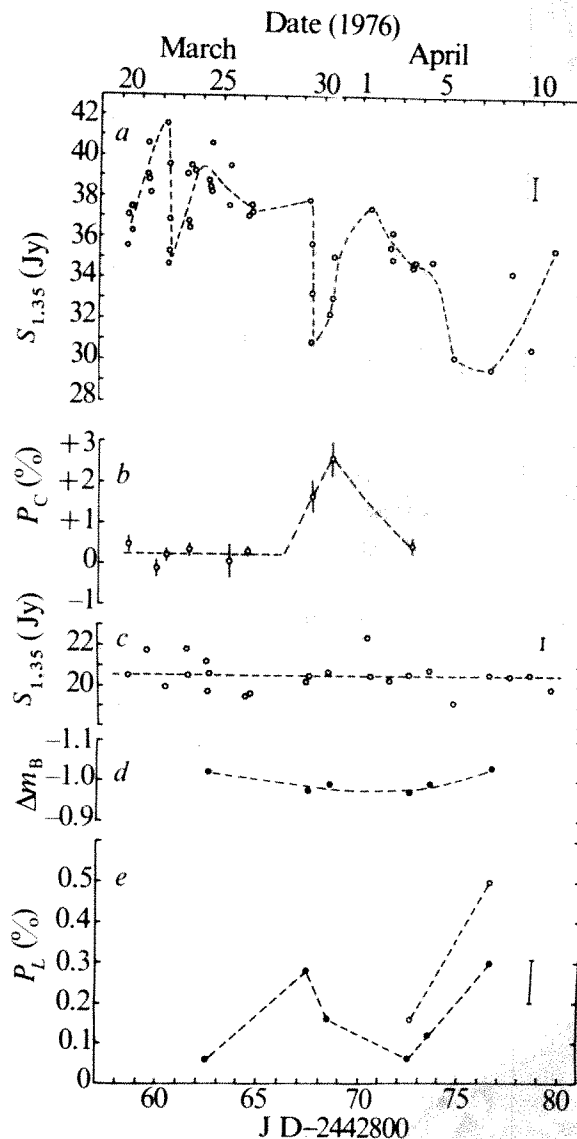
Total flux I and intensity V of circular polarised emission at 1.35 cm of the quasar 3C273 were measured during March–April 1976 using the 22-m radio telescope of the Crimean Astrophysical Observatory. The angular resolution of the radio telescope was $2.5' \times 2.6'$ and the sensitivity was about 0.7 Jy with a time constant of 1 s using a switched receiver with a maser as a high-frequency pre-amplifier. Beam switching in the azimuth plane was used to reduce the influence of fluctuations of atmospheric radio emission. The quarter-plate analyser was placed behind the central circular feed. The main and reference beams had orthogonal polarisation and measurements of flux I were made by an 'on-off' method similar to that described in ref. 6. A minicomputer operating on-line processed the output signals of the receiver and checked its amplification⁷. Flux density was measured relative to the standard sources Saturn (brightness temperature 128 ± 5 K) and the thermal source DR21 (flux density 19.5 ± 0.6 Jy). The water line at 1.35 cm lies in the received band, so the atmospheric attenuation may be variable. Therefore, to eliminate the influence of the atmospheric extinction variations the standard sources and the control source 3C274 were measured just before or after 3C273 and at about the same zenith distances. The corrections for extinction in this case did not exceed a few per cent. The r.m.s. error of the total flux determined with the aid of 3C274 does not exceed 2.5%. Zero level for parameter V was found by observation of DR21 which is expected to have zero circular polarisation; it showed no fluctuations in excess of the r.m.s. errors (its mean value found here is $-0.35 \pm 0.07\%$ where minus corresponds to left-handed polarisation).

Concurrently with radio observations of 3C273 optical observations were carried out with a polarimeter⁸ at the Cassegrain focus of the 2.6-m Crimean reflecting telescope. The optical intensity and the linear polarisation were recorded in B and V spectral bands with $\lambda_{\text{eff}} = 430$ and 540 nm respectively. The optical flux was measured with

respect to the comparison star 50" to the West of 3C273 and polarisation was calibrated with the aid of standard stars⁹.

The results of radio and optical measurements are shown in Fig. 1. They sometimes show rapid intensity fluctuations

Fig. 1 *a*, Variations of the radio flux of 3C273 at 1.35 cm; *b*, the degree of circular polarisation 3C273 at 1.35 cm; *c*, the radio flux of control source 3C274; *d*, the optical brightness 3C273 at the B band in magnitudes; *e*, the degree of linear polarisation of optical radiation in the B (●) and V (○) bands. Vertical bars show the value of r.m.s. error.



of radio emission of 3C273 in a period of one day (22 and 29 March, 5 and 7 April) whereas the optical brightness remains nearly constant. The total (peak-to-peak) amplitude of relative variations of the radio flux from 3C273 is 33%, twice as large as that of the control source 3C274. The ratio of corresponding dispersions is 11.3 and is statistically significant at the $<0.5\%$ level. On the two days 22 and 29 March, variations of the radio flux of 3C273 reaching 20% were observed over about 4 h, although the intensities of 3C274 measured at the beginning and at the end of these runs did not differ significantly. The rapid decrease of radio flux on 29 March was accompanied by an increase in the degree of circular radio polarisation p_c from the average value of $0.24 \pm 0.07\%$ to $2.1 \pm 0.2\%$ and by the appearance of the optical linear polarisation beyond the 3σ range. Another such fluctuation of optical linear polarisation took place near 7 April when the radio flux was also at a minimum. The planes of polarisation on these two occasions were quite different (see Fig. 2).

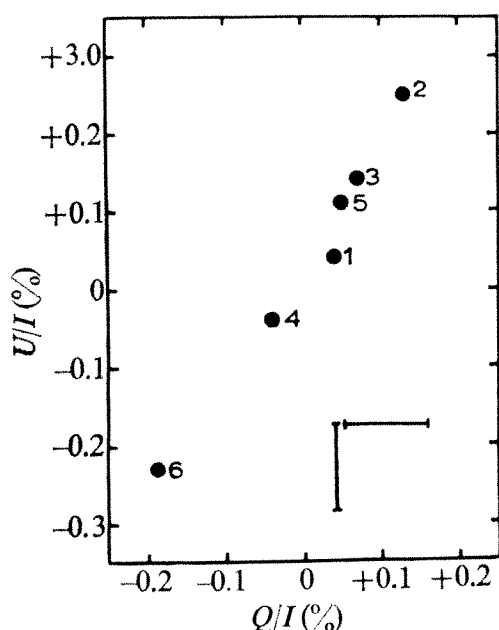


Fig. 2 Diagram of normalised Stokes parameters Q/I and U/I for optical linear polarisation of 3C273 at the B band. 1, 24 March; 2, 29 March; 3, 30 March; 4, 3 April; 5, 4 April; 6, 7 April 1976. Bars show the r.m.s. errors of each parameter.

A similar phenomenon, but with a slower decrease of the flux of 3C273 by 20%, was observed earlier at a wavelength of 3.5 cm on 23–27 May, 1969 (ref. 9). There is also a report about the decrease of the flux density at 7.2 cm of another source OJ287 of the same amount (20%) over about 2 h (ref. 10). It is remarkable that in OJ287 there is a similar correspondence between daily variations of its flux at 1.35 cm and that of optical linear polarisation as in the case of 3C273 mentioned here: optical emission is more polarised near the minimum radio flux^{11,12}.

The observed fluctuation of linear (in optical range) and circular (in radio range) polarisation could eventually be connected with possible rapid changes of transversal and longitudinal components of magnetic field in the quasar 3C273 during the periods of strong diminution of radio flux. This also agrees with the earlier observations of temporary appearance of circular polarisation in the optical range reported in ref. 5 and of the variations of the linear and circular polarisation of this quasar at radio wavelengths⁴.

We thank Professor A. B. Severny for drawing our attention to the possibility of a fast variability in 3C273 and for his interest in our investigation.

V. A. EFANOV
I. G. MOISEEV
N. S. NESTEROV
N. M. SHAKHOVSKOY

Crimean Astrophysical Observatory,
P/O Nauchny,
Crimea, 334413, USSR

Received 21 March; accepted 26 July, 1977.

- ¹ Pauliny-Toth, I. I. K. & Kellermann, K. I. *Astrophys. J.* **146**, 634–645 (1966).
- ² Burkhead, M. S. & Parvey, M. I. *Publ. astr. Soc. Pacific* **80**, 483–486 (1968).
- ³ Lyuty, V. M. *Astr. Zirc. (USSR)*, 1–3 (1976).
- ⁴ Seielstad, G. A. & Berge, G. L. *Astr. J.* **80**, 271–281 (1975).
- ⁵ Severny, A. B. & Kuvshinov, V. M. *Astrophys. J. Lett.* **200**, L 13–17 (1975).
- ⁶ Dent, W. A., Kapitzky, J. E. & Kojoian, G. *Astr. J.* **79**, 1232–1241 (1974).
- ⁷ Domin, S. L., Efanov, V. A., Korsensky, V. A., Moiseev, I. G. & Nesterov, N. S. *Izv. Crimean Astrophys. Obs.* **57** (in the press).
- ⁸ Shakhovskoy, N. M. & Efimov, Yu. S. *Izv. Crimean Astrophys. Obs.* **45**, 90–110 (1972); **54**, 99–119 (1976).
- ⁹ Gorshkov, A. G., Moiseev, I. G. & Soglasnov, V. A. *Izv. Crimean Astrophys. Obs.* **45**, 176–181 (1972).
- ¹⁰ Kikuchi, S. *et al. Publ. astr. Soc. Japan* **25**, 555–561 (1973).
- ¹¹ Efanov, V. A., Moiseev, I. G. & Nesterov, N. S. *Izv. Crimean Astrophys. Obs.* **56**, 68–74 (1977).
- ¹² Shakhovskoy, N. M. & Efimov, Yu. S. *Izv. Crimean Astrophys. Obs.* **56**, 39–51 (1977).

New high energy γ -ray sources observed by COS B

LOCALISED γ -ray sources contribute to the overall galactic emission; some of these sources have been identified with known astronomical objects^{1,2}, while several unidentified γ -ray sources have also been reported^{3,4}. We describe here a search for γ -ray sources using data from the ESA γ -ray satellite COS B which revealed 10 new unidentified sources. These sources seem to be galactic with typical γ -ray luminosities above 100 MeV in excess of 10^{35} erg s⁻¹.

The COS B satellite was launched in August 1975, and most of its (typically 1-month) observations have been devoted to a sensitive survey of the galactic plane. The characteristics of the instrument and the important features of the mission are described elsewhere^{5,6}. The data from four of the observation periods, for which manual analysis procedures⁶ are complete, have been analysed for sources of γ rays of energy > 100 MeV, using a method which yields an angular resolution of about 2.5° (FWHM). In each case the search has been limited to a region 40° wide in galactic longitude and 30° wide in latitude centred on the pointing direction. The method of analysis has been described in detail elsewhere⁷.

From the γ -ray intensity distributions the statistically most significant enhancements⁷ have been taken together with PSR0833–45, to compile the catalogue of γ -ray sources shown in Table 2. The nomenclature CG $n+m$ is introduced for a γ -ray source detected by COS B at longitude n° and latitude m° (truncated values). Table 2 lists the galactic coordinates, and the intensities above 100 MeV relative to the Crab (CG185–5). The indicated uncertainties in the relative intensities include systematic errors, reflecting possible changes in the instrument sensitivity, for which corrections are being derived. The intensity of CG185–5 above 100 MeV of $(3.5 \pm 1.0) \times 10^{-6}$ photon cm⁻² s⁻¹, is found to agree with other measurements⁸.

The latitude distribution of the detected sources, shown in Fig. 1, immediately indicates their galactic nature. The absence of sources between 7° and 15° is particularly significant because the visibility of sources increases with galactic latitude due to the

Table 1 Observations used in the search for localised sources

Pointing direction μ°	b°	From	To
185	-6	17 August	17 September 1975
74	0	28 November	24 December 1975
322	0	23 February	24 March 1976
126	1	24 June	25 July 1976

decrease in the background flux contributed by the galactic disk. The distribution suggests typical distances in excess of 1 kpc which, coupled with typical flux values of 10^{-9} erg cm $^{-2}$ s $^{-1}$, leads to individual luminosities in excess of 10^{35} erg s $^{-1}$.

The nature of this new class of astronomical objects is intriguing. The positional errors virtually exclude unique identification with a known object, and the finite angular resolution makes it impossible to distinguish between a compact object and an emission region of angular dimension up to 2°. Although our results may not be representative for the entire galaxy, the sources do contribute significantly to the overall γ -ray luminosity of the galaxy, in the anti-centre region even accounting for most of the emission⁹.

Table 2 γ -Ray sources detected by COS B

Source	$l^{\text{II}}(^{\circ})$	$b^{\text{II}}(^{\circ})$	Relative intensity	Remarks
CG64+0	64.5 ± 0.5	0.0 ± 1.0	$0.30^{+0.10}_{-0.05}$	
CG75+0	75.0 ± 1.0	0.0 ± 0.5	$0.45^{+0.15}_{-0.10}$	
CG78+1	78.5 ± 0.5	$+1.5 \pm 0.5$	$1.00^{+0.30}_{-0.20}$	
CG121+3	121.0 ± 1.0	$+3.5 \pm 1.0$	$0.20^{+0.10}_{-0.05}$	
CG135+1	135.5 ± 1.0	$+1.5 \pm 1.0$	$0.30^{+0.15}_{-0.10}$	
CG176-7	176.0 ± 1.0	-7.0 ± 1.0	0.50 ± 0.10	
CG185-5	185.3 ± 0.5	-5.6 ± 0.5	1.00	PSR0531+21 at $l^{\text{II}} = 184.6, b^{\text{II}} = -5.8$
CG189+1	189.0 ± 1.5	$+1.0 \pm 1.5$	0.40 ± 0.10	
CG195+4	195.9 ± 1.0	$+4.5 \pm 0.5$	0.90 ± 0.20	195+5 (SAS-2)
CG263-2	263.7 ± 0.3	-2.6 ± 0.3	3.30 ± 0.50	PSR0833-45 at $l^{\text{II}} = 263.6, b^{\text{II}} = -2.8$
CG312-1	312.0 ± 1.0	-1.5 ± 1.0	0.45 ± 0.15	
CG327-0	327.5 ± 1.0	-0.5 ± 1.0	0.35 ± 0.15	
CG333+0	333.5 ± 1.0	0.0 ± 1.0	0.70 ± 0.20	

The suggestion by Black and Fazio¹⁰, that interstellar clouds could be γ -ray sources when bombarded by cosmic-ray protons, has been investigated. If the density of cosmic rays is the same beyond 1 kpc as is measured locally, masses in excess of $10^6 M_{\odot}$ would be required to account for the observed source intensities. No such massive clouds have been observed near the locations of the reported γ -ray sources.

A search for possible X-ray counterparts of the γ -ray sources has also been made. The weak sources 4U0241+61 and 4U1416-62 (ref. 11) are within the error boxes of CG135+1 and CG312-1 respectively. The soft source Cyg X-7, associated with the supernova remnant DR4 (ref. 12) falls within the error box of CG78+1. In addition, hard X-ray emission from the region of CG135+1 has been reported¹³. Such positional coincidences, however, provide only a weak argument for correlation in view of the high density of X-ray sources and the size of the γ -ray error boxes. In any case the absence of strong X-ray counterparts implies that the new γ -ray sources have substantially higher luminosities above 100 MeV than in the 2-6-keV band. It is noted that CG78+1 is not positionally coincidental with Cyg X-3, for which strong periodic γ -ray emission at an earlier epoch has been reported¹. Moreover, a search for the 4.8-h periodicity in the γ -ray emission from this region has yielded a negative result¹⁴.

Of the 149 known radio pulsars only PSR0531+21 and PSR0833-45 are confirmed γ -ray emitters. Of the remainder only one (PSR0611+22) falls within the error box of one of the COS B sources (CG189+1), for which a search for pulsating γ -ray emission has been inconclusive¹⁵.

Future steps towards a better understanding of the results presented here should include: (1) sensitive searches for radio pulsars and weak X-ray sources near the positions of the unidentified sources and (2) searches for timing signatures from candidate counterparts; in this context it is noted that CG195+4 shows periodic variations¹⁻¹⁰. This search is far from complete, as only one-third of the galactic plane has been effectively studied. Further analysis of available COS B data will provide a galactic distribution of γ -ray sources which will permit a comparison with other galactic populations.

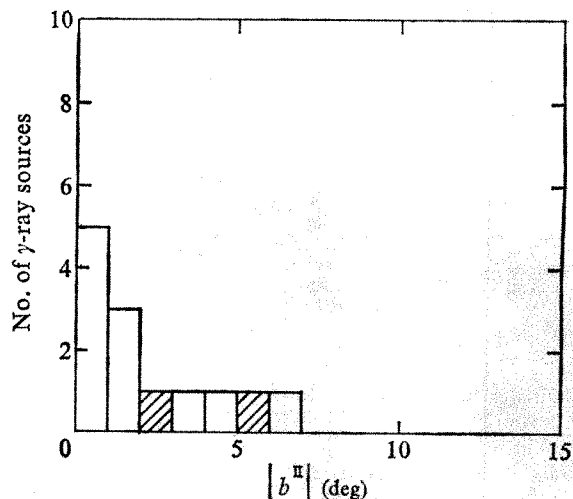


Fig. 1 Latitude distribution of γ -ray sources. The shaded area indicates the identified γ -ray sources PSR0833-45 and PSR0531+21.

The COS B mission is planned to continue until the end of 1978. Although much of the observation time is to be devoted to prolonged observations of the galactic plane from $l^{\text{II}} = 340^{\circ}$ to $l^{\text{II}} = 90^{\circ}$, special observations to check on the consistency of suggested counterparts for already detected sources may be considered.

G.G.L. acknowledges the receipt of an ESA Fellowship.

W. HERMSEN
B. N. SWANENBURG

*Cosmic Ray Working Group,
Huygens Laboratory,
Leiden,
The Netherlands*

G. F. BIGNAMI
G. BOELLA

*Laboratorio di Fisica Cosmica e
Tecnologie Relative del CNR,
Università di Milano,
Italy*

R. BUCCHERI
L. SCARSI

*Laboratorio di Fisica Cosmica e
Tecnologie Relative del CNR,
Università di Palermo, Italy*

G. KANBACH
H. A. MAYER-HASSELWANDER

*Max-Planck-Institut für
Extraterrestrische Physik,
Garching bei München, FRG*

J. L. MASNOU
J. A. PAUL

*Service d'Electronique Physique,
Centre d'Etudes Nucleaires de Saclay,
France*

K. BENNETT
J. C. HIGDON
G. G. LICHTI
B. G. TAYLOR
R. D. WILLS

*Space Science Department
of the European Space Agency,
ESTEC, Noordwijk,
The Netherlands*

Received 29 July; accepted 16 August 1977.

- Hartman, R. C. *et al. Proc. GSFC Symp.* 15-25 (NASA CP-002, 1977).
- Bennett, K. *et al. Astr. Astrophys.* (in the press).
- Kniffen, D. A. *et al. 14th Intu Cosmic Ray Conf., Munich* 1, 100-105 (1975).
- Bennett, K. *et al. Astr. Astrophys.* 56, 469-471 (1977).
- Bignami, G. F. *et al. Space Sci. Instr.* 1, 245-268 (1975).
- Scarsi, L. *et al. Proc. 12th ESLAB Symp.* 3-12 (ESA SP-124, 1977).
- Hermesen, W. *Proc. 12th ESLAB Symp.* 13-20 (ESA SP-124, 1977).
- Thompson, D. J., Fichtel, C. E., Hartman, R. C., Kniffen, D. A. & Lamb, R. C. *Astrophys. J.* 213, 252-262 (1977).
- Bennett, K. *et al. Proc. 12th ESLAB Symp.* 83-93 (ESA SP-124, 1977).
- Black, J. H. & Fazio, G. G. *Astrophys. J.* 185, L7-11 (1973).
- Forman, W. *et al. Astrophys. J. Suppl.* (submitted).
- Davidson, A. F. *et al. Astrophys. J.* 215, 541-551, (1977).
- Coe, M. J., Engel, A. R. & Quenby, J. J. *IAU Circ.* No. 3003 (1976).
- Bennett, K., Bignami, G. F., Hermesen, W., Mayer-Hasselwander, H. A., Paul, J. A. & Scarsi, L. *Astr. Astrophys.* 59, 273-274 (1977).
- Kanbach, G. *et al. Proc. 12th ESLAB Symp.* 21-32 (ESA SP-124, 1977).
- Masnou, J. L. *et al. Proc. 12th ESLAB Symp.* 33-37 (ESA SP-124, 1977).

Positions of galactic X-ray sources Cir X-1, TrA X-1 and 3U1626-67

THE positions of three celestial X-ray sources were measured with the rotating modulation collimators (RMC) on SAS-3 during a survey of the galactic plane¹ and are precise to $\leq 25''$ (ref. 2). One of these sources, Cir X-1 (ref. 3), is a highly variable X-ray source often compared with the black-hole candidate, Cyg X-1. Another, TrA X-1 = A1524-61, is a 1974 X-ray nova⁴, and the third, 3U1626-67 (ref. 5), is a 7.68-s X-ray pulsar⁶. The results reported here support recently proposed optical and radio identifications⁷⁻⁹ of these three sources. In two cases (TrA X-1 and 3U1626-67), they have been instrumental in bringing about the proposed identifications. Cir X-1 exhibits a binary periodicity¹⁰ of 16.6 d, extreme aperiodic variability on timescales of > 0.1 s (refs 11, 12) and 1-3 s (ref. 13), and flaring^{14,15} with time constants of a few ms. This variability and the absence of a shorter (spin) period^{13,15} have led to suggestions^{11,13-15} that this object may be similar to Cyg X-1. The absence of a compelling optical or radio counterpart, however, has prevented the further exploration of this hypothesis.

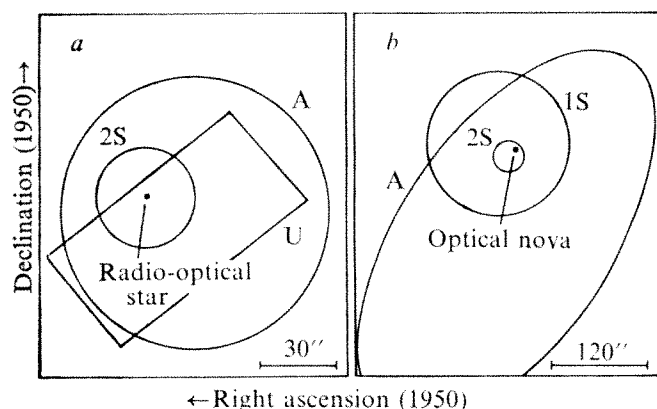


Fig. 1 X-ray positions and error regions (90% confidence) for *a*, Cir X-1 and *b*, TrA X-1. The Ariel V, A, (refs 19, 8); Uhuru, U, (ref. 11) and SAS-3, 1S, (ref. 21) results precede the present work, 2S. The locations of the optical and radio counterparts^{7,8} are indicated. (Finding charts are presented in refs 7 and 8).

A faint ($R \sim 16-17$, $B-R \sim 6$) optical star with strong H α emission¹⁶ and a flaring radio source^{17,18} have been proposed as counterparts to Cir X-1 on the basis of Uhuru^{5,11} and Ariel V¹⁹ X-ray positions. The radio source has been shown⁷ to lie within $2''$ of the H α star and the radio flares have been observed to occur 0.5-3.0 d after several of the periodic (16.6-d) X-ray eclipses. This establishes its identification and its present

2S1627-673

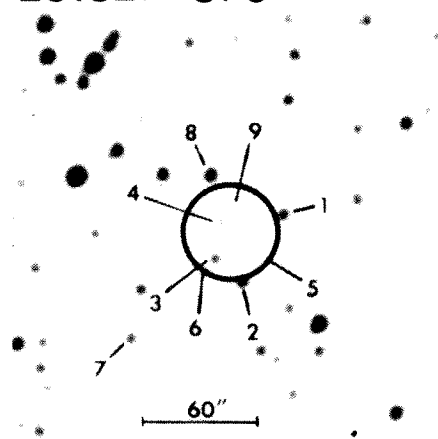


Fig. 2 Finding charts for the source 3U1626-67 = 2S1627-673. The optical candidate is star 4 (ref. 9).

position (Fig. 1, Table 1) removes any remaining doubt about the association of the X-ray source with the radio-optical star.

TrA X-1 was a classic X-ray nova first observed on 12 November 1974 with the Ariel V satellite⁴. It became as bright as the Crab Nebula on ~ 3 December 1974 and decayed with a time constant of ~ 2 months²⁰. SAS-3 was launched in May 1975, and the source was detected with the RMC system in June 1975. Preliminary analysis of the SAS-3 data yielded a position²¹ accurate to $1.5'$. This together with a revised Ariel V position has made possible the recent discovery⁸ of an optical nova that was observed at $B = 17.5$ on a plate taken 15 December 1974 with the Anglo-Australian Telescope. The star subsequently faded with a time constant of ~ 117 d. Earlier limits of $B > 19.5$ and $R > 19$ were obtained 20 and 5 months respectively before the X-ray maximum⁸. The present refined SAS-3 position, accurate to $20''$, lies $14''$ from the position of the optical nova (Fig. 1).

The source 3U1626-67 recently was found⁶ to be a 7.68-s pulsar with no evidence for a binary Doppler shift ($P_{orb} \lesssim 0.5$ d or $P_{orb} \gtrsim 35$ d). It has a hard X-ray spectrum and is variable⁶ by a factor 3 on timescales of 100-300 s. Shortly after the discovery of the periodicity, the position of the X-ray source was measured with SAS-3. The celestial area defined by the measurement is smaller by a factor of 50 than that previously obtained⁵ with Uhuru. An optical study of this SAS-3 error region led to the suggested identification⁹ of a $V = 18.5$ star (4 in Fig. 2) as the counterpart because of its large ultraviolet excess ($U-B = -1.2$ and $B-V = +0.2$).

The existence of correlated temporal behaviour has firmly established the identifications of two of the sources, Cir X-1 (ref. 7) and TrA X-1 (ref. 8). The proposed identification⁹ of 3U1626-67 is probably correct because of the small X-ray error

Table 1 Celestial positions

SAS-3 designation	Other designations	Position (1950) α	δ	I^{11} b^{11}	Error radius (90%)	Flux density* (μ Jy)	Comments
2S1524-617	TrA X-1 A1524-61	15h24m06.9s 231. ^o 0288	$-61^{\circ}42'41''$ $-61.^{\circ}7114$	320.3 -4.4	20''	60	X-ray nova ⁴ Optical nova ⁸
2S1516-569	Cir X-1 3U1516-56	15 16 48.6 229.2025	$-56^{\circ}59'14''$ -56.9872	322.1 0.0	20	25-850	Radio-optical star ⁷
2S1627-673	3U1626-67	16 27 13.6 246.8067	$-67^{\circ}21'23''$ -67.3564	321.8 -13.1	25	22	7.68-s X-ray pulsar ⁶ Ultraviolet excess star ⁹

*1.0 μ Jy = 2.2×10^{-11} erg cm $^{-2}$ s $^{-1}$ (2-11 keV) 1,2 .

region and the marked ultraviolet excess of the proposed star. In all cases the identifications were brought about by $\lesssim 1.0'$ X-ray and radio positions^{7,11,17,19} and the optical counterparts have turned out to be quite faint ($B > 17.5$).

We thank J. McClintock, G. Spada, and S. Rappaport for helpful conversations, and the staffs of the Center for Space Research at M.I.T. and the Goddard Space Flight Center. This work was supported, in part, by the US NASA. This is the third of a series of articles on determining the positions of X-ray sources using SAS-3.

H. V. BRADT
K. M. V. APPARAO*
R. DOWER
R. E. DOXSEY
J. G. JERNIGAN
T. H. MARKERT

Department of Physics and
Center for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received 19 July; accepted 8 August 1977.

*Permanent address: Tata Institute of Fundamental Research, Bombay, India.

- ¹ Bradt, H. V. *et al.* *Nature* (in the press).
- ² Doxsey, R. E. *et al.* *Nature* (in the press).
- ³ Margon, B., Lampton, M., Bowyer, S. & Cruddace, R. *Astrophys. J. Lett.* **169**, L23-L25 (1971).
- ⁴ Pounds, K. A. *IAU Circ. No.* 2729 (1974).
- ⁵ Giacconi, R. *et al.* *Astrophys. J. Suppl.* **27**, 37-64 (1974).
- ⁶ Rappaport, S. *et al.* *Astrophys. J. Lett.* (in the press).
- ⁷ Whelan, J. A. J. *et al.* *Mon. Not. R. astr. Soc.* (in the press).
- ⁸ Murdin, P. *et al.* *Mon. Not. R. astr. Soc.* **178**, 27-32p (1977).
- ⁹ McClintock, J. *IAU Circ. No.* 3088 (1977).
- ¹⁰ Kaluzienski, L. S., Holt, S. S., Boldt, E. A. & Serlemitsos, P. J. *Astrophys. J. Lett.* **208**, L71-L75 (1976).
- ¹¹ Jones, C., Giacconi, R., Forman, W. & Tananbaum, H. *Astrophys. J. Lett.* **191**, L71-L74 (1974).
- ¹² Forman, W., Jones, C. & Tananbaum, H. *Astrophys. J.* **208**, 849-862 (1976).
- ¹³ Spada, G. *et al.* *Astrophys. J. Lett.* **190**, L113-L115 (1974).
- ¹⁴ Toor, A. *Astrophys. J. Lett.* (in the press).
- ¹⁵ Dower, R., Bradt, H. & Canizares, C. *Bull. Am. Astr. Soc.* **9**, 297 (1977).
- ¹⁶ Mayo, S. K., Whelan, J. A. J. & Wickramasinghe, D. T. *IAU Circ. No.* 2957 (1976).
- ¹⁷ Clark, D. H., Parkinson, J. H. & Caswell, J. L. *Nature* **254**, 674-676 (1975).
- ¹⁸ Haynes, R. F., Caswell, J. L. & Simons, L. W. *IAU Circ. No.* 2977 (1976).
- ¹⁹ Wilson, A. M. & Carpenter, G. F. *IAU Circ. No.* 2965 (1976).
- ²⁰ Kaluzienski, L. J. *et al.* *Astrophys. J.* **201**, L121-L124 (1975).
- ²¹ Jernigan, J. G. *IAU Circ. No.* 2817 (1975).

Atmospheric waves in the ionosphere due to total solar eclipse

CHIMONAS¹ predicted that the 'cooling spot' of the lunar shadow of an eclipse, which travels at supersonic speeds through the atmosphere, is a continuous source of atmospheric gravity waves. These gravity waves show up by their interaction with the ionosphere and can be detected using standard radio techniques. Total electron content measurements² in the USA at a distance of several thousand km from the path of totality, detected oscillations which were attributed to a solar eclipse in agreement with the theory of Chimonas³. Measurements made during the 1973 central African eclipse⁴ failed to detect any oscillations, although these results may have been obscured by the occurrence of a magnetic storm at the same time. We present here the results of measurements made on the angle of arrival of a high frequency radio wave inside the path of totality of an eclipse, which took place on 23 October 1976.

The angle of arrival of a continuous wave (CW) obliquely propagated signal on 2.5 MHz was used over a short path of 71.4 km, from Lyndhurst (38°33'S, 145°16'E) to Beveridge (37°28'S, 144°56'E). On 23 October 1976, a total eclipse of the Sun occurred. The radio path was in the path of totality. Two different ionosondes were operated at Beveridge on the eclipse day. A phase path ionosonde, gating at about 100 km, gave group and phase path variations on 2.1, 2.4 and 2.9 MHz and results obtained from this ionosonde indicated that there were always E-region reflections during the eclipse which did not vary by more than ± 5 km. A digitised ionosonde, which records the strongest returned signal, gave ionograms every 45 s. These

ionograms indicated that E_s at a group height of 101 km appeared with a critical frequency $f_o E_s$ of 2.2 MHz at 1619, and at 1621 LT this E_s had an $f_o E_s$ of 2.4 MHz (this is just above the equivalent vertical frequency of 2.35 MHz, assuming a group height of reflection of 100 km). At 1624 LT $f_o E_s$ was 2.2 MHz and at 1625 LT it was 2.7 MHz and gradually increased to over 3.1 MHz at 1630 and remained so until after 1730 LT.

At the start of totality (~ 1642 LT) distinct oscillations with a period of 6-7 min were observed in the angle of arrival. Assuming that the oscillations observed were due to changes in the tilt angle of the sporadic E layer and to a minor extent height variations, it can be shown that the tilt angles along, γ_L , and across, γ_C , the propagation path, are given by

$$\gamma_L = \frac{1}{2} \left\{ \phi' - \frac{\pi}{2} + \tan^{-1} \left(\frac{1}{2 \tan \phi_0 - \tan \phi'} \right) \right\}$$

$$\gamma_C = \tan^{-1} \{ \sin(\theta - \theta_0) \tan \phi \}$$

where $\phi' = \tan^{-1} \{ \tan \phi \cos(\theta - \theta_0) \}$, θ and ϕ are the measured azimuth (bearing) and zenith angles and θ_0 and ϕ_0 are the angles expected for the case of a simple horizontally stratified ionosphere (including a horizontal sporadic E layer). Using the theorem of Breit and Tuve⁵, ϕ_0 was obtained from the group height of the sporadic E layer (assumed to be constant during the relevant period) and γ_L and γ_C were determined. They are shown as a function of time in Fig. 1a and b, several points are

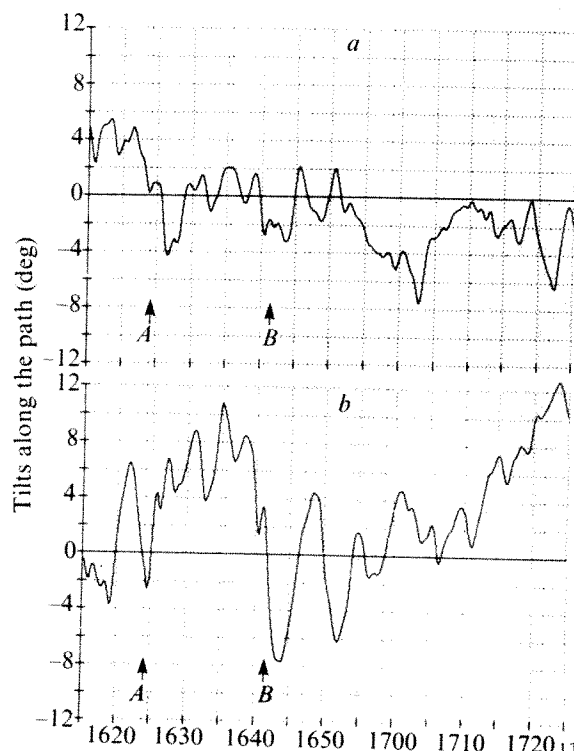


Fig. 1 Variation of the tilt angle of the sporadic E layer with time: a, along the propagation path; b, across the propagation path. A, E_s appeared; B, onset of totality.

evident: (1) between 1625 LT and totality, the E_s layer had an apparent average systematic tilt in γ_L (along the path) of about 6° with a small oscillation superimposed on it. (2) Just after totality this apparent tilt changes considerably and returned to its previous value slowly over about 40 min. (3) During this return, several cycles of a damped oscillation initially of large amplitude occurred of period 6-7 min. (4) No such variations were observed in γ_C (that is across the path).

Between 1619 and 1625 LT, an apparent single oscillation also occurred. As mentioned above, during this period the E_s layer was forming. The digitised ionosonde records indicated that we were only getting strong E_s reflections near 1621–22 LT. The phase ionosonde records indicated that we were still getting E-region reflections near 1619–20 and 1623–24 LT. Since before 1620 reflections from the normal E-region indicated a tilt of $1-2^\circ$, the excursion at 1621–22 is probably due to a reflection from the E_s during its formation.

Chimonas¹ applied his theory to the free bow-wave in the atmosphere outside the penumbra, where the wavefronts of the gravity waves in a previously still atmosphere were expected to be emitted at an angle η to the eclipse where η is given by

$$\tan \eta = V_G/V_E$$

and V_G is the velocity of the atmospheric wave at the altitude concerned (101 km). Taking $V_G = 300 \text{ ms}^{-1}$ and V_E as the velocity of the Moon shadow the wave would be expected to be propagated in a direction $\sim 166^\circ$ south of north (geographic) which is almost along the path between Lyndhurst and Beveridge (it being $\sim 157^\circ$ south of north (geographic)). Hence, outside the shadow, the oscillations due to the eclipse might be expected to be only in γ_L for our geometry. (It might be expected that the changes in the intensity of the solar radiation near totality would cause changes in the electron density gradients and cause changes in the angle of arrival. But, one would expect them to be evident both in the components across and along the path, and there is no reason to expect them to be oscillatory.) The results presented here are interesting since they are in the path of totality, (but south of the centre line), and in fact the oscillations in γ_L decay in amplitude and γ_L returns to near its original value after a few cycles, as may be expected for a fully developed bow wave. But this dominant oscillation only in γ_L inside the shadow is unexpected. The only other angle of arrival measurements, to our knowledge, made during an eclipse⁶ showed that although in the F region a tilt was observed which was consistent with that expected from a consideration of the geometry of the eclipse path, no tilt or oscillation was observed in the normal E region or in an E_s layer which appeared 11 min after the maximum phase of the eclipse and lasted 9 min. These results, however, were not taken in the path of totality, but in a path that experienced only 0.72 obscuration at the maximum phase.

R. N. E. BAULCH
E. C. BUTCHER

Division of Theoretical and Space Physics,
La Trobe University,
Bundoora, Victoria, 3083, Australia

Received 17 May; accepted 11 August 1977.

¹ Chimonas, G. J. *Geophys. Res.* **75**, 5545 (1970).

² Davis, M. J. & Da Rosa, A. V. *Nature* **226**, 1123 (1970).

³ Chimonas, G. & Hines, C. O. *J. geophys. Res.* **76**, 7003 (1971).

⁴ Schoedel, J. P., Klostermeyer, J. & Roettger, J. *Nature* **245**, 87 (1973).

⁵ Breit, G. & Tuve, M. A. *Phys. Rev.* **28**, 554 (1926).

⁶ Bramley, E. N. *J. atmos. terr. Phys.* **8**, 98 (1955).

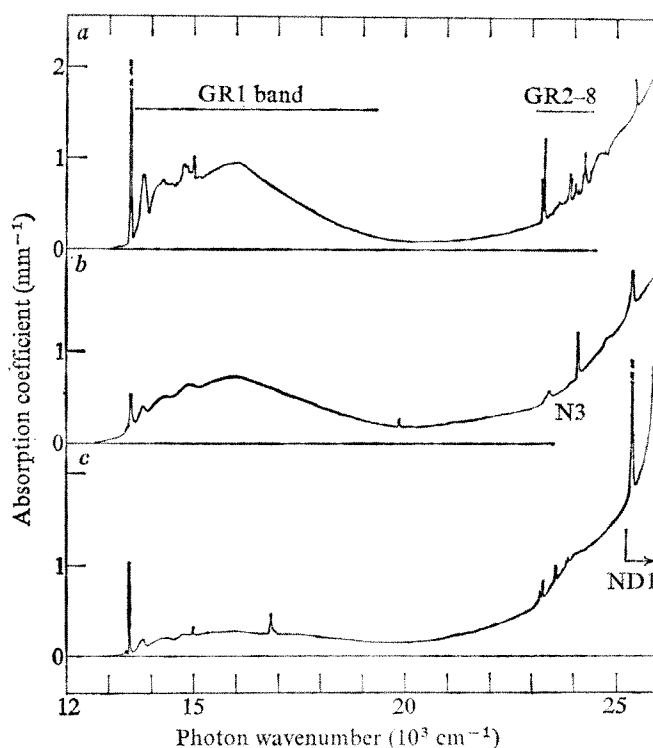


Fig. 1 Visible absorption spectra, measured at liquid nitrogen temperature, of representative diamonds following irradiation at room temperature with 2-MeV electrons to approximately the same dose ($\sim 10^{22}$ electrons m^{-2}). *a*, Relatively pure type IIB diamond, containing about 10^{24} m^{-3} B atoms—the damage level greatly exceeding this³⁶; $G/N = 18$. *b*, Type IA diamond with $4 \times 10^{26} \text{ m}^{-3}$ nitrogen atoms in small aggregates of donor binding energy $E_D \approx 4 \text{ eV}^{18}$, $G/N = 1.3$. *c*, Type IB diamond containing $\sim 5 \times 10^{24} \text{ m}^{-3}$ isolated substitutional atoms of $E_D \approx 1.7 \text{ eV}^{16}$; $G/N \ll 0.1$. For each diamond G/N gives the ratio of integrated strengths (wavenumber \times absorption) of the GR1 and ND1 zero phonon lines. The value for the type IA diamond differs from ref. 37 due to measurement in unfiltered tungsten light. The large linewidths in the type IA diamond are caused by its high nitrogen content³⁸.

previously except that the $25,410 \text{ cm}^{-1}$ (ND1) centre is postulated here to be the negative vacancy, V^- , and not the interstitial nitrogen atom as originally suggested². The reasons for this re-interpretation are as follows. After irradiation, all diamonds show the 'GR' absorption bands (Fig. 1, Table 1), which all occur at the same tetrahedral centre³⁻⁵. Since the GR bands are observable after high temperature annealing ($T > 1,200 \text{ K}$ in relatively pure specimens⁶), it is assumed they occur at the vacancy⁷ because during irradiation, the host interstitial in Group IV semiconductors seems to be highly mobile⁸⁻⁹. The lack of electron paramagnetic resonance (EPR) associated with the GR centre¹⁰ then implies a neutral vacancy V^0 or less likely multiple charged centres (V^{2+} , V^{4+} and so on).

The GR1 band is an E to T transition^{4,5} with its centroid at $(15,920 \pm 150) \text{ cm}^{-1}$ at 30 K. Many-electron LCAO calculations on V^0 predict a 1E or 3T_1 ground state, the two being almost degenerate¹¹⁻¹³. The first allowed transition from 1E , assuming it to be the ground state, is typically at $16,000 \text{ cm}^{-1}$ to a 1T_2 excited state. The excellent agreement with experiment on the irreducible representations of the states, their relative energies and their spin states supports the use of LCAO calculations rather than the alternative techniques which have not yet given even qualitatively correct energy level schemes^{14,15}.

Since the GR bands are the dominant product of irradiating relatively pure diamond (types IIA and IIB, see Fig. 1*a*), it seems that V^0 is the stable form of the vacancy in pure diamond.

It is important to consider the effect of irradiating type IB diamonds, whose dominant impurity is isolated substitutional nitrogen atoms with donor binding energies of about 1.7 eV (ref. 16).

Charge states of the vacancy in diamond

KNOWLEDGE of radiation damage processes in diamond has increased substantially in the past few years. I present here a concise summary of these data, and suggest that the $25,410 \text{ cm}^{-1}$ (ND1) absorption band occurs at a negatively charged vacancy. It will be shown that with this interpretation, the optical effects of radiation damage fall into a self-consistent pattern.

Optical studies of radiation effects in diamond have been carried out mainly in the visible spectrum following room-temperature irradiation with 1–2-MeV electrons. (For neutron and γ -ray effects see Clark *et al.*¹.) Table 1 summarises the results. The models given in the Table are as suggested

The GR bands are produced only weakly while the 25,410 cm⁻¹ (ND1) band, which also occurs at a T_d centre², is extremely strong (Fig. 1c). The simplest explanation is that the vacancies which must be produced by the irradiation are converted from V⁰ to V⁻ by charge transfer from the nitrogen donors: Loubser (personal communication) has observed a decrease in the EPR of these donors as the irradiation proceeds.

LCAO calculations, which seemed to be accurate for V⁰, predict that the first absorption band of V⁻ should occur between about 20,000 and 38,000 cm⁻¹ in an ⁴A₂ to ⁴T₁ transition¹¹⁻¹³. Experimentally the ND1 transition has been found to occur from an A to a T state², the centroid of the band being at (27,500 ± 200) cm⁻¹. The theoretical ⁴A₂ ground state implies an EPR active centre. No quantitative experimental study of this point has yet been made. A single isotropic line with *g* ≈ 2.0 has been observed in the three specimens inspected here, and also in measurements by Dyer and du Preez¹⁷. This EPR and the ND1 optical absorption show strikingly similar cyclic behaviour when the diamonds are subjected to alternate heating and bleaching cycles. However, Dyer and du Preez observed a decrease in the EPR signal and an increase in ND1 absorption after a 500 °C anneal in one specimen, suggesting that the absorptions arise at different centres, but these com-

parisons are not straightforward in view of the photochromic behaviour of the ND1 centre¹⁸. A detailed study of this EPR is required.

The assignment of the 25,410 cm⁻¹ (ND1) band to V⁻ produces two simplifications. First, reversible photochromic effects occur between the 13,495 cm⁻¹ (GR1) and 25,410 cm⁻¹ (ND1) bands even when the vacancy concentrations are ≈ 10⁻³ of the impurity content of the diamonds^{17,18}. If GR1 and ND1 occur at chemically indistinguishable sites a mechanism of charge transfer between defects of minor concentrations has to be designed. In terms of the present model, photoionisation of V⁻ (ND1) leads necessarily to V⁰ and hence GR1 absorption. Presumably the released electron produces the observed photoconduction¹⁹. Conversion of GR1 (V⁰) to ND1 (V⁻) by blue light¹⁸ (23,250 cm⁻¹ < *hν* < 25,400 cm⁻¹) is then seen to be by hole emission (V⁰ → V⁻ + h⁺), explaining the photoconductivity observed experimentally²⁰.

Second, in nitrogen-containing diamonds, thermal annealing destroys the 13,495 and 25,410 cm⁻¹ bands (GR1 and ND1) producing new optical bands (for example, at 15,690 and 19,865 cm⁻¹) depending on the form of nitrogen^{21,22}. Recent data imply that the new bands are formed when the nitrogen traps a vacancy²³⁻²⁵. In some experiments (ref. 23 and M. H. Nazaré, personal communication) the rate of growth of the new bands matches the destruction of GR1, in others^{22,26} it matches the removal of ND1. This is rationalised if both GR1 and ND1 occur at centres of the same chemical species (such as V⁰ and V⁻), and if we assume a charge redistribution can occur, if necessary, after trapping.

All our present optical data on irradiated diamond seem to be consistent with ND1 being V⁻. In principle, this model can be tested using a photo-Hall experiment to identify the carrier emitted when GR1 and ND1 centres are photoionised. The model predicts

$$h\nu + \text{GR1 (V}^0\text{)} \rightarrow \text{ND1 (V}^-\text{)} + h^+ \quad (1)$$

$$\text{and } h\nu + \text{ND1 (V}^-\text{)} \rightarrow \text{GR1 (V}^0\text{)} + e^-$$

In contrast, if GR1 and ND1 occur at chemically different sites, the same charge must be liberated when the GR1 and ND1 centres are photoionised, so that the observed cyclic processes can occur. The available data are not inconsistent with equation (1)²⁷.

The earlier suggestion² that the ND1 centre was an interstitial nitrogen atom was reached by arguing that defects in diamond have highly localised orbitals. In this limit it was thought reasonable to 'assume that it is unlikely that the effect of the presence of nitrogen (as an impurity in a diamond) is to produce charge compensation'². However, the data now available for type IB diamond (see Fig. 1c) show that the absorption strength of chemically stable defects like the GR1 centre is in fact strongly affected by the presence of the deep nitrogen donor (*E_D* ≈ 1.7 eV), an effect most likely to occur as a result of charge compensation.

Finally, the fate of the carbon interstitials, produced by the irradiation, seems to be that they are highly mobile, as in silicon²⁸, and become trapped at unknown crystal defects, producing complex EPR centres²⁹.

I thank A. M. Mainwood, C. M. Welbourn and J. H. N. Loubser for helpful discussions. Equipment was made available by de Beers Industrial Diamond Division Pty. Ltd and the Royal Society. This work was supported in part by the SRC.

GORDON DAVIES

Wheatstone Physics Laboratory,
King's College,
London WC2, UK

Received 1 June; accepted 2 August 1977.

- Clark, C. D., Ditchburn, R. W. & Dyer, H. B. *Proc. R. Soc. A* **234**, 361 (1956).
- Davies, G. & Lightowler, E. C. *J. Phys. C* **3**, 638 (1970).
- Walker, J., Vermeulen, L. A. & Clark, C. D. *Proc. R. Soc. A* **341**, 253 (1974).
- Clark, C. D. & Walker, J. *Proc. R. Soc. A* **334**, 241 (1973).
- Davies, G. & Penchina, C. M. *Proc. R. Soc. A* **338**, 359 (1974).

Table 1 Major visible absorption bands

Optical band* (cm ⁻¹)	(eV)	Nature of transition†	Ref.‡
10,080 H2	1.25	End product of annealing radiation damage in diamonds containing nitrogen	21
13,495 GR1	1.673	E → T at T _d centre V ⁰	4, 5
13,590	1.685	A → A transitions at a tetragonal centre?	30
15,000	1.860	A → E at trigonal centre V-N	23
15,690	1.945	End product of annealing radiation damage in diamonds containing nitrogen; complex response to uniaxial stress	§
16,825	2.086		
17,390	2.156	E → A at trigonal centre	31
19,495	2.417	<110> electric dipole at monoclinic I centre	32
19,860	2.462	<110> electric dipole at C _{2v} centre	33
3H			
19,865	2.463	<110> electric dipole at C _{2v} centre	34
H3		N-V-N	25
20,150	2.498	<110> electric dipole at C _{1h} centre; V plus an aggregate of nitrogen	32
H4			
20,510	2.543	V-V?	21
21,280	2.638	σ dipole at monoclinic I centre	33
TR12			
23,240	2.881	E → T transitions at 13,495 cm ⁻¹ centre (V ⁰)	3
23,295	2.888		
GR2, GR3			
23,410	2.902	E → ? transitions at 13,495 cm ⁻¹ centre (V ⁰)	3
23,710	2.940		
23,860	2.958		
24,000	2.976		
24,200	3.00		
GR4,5,6,7,8			
23,550	2.920	E → A transition at trigonal centre	31
24,080	2.985	A → E transition at C _{3v} centre, probably 3 nitrogen atoms	35
N3			
25,410 ND1	3.150	A → T transition at T centre (V ⁻)	2
			See text

*Bands are labelled by their zero phonon lines, in cm⁻¹ and eV, and by their common names.

†The order of the irreducible representations for the transitions is ground → excited state.

‡Only the latest relevant references are given.

§M. H. Nazaré, personal communication.

- 6 Palmer, D. W. thesis, Univ. Reading (1961).
- 7 Mitchell, E. W. J. in *Science and Technology of Industrial Diamonds* 1, 17 (Industrial Diamond Information Bureau, London, 1967).
- 8 Norris, G. B., Brower, K. L. & Vook, F. L. *Rad. Effects* 18, 1 (1973).
- 9 Hiraki, A., Cleland, J. W. & Crawford, J. H. J. *appl. Phys.* 38, 3519 (1967).
- 10 Clark, G. D., Duncan, I., Lomer, J. N. & Whippley, P. W. *Proc. Br. ceram. Soc.* 1, 85 (1964).
- 11 Coulson, C. A. & Kearsley, M. J. *Proc. R. Soc. A* 241, 433 (1957).
- 12 Yamaguchi, T. *J. phys. Soc. (Japan)* 17, 1359 (1962).
- 13 Coulson, C. A. & Larkins, F. P. *J. phys. Chem. Solids* 32, 2245 (1971).
- 14 Rouhani, M. D., Lannoo, M. & Lenglet, P. *J. Phys. (France)* 31, 597 (1970).
- 15 Messmer, R. P. & Watkins, G. D. in *Radiation Effects in Semiconductors* (eds Corkett, J. W. & Watkins, G. D.) 23 (Gordon and Breach, London, 1971).
- 16 Farrer, R. G. *Solid St. Commun.* 7, 685 (1969).
- 17 Dyer, H. B. & du Preez, L. *J. chem. Phys.* 42, 1898 (1965).
- 18 Davies, G. *Chemistry and Physics of Carbon* (eds Philips, P. W. & Turner, P. A.) 13, 1 (Dekker, New York, 1977).
- 19 Farrer, R. G. & Vermeulen, L. A. *J. Phys. C* 5, 2762 (1972).
- 20 Vermeulen, L. A., Clark, C. D. & Walker, J. *Proc. Freiburg Conf.* 294 (Conf. Ser. no. 21 Institute of Physics, London, 1974).
- 21 Clark, C. D., Ditchburn, R. W. & Dyer, H. B. *Proc. R. Soc. A* 237, 75 (1956).
- 22 du Preez, L. thesis, Univ. Witwatersrand, (1965).
- 23 Davies, G. & Hamer, M. F. *Proc. R. Soc. A* 348, 285 (1976).
- 24 Loubser, J. H. N. & van Wyk, J. A. *Diamond Research 1977* 11 (de Beers Industrial Diamond Div. Pty Ltd, Ascot, 1977).
- 25 Davies, G. *J. Phys. C* 9, L537 (1976).
- 26 Burton, B. thesis, Univ. London (1976).
- 27 Vermeulen, L. A. *Diamond Research* 22 (Industrial Diamond Information Bureau, Ascot, 1976).
- 28 Watkins, G. D. in *Defects and their Structure in Nonmetallic Solids* (eds Henderson, B. & Hughes, A. E.) 203 (Plenum, New York, 1976).
- 29 Lomer, J. N. & Welbourn, C. M. *Diamond Research* 5 (de Beers Industrial Diamond Div. Pty Ltd, Ascot, 1977).
- 30 Walker, J. *J. Phys.* (in the press).
- 31 Davies, G. *Diamond Conference*, Reading (1977).
- 32 de Sa, E. S. & Davies, G. *Proc. R. Soc.* (in the press).
- 33 Walker, J. *Inst. Phys. Conf. Series* no. 23, 317 (1975).
- 34 Davies, G., Nazaré, M. H. & Hamer, M. F. *Proc. R. Soc. A* 351, 245 (1976).
- 35 Kaplyanskii, A. A., Kolyshkin, V. I. & Medvedev, V. N. *Sov. Phys. Solid St.* 12, 1193 (1970).
- 36 Dyer, H. B. & Ferdinando, P. *Br. J. appl. Phys.* 17, 419 (1966).
- 37 Davies, G. *Proc. R. Soc. A* 336, 507 (1974).
- 38 Davies, G. *J. Phys. C* 3, 2474 (1970).

Alkali cations and direct reduction of wustite

THERE are few reports^{1,2} on the direct reduction of wustite by carbon according to an autocatalytic mechanism, and none deal with its quantitative analysis in terms of any kinetic model. Eck³ has postulated that this mode of reduction is indicative of the increased role of processes such as diffusion and nucleation in the solid state, relative to the gasification of carbon by the Boudouard reaction—generally regarded as the rate-controlling step in the reduction⁴. We describe here a quantitative analysis of autocatalytic reduction in terms of the Prout-Tompkins⁵ mechanism for the growth of a product phase in the lattice of the unreacted phase. We derive a rate constant characterising the growth of the metallic phase and use it to evaluate the catalytic effects of small admixtures of alkali salts (ref. 6 summarises the literature on catalysed reduction).

Reduction of wustite in mixtures comprising precipitated red ferric oxide (Hopkin and Williams), spectrographic graphite (Union Carbide) and varying amounts of alkali salts (cation/Fe from 0.5% to 3.6% depending on catalytic effect) was studied at constant temperature by a Mettler Thermoanalyser. Samples were heated from room temperature at a rate of 25 °C min⁻¹ to the pre-selected temperature; simultaneous values of temperature, cumulative mass change (TG) and rate of mass change (DTG) were recorded.

The start of the wustite reduction (which always occurred after the reaction temperature was reached) was fixed at that value of mass loss which corresponds to the complete conversion of ferric oxide to wustite, assuming it to take place in a self-generated CO/CO₂ atmosphere in equilibrium with Fe₂O₃/Fe₃O₄. X-ray diffraction analyses of partially reacted samples confirmed the two-stage nature of reduction: Fe(III)—and Fe(III, II)—oxides were no longer detected when metallic iron appeared in wustite. The reductions were all carried to completion (as confirmed by stoichiometry and cessation of mass loss). Fractional conversion (α) of wustite to metallic iron was calculated from the TG data as fractional mass change values. Representative reduction curves are shown in Fig. 1.

The average mass change (29 measurements) was 38% relative to the starting mass of wustite. This suggests that >90% of wustite oxygen was abstracted as CO and that the composition of the reduction atmosphere lay closer to the Boudouard equi-

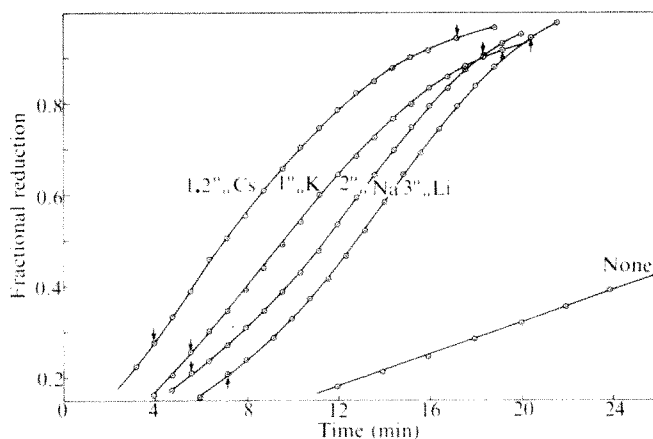


Fig. 1 Reduction of wustite to metallic iron by graphite in a dynamic argon atmosphere (flow rate 50 ml STP min⁻¹) at 960 °C in the presence of alkali catalysts. Alkalis were added as dry powders in the form of carbonates except for Rb (nitrate). TG data points were hand-read from a chart recording for the construction of reduction curves. Samples mass: 80 mg Fe₂O₃ + 15 mg C. Particle sizes: FeO ~ 1 µm (by scanning electron microscopy); graphite, 144 + 105 µm. Sample container: cylindrical alumina crucible (length, 20 mm; diameter 8 mm) resting on a Pt/Rh thermocouple junction. Sample loose-packed (bulk density ~ 0.5 g cm⁻³). Arrows indicate range of α for which the curves conform to equation (1).

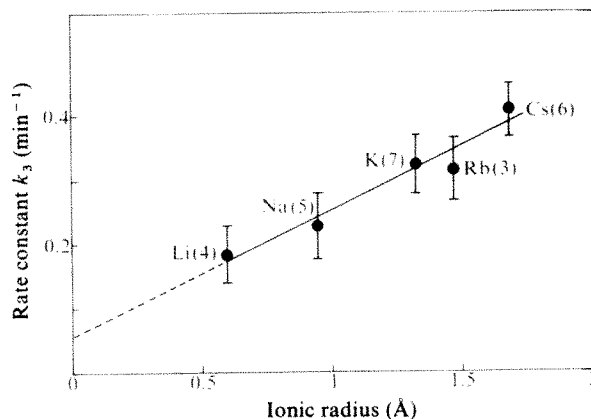
librium (~ 98% CO) than to the wustite-iron-gas equilibrium (~ 70% CO).

We have estimated the rate constants characterising the reduction curves of Fig. 1 by fitting the curves in the range 0.2 < α < 0.95 to a modified form of the Prout-Tompkins equation for nucleation phenomena. The modification takes into account our experimental observation that the curves are in general not symmetric

$$\ln \frac{\alpha}{1 - (\alpha/2\alpha_i)} = k_3 t + \text{constant} \quad (1)$$

α_i is the value of α for which the rate of reduction (traced by the DTG curve) attains its maximum. k_3 is the so-called branching coefficient describing the appearance of nucleated iron by a

Fig. 2 Empirical relationship between the Prout-Tompkins branching coefficient k_3 and the Pauling ionic crystal radii of alkalis. Numbers in parentheses indicate the number of determinations of k_3 . Error bars denote the 95% confidence intervals (Student's *t* distribution). Line through data points is the least-squares fit. a (intercept) = 0.055 ± 0.025 , m (slope) = 0.195 ± 0.021 . Errors correspond to 1σ . The best-fit value of intercept is identified with the uncatalysed value of k_3 : 0.066 ± 0.016 min⁻¹ (four determinations).



chain mechanism. (See ref. 5 for the derivation of equation (1) when $\alpha_i = 0.5$.) In this interpretation of reduction, autocatalysis is essentially a consequence of the proportionality of the rate of reduction to the number of iron nuclei. Justification for this proportionality is provided by observations⁷ that the rate of abstraction of oxygen from wustite is much higher at the triple phase boundary wustite-iron-gas than elsewhere. We interpret deviations from the Prout-Tompkins mechanism for $\alpha < 0.2$ as resulting from the significant contribution made by the (unknown) initial nucleation law to the formation of metallic iron; derivation of equation (1) assumes this contribution to become negligible at some (low) value of α .

A summary of k_3 values derived by means of equation (1) (and recalculated by linear interpolation or extrapolation at cation/Fe = 2% to facilitate comparison) is given in Fig. 2. This also demonstrates a linear relationship ($r = 0.972$) between the catalytic effects of alkali cations and their ionic sizes.

We conclude first, that autocatalytic reduction of wustite yielding sigmoid-shaped reduction curves can be explained by invoking the Prout-Tompkins (branching chains) mechanism for the formation of metallic iron; and second, that alkali cations enhance the rate of this mechanism in direct proportion to their ionic sizes.

We are grateful to the Anglo American Corporation of South Africa Limited for providing laboratory facilities.

THOMAS SZENDREI

Anglo American Research Laboratories,
P.O. Box 106, Crown Mines, 2025,
Republic of South Africa

P. C. VAN BERGE

Department of Chemistry,
Rand Afrikaans University,
Johannesburg,
Republic of South Africa

Received 12 August; accepted 3 August 1977.

¹ Lisnyak, S. S. & Chufarov, I. Zh. fiz. Khim. 33, 1840-1845 (1959).

² Zhuravleva, M. G., Chufarov, I. & Khromykh, L. G. Dokl. Akad. Nauk SSSR 135, 385-388 (1960).

³ Eck, R. Berg-u hüttem. Mh. 113, 11-26 (1968).

⁴ von Bogdandy, L. & Engell, H.-J. The Reduction of Iron Ores 100-104 (Springer-Verlag, Berlin and New York, 1971).

⁵ Jacobs, P. W. M. & Tompkins, F. C. in Chemistry of the Solid State, ch. 7 (Butterworths, London, 1955).

⁶ Piepenbrock, R., Koch, K., Tromel, G., Zellerfeld, C. & Fix, W. Arch. Eisenhüttenwes. 47, 141-146 (1976).

⁷ Pluschkell, W. & Yoshikoshi, H. Arch. Eisenhüttenwes. 41, 715-721 (1970).

Pacific sea-surface temperature related to rain in California

CALIFORNIA is experiencing the worst drought in modern history, and there is much concern and speculation about future rainfall prospects. We recently succeeded in relating South Atlantic sea surface temperatures (SST) to rain in northeastern Brazil¹, and here we use similar methods to relate sea surface temperatures over the North Pacific Ocean to rainfall in California. We found December SST in the north-east Pacific off Washington to have a limited but definite predictive value, and to account for about 45% of the variation in California rainfall for the period February-April.

Namias has published several papers relating SST in the Pacific to atmospheric circulation². In our approach, aimed at obtaining a useful forecasting tool, we go directly from a contributory cause (SST) to an ultimate effect (rainfall), bypassing intervening physical processes. We justify this by the belief that rainfall is an integrator of the multitude of ocean-atmospheric interactions and processes that occur simultaneously on several scales of space and time.

We divided the rainy season into two parts, early rains (November-January), and the late rains (February-April). The months May-October are usually dry in California,

and when rains do occur they are influenced by factors apart from the normal November-April rains. We did not investigate the effect of SST on rains in May-October, although SST off Baja California is almost certainly related to the occurrence of September and October rains originating from decaying *chubascos*, or west coast Mexican hurricanes.

First, we established rainfall indices (RFI) for California. Rainfall is quite highly correlated among stations over most of the state. We chose three stations: Sacramento, Fresno and Los Angeles. All have long records, and are about equally spaced along the length of California. All three stations respond only to synoptic situations that are definitely wet, and receive little moisture from marginal situations. For November-April all three stations have high correlation with the November-April RFI, r being 0.90, 0.92 and 0.89, for Sacramento, Fresno and Los Angeles, respectively. To give each station equal weight in the index, multipliers of 0.86, 1.26 and 0.96, derived from 1930-60 mean annual rainfall values, were used for Sacramento, Fresno and Los Angeles, respectively. The RFI for a given period is the sum of weighted rainfall for that period, in millimetres.

Interestingly, for the period 1889-1972 there is no correlation between early and late rainfall indices. Kendall's parameter τ equal to -0.07 and r to -0.02, are not significant. Thus failure of early rains does not indicate that late rains will also be deficient.

Our SST values were mean temperatures developed by NMFS Pacific Environmental Group from ship observations assembled by the National Climatic Center. These values are means of all observations taken in a given 5° quadrant in a Marsden Square, during a given month and year. They have not been corrected for space or time bias, and it would be expected that correlations obtained from them would be improved if corrections could be made. We used monthly averages of available ship observations of SST in 5° quadrants, 1931-72, for the Pacific Ocean north of 15°N latitude. Ship reports for three Marsden Squares (90, 128, 130) were not conveniently available, so these areas were not considered. The area to the south-east of Hawaii and equatorwards of 15° is probably influential in California rainfall, but SST data there are too sparse for analysis. Elsewhere, data exist in most quadrants for most years, except 1941-46. In preparing our correlation maps, we used all values that were averages of four or more observations in a given month and quadrant, except a few extreme coldest and warmest values based on less than 10 observations were excluded.

In our work relating rain in Brazil to SST in the South Atlantic Ocean we found a slight warming trend of 0.0078 °C per yr for the period 1907-72, and had assumed that this warming was due to instrumental error resulting from evaporative cooling from bucket samples in early years, and heating by ships' engines in injection temperatures in later years. Removal of this trend improved correlations with rainfall. We had originally intended to adjust the North Pacific SST data for the same reason, but soon found that the trend was generally much larger and more variable in space and time than could be accounted for by instrumental factors. We therefore added an arbitrary 0.01 °C per yr before 1972, to compensate partially for the warming trend. Correlations obtained when temperatures are so adjusted are higher than when the raw data are used, which suggests that some degree of instrumental warming may be real.

We found little significant or useful correlation between Pacific SST and November-April rainfall, although there is a tendency towards negative correlation extending from the central Pacific to the region south-east of Japan. In general, the cold water there tends to make California rainy.

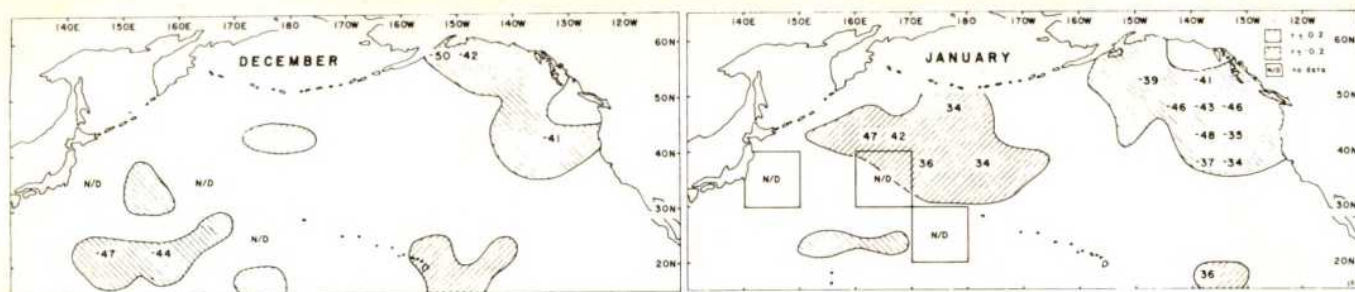


Fig. 1 Correlation between SST and November-January rainfall in California. Correlation coefficients plotted when significant above the 0.05 level.

We found no useful forecasting relation between Pacific SST and November-January rainfall, however, these rains are accompanied by negative correlation—cold water off the coast (Fig. 1). This substantiates Namias' view that warm water off the coast was involved in the 1976-77 winter drought in California³.

A useful forecasting relation seems to exist between SST and February-April rainfall. The situation begins with negative correlation (cold water) in the western and central parts of the Pacific in October. Later in November, December and January, positive correlation (warm water) appears in the Gulf of Alaska. This progresses southwards in February and March, while cold water remains a short distance out to sea (Fig. 2). The area of positive correlation in the north-east Pacific off Washington in December seems to offer the most promise in forecasting February-April rains. In this area MS 158-4 has a correlation coefficient (r) of 0.63 between December SST and February-April rainfall, significant above the 0.001 level. It seems that processes induced by cold water in the central and western Pacific in October, November and December,

when followed by warm water west of Washington in December, are favourable to February-April rains in California. This implies that SST gradient between the two areas is important although we found that this gradient does not yield correlation coefficients as high as using SST at MS 158-4 directly.

Mean SST values for a given month and quadrant contain real SST variations, as well as considerable noise due to non-random distribution of observations in space and time and to various instrumental errors. This noise can be reduced by using a median anomaly from three adjacent quadrants, using those quadrants most nearly aligned with the isotherms of SST. The median is a better measure than the mean because of the effect of extreme values on the mean. The three-quadrant median deviation is taken as the corrected deviation from normal for the central quadrant. Table 1 illustrates the derivation of corrected SST values for MS 158-4.

When monthly SST values in MS 158-4 are refined as stated above, by introducing data from adjacent quadrants, $r=0.68$ and $\tau=0.50$, both with massive significance. This

Fig. 2 Correlation between SST and February-April rainfall in California. Correlation coefficients plotted when significant above the 0.05 level.

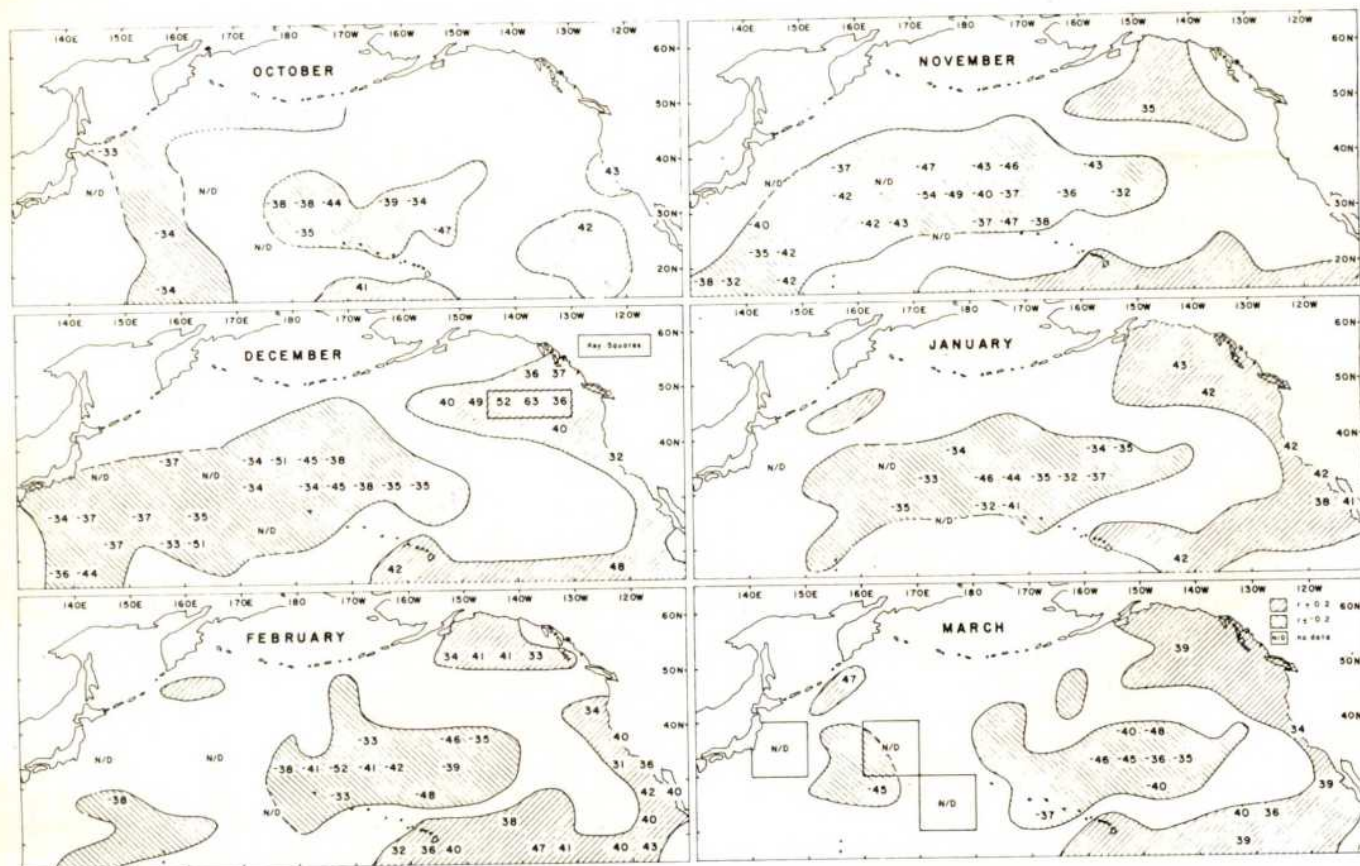


Table 1 Derivation of corrected SST for MS 158-4 ($^{\circ}\text{C} \times 100$)

Year	SST for MS 158-4		Deviation	Deviation	Deviation	Correction†	Corrected SST MS 158-4
	Raw value	Adjusted*	MS 159-3	MS 158-4	MS 158-3		
1931	824	865		-48	22		
32	750	790	-126	-123	-7	0	790
33	736	775	-80	-138	-110	28	803
34	900	938	94	25	62	37	975
35	894	931	14	18	95	0	931
1936	1018	1054	158	141	154	13	1067
37	1029	1064	212	151	-42	0	1064
38	797	831	116	-82	-154	0	831
39	829	862	58	-51	-76	0	862
40	900	932	35	19	43	16	948
1941	—	—	—	—	—	—	—
42	—	—	—	—	—	—	—
43	—	—	—	—	150	—	—
44	—	—	—	—	190	—	—
45	916	943	116	30	-28	0	943
1946	—	—	165	—	—	—	—
47	—	—	—	—	—	—	—
48	—	—	-93	—	-209	—	—
49	947	970	8	57	94	0	970
50	897	919	-65	6	-91	-71	848
1951	906	927	-24	14	-38	-38	889
52	843	863	-44	-52	7	6	869
53	831	850	-39	-63	-27	24	874
54	877	895	-46	-18	-32	-14	881
55	823	840	2	-73	-90	0	840
1956	844	860	29	-53	-32	21	881
57	1034	1049	209	136	137	1	1050
58	953	967	-3	54	51	-3	964
59	1012	1025	33	112	75	-37	988
60	848	860	-120	-53	104	0	860
1961	1078	1089	12	176	70	-106	983
62	1079	1089	-22	176	35	-141	948
63	787	796	-35	-117	-21	82	878
64	958	966	-93	53	-41	-94	872
65	878	885	-44	-28	10	0	885
1966	901	907	8	-6	-11	0	907
67	798	803	-125	-110	-78	0	803
68	871	875	-105	-38	-22	0	875
69	867	870	-63	-43	-8	0	870
70	878	880	-70	-33	-67	-34	846
1971	845	846	-52	-67	-93	0	846
72	948	948	-21	35	-22	-56	892
<i>n</i>	35	35	36	35	38		34
Mean SST	894	913	862	913	996		904
<i>r</i>	0.593	0.628					0.668
<i>t</i>	4.23	4.63					5.07
α''	<0.001	<0.001					<0.001
ϕ	0.441	0.482					0.509
<i>z</i>	3.73	4.07					4.24
α''	<0.001	<0.001					<0.001

Median values are italicised.

*0.21 $^{\circ}\text{C}$ added for each year before 1972.

†Difference between MS 158-4 deviation from normal and three square median deviation from normal.

provides the basis for a February–April rainfall forecast using December SST (Fig. 3). Normal is defined as the mean for the 34 yr during the period 1931–72 when data exist in all three quadrants. When SST is normal, the rainfall forecast will be for 100% of normal, with 95% confidence limits between 10% and 190% of normal. If SST is 1°C above normal, the rainfall forecast is for 160% of normal, with 95% confidence limits between 65% and 250%. If SST is 1°C below normal the rainfall forecast is for 40% of normal, with 95% confidence limits between 0% and 135% of normal. The confidence limits are unfortunately wide, but probably reflect with reasonable accuracy the limits set by SST upon rainfall. Although imprecise, it is an improvement over a forecast based on climatic normals, which would be for 100% of normal with 95% confidence limits between 10% and 190% of normal, regardless of SST.

The τ value of 0.50 implies that the odds are three to

one that a change in rainfall will match the sign change in SST.

We believe that, early in the season, cold water in the central Pacific induces greater-than-normal troughing in the overlying atmosphere. This induces an increased frequency of south-westerly winds that push the SST isotherms northwards in the Gulf of Alaska. As winter advances the cold anomaly in the central Pacific is advected slowly eastward. Troughing in the atmosphere may likewise move eastward, and act to decrease the intensity of the surface subtropical high pressure cell off the California coast. The resultant decreased north-westerly winds along the coast allows a warm SST anomaly to form by decreased upwelling and by reduced southward flow of the California Current. The warm water moves southward, and then westward with the North Equatorial Current as the season advances, to create a positive anomaly of SST near Hawaii. Namias has shown that warm water in this area is important for

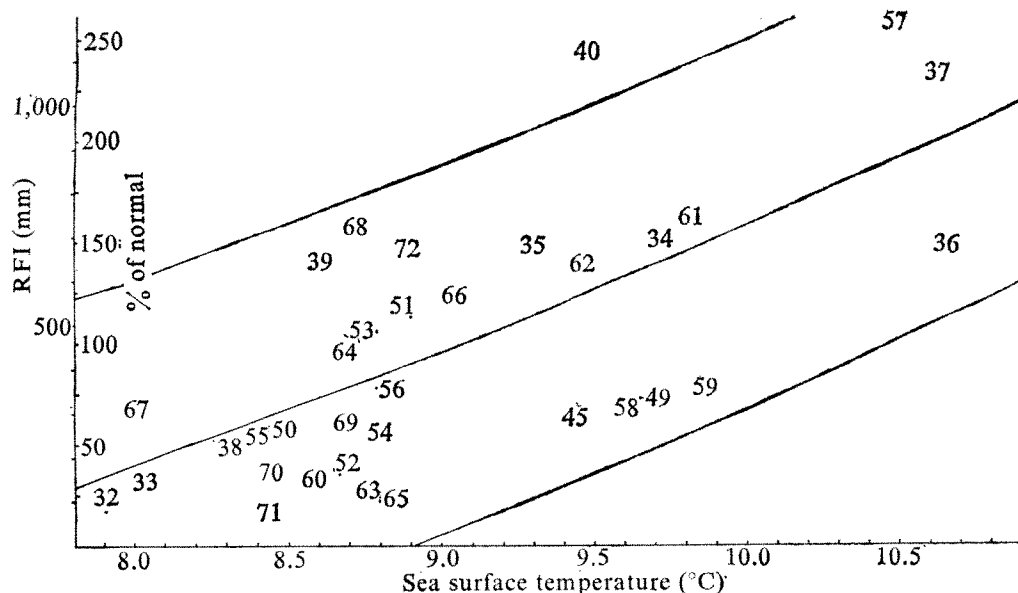


Fig. 3 February–April California rainfall indices (RFI) plotted against corrected December SST in Marsden Square 158–4. Normal SST is 9.04 °C and RFI is 459 mm. Years indicated are for SST. Second degree regression line with 95% confidence limits is shown.

California moisture⁴. Our speculation is tenuous, but we hope that other researchers will study the cause and effect relationships underlying what seems to be real correlation between SST and California rainfall.

We thank Jerome Namias for comments, Jim Canfield for cartography and Martha Raught for typing.

CHARLES G. MARKHAM

Geography Department,
California State University, Fresno,
Fresno, California 93740

DOUGLAS R. McLAIN

*Pacific Environmental Group,
National Marine Fisheries, NOAA,
Fleet Numerical Weather Center,
Monterey, California 93940*

Received 20 June; accepted 31 August 1977.

- 1 Markham, C. G. & McLain D. R. *Nature* **265**, 320–323 (1977).
- 2 Namias, J. *Mon. Weather Rev.* **104**, 1107–1121 (1976); *Preprint Volume Sixth Conference on Weather Forecasting and Analysis*, 13–16 (American Meteorological Society, 1976); *Proc. WMO/IMAP Symp. Long-term Climatic Fluctuations*, 331–340 (WMO, 1976); *J. mar. Res.* **33**, 53–60 (1975).
- 3 *Sci. News* **111**, 100–101 (1977).
- 4 Namias, J. *J. phys. Oceanogr.* **1**, 65–81 (1971).

Direct measurements of secondary currents in river bends

FLUIDS flowing through pipes or channels can develop secondary currents. These are defined as currents which occur in the plane normal to the local axis of the primary flow. Their development in straight channels has been ascribed to anisotropic turbulence and the non-uniform distribution of boundary shear stress¹⁻³ but in meander bends they are generally caused by skewing of the flow³⁻⁵. Secondary currents distort the distributions of primary isovels and boundary shear stress from those expected in simple flows and, therefore, have important implications for bed and bank erosion and for resistance to flow. We report here measurements of longstream and cross stream velocities carried out across sections of a river perpendicular to the outer banks of several bends using an electromagnetic flow meter.

At meander bends fully developed secondary currents appear as a single flow cell, carrying surface water towards the outer

bank and bed water towards the inner bank⁶⁻⁹. Measurements in flumes^{10,11}, however, and in rivers^{12,13} have indicated the existence of a small cell of reverse circulation at the outer bank. A similar feature seems to exist in pipes¹⁴. As this reverse cell has been observed at single bends it is not connected with the reverse circulation of a relict cell from an upstream bend^{15,16}. Rozovskii¹¹ concluded that it is a wall effect, extending over a region of perhaps one or two depths from the bank, but negligible in bends with large ratios of width to depth. Hey and Thorne¹² proposed a new theory of secondary flow at bends, incorporating two cells in place of the classical single cell.

Identification of the outer bank cell in river bends has been based on only a few direct measurements of secondary flows^{12,13}. Because of a lack of suitable instrumentation, these measurements were relatively inaccurate and more detailed information is therefore required. The development of the electromagnetic flow meter has enabled such measurements to be made. This meter measures velocity in two directions simultaneously and so enables the components of horizontal flow to be defined. Measurements can be made to within 1 cm s^{-1} (refs 17 and 18).

In this investigation, measurements were made with an electromagnetic flow meter along sections perpendicular to the outer banks of several bends. The meter was positioned to measure the longstream velocity (parallel to the outer bank) and the cross stream velocity (perpendicular to the outer bank.) These are not necessarily the primary and secondary velocities but the following theory allows calculation of those components.

Secondary currents at any vertical through the flow are defined to occur in the plane normal to that containing the mean primary velocity vector at that vertical. It is assumed that in steady conditions, the average secondary flow in the plane is zero, that is, the secondary discharge in one direction is balanced by the discharge in the other. The longstream and cross stream velocities (those to which the electromagnetic meter responds) are defined by \mathbf{u} and \mathbf{w} respectively. The resultant velocity \mathbf{q} at any depth y is defined to be at a horizontal angle θ to the longstream axis so that

$$u = q \cos \theta \quad (1)$$

$$\mathbf{w} = \mathbf{q} \sin \theta \quad (2)$$

The mean primary velocity U at the vertical is at the fixed angle $\mathbf{1}$ to the longstream axis. $\mathbf{1}$ defines the vector of the

primary flow so the primary velocity U_p at any depth is found by resolving in that direction

$$U_p = q \cos(\theta - \phi) = u \cos \phi + w \sin \phi \quad (3)$$

The secondary velocity U_s is obtained by resolving in the plane normal to that containing the primary velocity vector

$$U_s = q \sin(\theta - \phi) = w \cos \phi - u \sin \phi \quad (4)$$

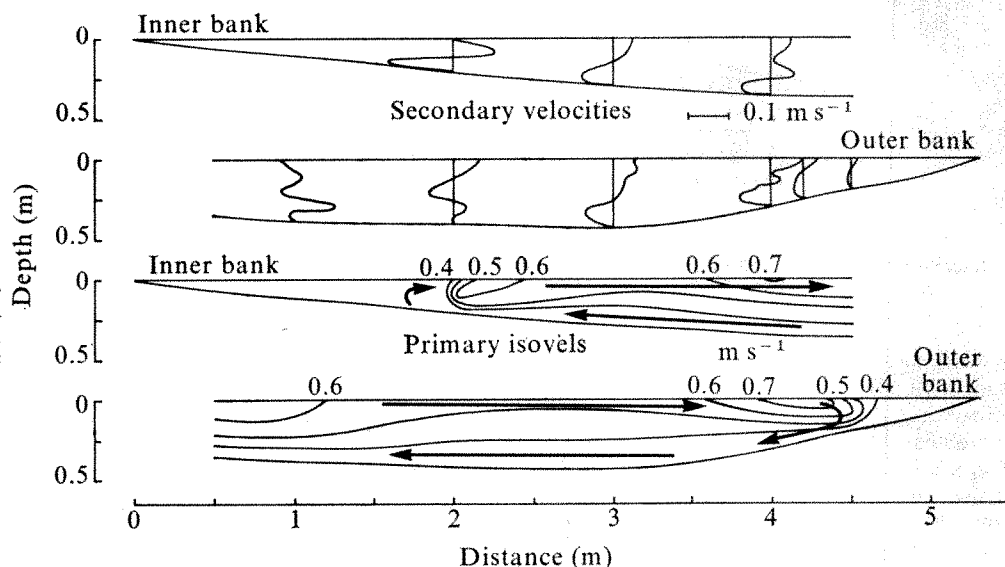
ϕ is unknown but is found by integrating the secondary

as far as was necessary to establish the presence of the main cell (Figs. 2 and 3).

The single classical cell does not always continue to the outer bank. Reverse cells are evident up to one depth from the bank at Rickety Bridge Bend and at Maes Mawr Bend. This pattern is reflected in the distortion of the primary isovels. However, the outer bank cells do not appear at Llandinam Bend.

The outer bank cells probably result from interaction of the main cell with the outer bank. The flow of this cell has a component perpendicular to the bank and, in an ideal fluid, the boundary streamlines of this component would follow exactly the water surface and the boundary of the channel. In a real fluid, this is not possible since it requires an infinite acceleration

Fig. 1 Primary and secondary velocities at Llandinam Bend—River Severn. Channel width 10 m; radius of curvature 75 m; bend arc angle 62°; discharge 1.1 m³ s⁻¹.



velocities over the depth, d . As their mean is defined to be zero,

$$\int_0^d q \sin(\theta - \phi) dy = 0 \quad (5)$$

so that

$$\tan \phi = \frac{\int_0^d q \sin \theta dy}{\int_0^d q \cos \theta dy} = \frac{\int_0^d w dy}{\int_0^d u dy} \quad (6)$$

w and u are the velocities measured by the electromagnetic meter. Substitution of the value of ϕ into equations (3) and (4) allows the secondary and primary velocities at any depth to be calculated.

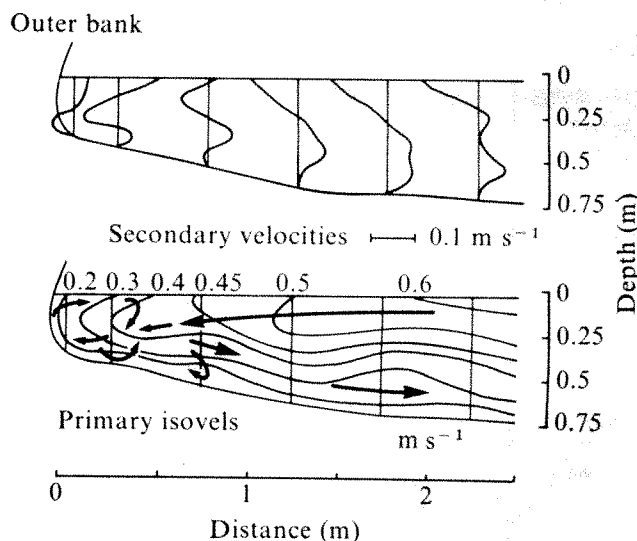
Measurements were made at three bends on the Upper River Severn. Llandinam Bend is a single bend at a shallow section of channel. Rickety Bridge Bend is a slight bend just downstream from a riffle which directs the flow strongly against the outer bank. Maes Mawr Bend is a conventional meander bend, on a pool and downstream of a shallow reach.

A complete survey was made at Llandinam Bend at a low discharge and this shows the classical cell across the whole channel (Fig. 1). At the other sites, velocity profiles were taken at progressively greater distances from the outer bank but only

at the junction of bank and water surface, so a breakdown of the ideal pattern is expected.

The occurrence of the reverse cells seems to depend on the form of the bank. They do not occur where the bank shelves but do where the bank is perpendicular to the water surface. This is verified by several other surveys made at high and low discharges and at shelving and steep banks at Maes Mawr bend. Where the outer bank is steep, the junction

Fig. 2 Primary and secondary velocities at Rickety Bridge Channel—River Severn. Channel width 8 m; radius of curvature 50 m; bend arc angle 50°; discharge 1.7 m³ s⁻¹.



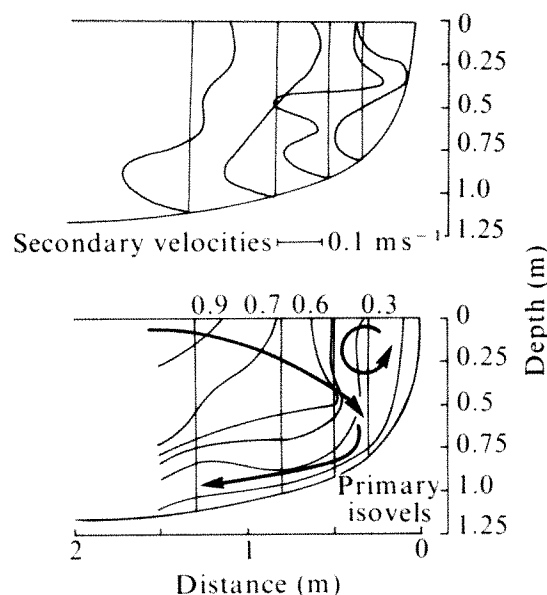


Fig. 3 Primary and secondary velocities at Maes Mawr Bend—River Severn. Channel width, 21 m; radius of curvature, 70 m; bend arc angle, 38°; discharge $9 \text{ m}^3 \text{ s}^{-1}$.

of bank and water surface (seen in section) corresponds to a stagnation point. At this point, water would be expected to pile up and it is possible that the resulting upwelling at the surface, combined with the weight of the superelevated water, could trigger a more general reversal of secondary flow in the region of the bank. This process might be aided by the local surging of flow which results from the passage of turbulent eddies.

If this theory is correct, the strength of the outer bank cell should be proportional to the component of flow towards the outer bank. This seems to be confirmed because the cell at Rickety Bridge Bend, where the flow is directed against the bank, is stronger than at Maes Mawr Bend.

Direct measurements of outer bank cells confirm that the classical cell oversimplifies the pattern of secondary flow in some river bends. This has important repercussions for the evaluation of flow resistance, the distribution of boundary shear stress, the movement of bed load and thence bank erosion and meander migration.

J. C. BATHURST

Engineering Research Center,
Colorado State University,
Fort Collins,
Colorado 80523

C. R. THORNE
R. D. HEY

School of Environmental Sciences,
University of East Anglia,
University Plain,
Norwich, UK

Received 10 June; accepted 10 August 1977.

- Brundrett, E. & Baines, W. D. *J. Fluid Mech.* **19**, 375–394 (1964).
- Gessner, F. B. & Jones, J. B. *J. Fluid Mech.* **23**, 689–713 (1965).
- Perkins, H. J. *J. Fluid Mech.* **44**, 721–740 (1970).
- Squire, H. B. & Winter, K. G. *J. Aero. Sci.* **18**, 271–277 (1951).
- Prandtl, L. *Essentials of Fluid Dynamics* (Blackie, London, 1952).
- Thomson, J. *Proc. R. Soc.* **25**, 5–8 (1876).
- Hawthorne, W. R. *Proc. R. Soc. A* **206**, 374–387 (1951).
- Fox, J. A. & Ball, D. J. *Proc. Instn Civ. Engrs* **39**, 467–475 (1968).
- Quick, M. C. *Proc. Am. Soc. Civ. Engrs J. Hydraul. Div.* **100**, 741–753 (1974).
- Einstein, H. A. & Harder, J. A. *Trans. Am. geophys. Un.* **35**, 114–120 (1954).
- Rozovskii, I. L. *Flow of Water in Bends of Open Channels* (transl. by Israel Program for Scientific Translations, Jerusalem, 1961).
- Hey, R. D. & Thorne, C. R. *Area* **7**, 191–195 (1975); **8**, 234–236 (1976).
- Bridge, J. S. & Jarvis, J. *Earth Surf. Process.* **1**, 303–336 (1976).
- Rowe, M. J. *J. Fluid Mech.* **43**, 771–783 (1970).
- Chacinski, T. M. & Francis, J. R. D. *Trans. Am. geophys. Un.* **33**, 771–773 (1952).
- Toebes, G. H. & Sooky, A. A. *Proc. Am. Soc. Civ. Engrs J. Water Harb. Div.* **93**, 213–236 (1967).
- Tucker, M. J. *Proc. Soc. Underwater Technol.* **2**, 53–58 (1972).
- Kanwisher, J. & Lawson, K. *Limnol. Oceanogr.* **20**, 174–182 (1975).

Prehistoric dental calculus gives evidence for coca in early coastal Ecuador

LOS CERRITOS, a late Chorrera (Engoroy) cemetery on the Santa Elena Peninsula of Ecuador yielded artefacts and radiocarbon assay which indicate that use of the site began about 840 BC and continued for several years within the Late Formative¹. The human skulls from the site are of particular interest because many showed heavy accumulations of supragingival dental calculus (tartar), which contrast with the relatively slight calculus accumulations on the teeth of skulls recovered from the earlier Valdivia (3000–1500 BC) site of Real Alto², also on the Santa Elena Peninsula. We argue here that the heavy calculus accumulations are the result of habitual, secular coca chewing in late Chorrera times. This diagnosis, in turn, supports the hypothesis of prehistoric trade in coca, probably in exchange for mollusc shells, with inhabitants of the eastern Andean slopes.

Of 27 adult human skulls from Los Cerritos with dentitions sufficiently intact to be evaluated, 17 had calculus deposits which ranged from slight to extreme (Table 1). This scale was devised to accommodate the range of variation in the material; even 'slight' would be considered heavy by modern standards.

According to ethnographic accounts^{3–5}, coca leaves were commonly chewed with lime in western South America. The lime is responsible for the dental deposits. Mehta *et al.*⁶ noted that heavy calculus in modern India correlated with chewing betel with lime. Leigh⁷ reported large dental accretions from prehistoric Guam, which he attributed to the habit of chewing betel nut with lime. He concluded that similar deposits from prehistoric Peru resulted from coca chewing⁸. Moodie⁹ also observed excessive calculus among pre-Columbian Peruvians. In the prehistoric examples, as in the Chorrera material, the deposits were on the buccal tooth surfaces of both maxilla and mandible, which is in accord with the practice of holding the quid between the cheek and teeth. In contrast, the sites most affected in modern populations are those near the openings of the salivary ducts—the lingual surfaces of mandibular incisors and the buccal surfaces of maxillary molars.

Lime has been implicated as an aetiological factor in dental deposits, but the nature of the deposits is uncertain. Mehta *et al.*⁶ called the deposits calculus, but Leigh⁸ referred to them as lime. The chemical composition not only provides an accurate description of the pathology, but can reveal clues about the mechanism and conditions of the depositions. Six samples of dental accretion from six Los Cerritos skulls were subjected to elemental and X-ray diffraction analysis. The samples were cleaned only by dry-brushing.

The major and minor elemental analysis of the deposits was performed by standard X-ray fluorescence techniques as developed by Rose *et al.*¹⁰. To approach a total composition of the material mean values are reported in Table 2 as the percent of oxides, even though the elements actually occur in mineral form. The sum of the components is less than

Table 1 Calculus deposits on the teeth of adult human skulls from Los Cerritos

Extent of accumulation	Probably male	Probably female	Sex indeterminate
Extreme	1	0	0
Very heavy	4	0	0
Moderately heavy	5	2	0
Slight	1	4	0
None	2	7	1

100% because very limited sample sizes precluded determination of carbon dioxide, moisture, and ignition loss.

X-ray diffraction patterns of each sample revealed a poorly-crystalline apatite-like phase representing hydroxyapatite, $\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, a major component of calculus¹¹⁻¹³. Considerable quartz was also present, together with some calcium silicate, $\text{Ca}_3\text{Si}_2\text{O}_7$, or calcium aluminium silicate, $\text{CaAl}_2\text{Si}_2\text{O}_8$. The similarity of the compositions indicates that the teeth were in contact for long periods with ground water from which silica precipitated within the relatively porous calculus structure.

Table 2 Major and minor elemental analysis of dental deposits

Element	% of oxides
SiO_2	40.02 ± 10.78
Al_2O_3	6.17 ± 2.02
CaO	26.01 ± 8.88
K_2O	0.42 ± 0.15
MgO	2.02 ± 0.87
Na_2O	1.08 ± 0.56
Fe_2O_3	3.96 ± 1.20
TiO_2	0.77 ± 0.21
P_2O_5	16.13 ± 5.33

Values are means \pm s.d. for six samples.

Brushite, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, and octacalcium phosphate, $\text{Ca}_8\text{H}(\text{PO}_4)_3 \cdot 2\text{H}_2\text{O}$, which are two crystalline forms known to occur in some calculus samples¹¹⁻¹³, were not discernible. It was not clear whether magnesium whitlockite, $(\text{Ca}, \text{Mg})_3(\text{PO}_4)_2$, another crystalline form found in some calculus, was present in any of the samples since certain major diffraction peaks for this substance fall in the same region as those characterising hydroxyapatite which was present. Insufficient sample sizes precluded fluoride analyses.

The presence of hydroxyapatite plus percentages of calcium and phosphorus similar to those reported for calculus¹¹⁻¹⁴ suggest, but do not confirm, that the accretions were originally laid down as dental calculus, not as lime or other simple calcium salts. Variations in the relative ratios of calcium, phosphorus, and magnesium indicate that some degradation has taken place on the calculus itself. This, together with the postmortem contamination, indicates that preservation of the material has been affected by environment, but not sufficiently to invalidate the basic conclusion.

The magnesium concentration in the samples was higher than that reported for modern calculus¹¹⁻¹⁴ but is compatible with the chewing of coca leaves since magnesium is a constituent of chlorophyll and a cofactor in some plant enzymes.

Since the lime was responsible for the deposits, the possibility that the people were chewing tobacco with lime cannot be ruled out altogether. Nevertheless, there is circumstantial evidence which argues in favour of coca use. In South America the historic distribution of chewing tobacco with lime was much more restricted than that of chewing coca with lime³. Furthermore, in the early historic period tobacco was used pre-eminently in ritualistic context whereas coca use was commonly secular and hedonic³. The huge calculus deposits imply habitual and, therefore, secular use. At Los Cerritos calculus deposits were more severe among males than females, suggesting that coca consumption by males was either more frequent or began at an earlier age.

Cultural evidence for the chewing of coca in coastal Ecuador appears as early as Valdivia times. Small ceramic lime pots have been found at Valdivian sites¹⁵, including Real Alto. In addition, a figurine recovered from the Valdivia-type site clearly depicts a quid in the cheek of a woman¹⁵.

Why, then, do the skeletons from Real Alto not show heavy deposits on the teeth? Presumably coca chewing expanded from a restricted, probably shamanistic, role in the Early Formative to a more popular, secular role in the Late Formative.

Since the climate of the Santa Elena Peninsula precludes the growing of coca, which thrives on the well-watered eastern slopes of the Andes, coca must have reached the coast by trade. Although there is evidence for trade routes as early as the Valdivia period^{16,17} commerce had become much more extensive by late Chorrera times. Paulsen¹⁸ presents evidence for the export of thorny oyster and conch shells from the Ecuadorian coast to the sierra beginning as early as 2800 BC. She concludes that the limited trade of the Early Formative had expanded to the Peruvian Andes by the first millennium BC. We propose that increased importation of coca to the south coast of Ecuador accompanied expanded exportation of mollusc shells to the sierra. In times of limited trade imported items would be at a premium and probably reserved for religious or curing purposes; expanded trade would make the exotic item, coca, available to a wider segment of the population for temporal use, in much the same manner as it is used by modern Andean peoples.

We thank C. Zevallos M. and R. Parducci M. for permission to study the Los Cerritos skulls and to take calculus samples; J. Zeidler for obtaining the samples, and D. B. Shimkin, J. G. Marcos, and D. W. Lathrap for valuable advice.

LINDA L. KLEPINGER

Department of Anthropology,
University of Illinois,
Urbana, Illinois 61801

JOHN K. KUHN

JOSEPHUS THOMAS, JR

Illinois State Geological Survey,
Urbana, Illinois 61801

Received 22 February; accepted 4 August 1977.

- 1 Zevallos, M. C. *Revista del Museo Nacional, Lima, Peru* 34, 20-27 (1965-66).
- 2 Lathrap, D. W. & Marcos, J. G. *Revista de la Universidad Católica, Pontificia Universidad Católica del Ecuador, Quito*. Numero Monografico: Arqueologia Ano III, no. 10, 41-64 (1975).
- 3 Cooper, J. M. in *Handbook of South American Indians* (ed. Steward, J. H.), 525 (Smithsonian Institution, Bureau of American Ethnology, Bulletin 143, Washington, DC, 1949).
- 4 Mortimer, W. G. *Peru. History of Coca: The Divine Plant of the Incas* (Vail, New York, 1901).
- 5 Towle, M. *The Ethnobotany of Peru* (Wenner-Gren Foundation, New York, 1961).
- 6 Mehta, F. S., Sanjara, M. K., Bareto, M. A. & Doctor, R. J. *Am. dent. Ass.* 50, 531-536 (1955).
- 7 Leigh, R. W. *Mem. Bernice P. Bishop Mus.* 11, 255-273 (1930).
- 8 Leigh, R. W. *Am. J. Phys. Anthropol.* 22, 267-296 (1937).
- 9 Moodie, R. L. *Pacif. Dent. Gaz.* 39, 24-29 (1931).
- 10 Rose, H. J., Alder, I. & Flanagan, F. J. *U.S. Geol. Surv. Prof. Pap.* 450-B, 80-85 (1962).
- 11 Mukherjee, S. J. *periodont. Res. Suppl.* 2, 1-35 (1968).
- 12 Glickman, I. *Clinical Periodontology*, 4th edn (Saunders, Philadelphia, 1972).
- 13 Schroeder, H. E. *Formation and Inhibition of Dental Calculus* (Huber, Berne, 1969).
- 14 Hodge, H. C. & Leung, S. W. *J. Periodont.* 21, 211-221 (1950).
- 15 Lathrap, D. W., Collier, D. & Chandra, H. *Ancient Ecuador: Culture, Clay and Creativity 3000-300 B.C.* (Field Museum of Natural History, Chicago, 1975).
- 16 Myers, T. P. *Am. Antiq.* 41, 353-360 (1976).
- 17 Lathrap, D. W. *World Archaeol.* 5, 170-186 (1973).
- 18 Paulsen, A. C. *Am. Antiq.* 39, 597-607 (1974).

High levels of cadmium in Atlantic seabirds and sea-skaters

THE possibility that cadmium may be an environmental pollutant has caused concern because it has toxic effects on many animal species including man, rat, rabbit, chicken, quail, and pigeon¹⁻⁴. The occurrence of cadmium in wildlife is not unusual and samples of marine species taken from around the British coast contain low levels (less than 0.5 mg per kg in fish and less than 2 mg per kg in most shellfish); however, levels of up to 93 mg per kg were present

in limpets, *Patellasp.*, collected from areas of natural mineralisation or industrial sources of cadmium⁵. Seabirds generally contain higher residues than marine invertebrates. For example, Anderlini *et al.*⁶ examined the amounts of nine metals in the livers of seven Antarctic and Pacific seabird species and noted that the maximum mean cadmium residue occurred in 10 ash petrels *Oceanodroma homochroa* collected from their breeding grounds in California (mean residue was 53.2 ± 20.5 mg per kg). Parslow *et al.*⁷ found up to 22.3 mg per kg dry wt cadmium in livers of eight puffins *Fratercula arctica* collected live from various British breeding colonies. It was suggested that pelagic birds might contain higher residues than coastal living species because a single fulmar *Fulmarus glacialis* examined by them contained 159 mg per kg cadmium. Anderlini *et al.* concluded that there was a correlation between increased concentration of cadmium in birds and exposure to industrial influences. Parslow *et al.* did not attempt to explain the residues they found in puffins, but Bourne⁸ concluded that individual birds were becoming increasingly contaminated through feeding near areas of local pollution around the British coast. It is debatable whether cadmium should be regarded as a pollutant to seabirds. We report here the occurrence of cadmium residues in the tissues of apparently healthy, breeding seabirds which are considerably higher than any previously found. More important, we conclude that the cadmium has a natural rather than an industrial origin, since we have found high cadmium residues in a marine insect, *Halobates*, which is widely distributed in tropical regions far from sources of industrial cadmium. This insect is only one example of the sources from which birds obtain their cadmium residues.

Table 1 Cadmium residues in individual St Kilda seabirds

Species	Sex	Cadmium concentration (mg per kg dry wt)	
		Liver	Kidney
Fulmar <i>Fulmarus glacialis</i>	1* ♂	9.1	46.7
	2* ♀	50.1	184
	3 ♂	7.7	32.8
	4 ♀	49.4	240
Manx shearwater <i>Puffinus puffinus</i>	1 ♂	26.0	133
	2 ♀	39.9	231
	3 ♂	14.6	67.0
	4 ♀	15.6	113
Puffin <i>Fratercula arctica</i>	1 ♂	18.9	109
	2 ♀	14.1	75.1
	3 ♂	29.4	125
	4 ♀	57.0	68.5
Leach's petrel <i>Oceanodroma leucorhoa</i>	1 ♂	21.5	128
	2 ♀	20.6	80.0
	3 ♂	9.2	30.2
	4 ♀	14.4	52.9
Storm petrel <i>Hydrobates pelagicus</i>	1 ♂	20.8	37.7
	2 ♀	26.5	36.7
	3 ♂	2.4	14.6
	4 ♀	1.8	16.0
Razorbill <i>Alca torda</i>	1 ♂	1.4	18.2
	2 ♀		

Birds were killed by exsanguination and their tissues immediately frozen using solid CO₂ and kept below -20 °C until analysed for heavy metals. Metal analyses were performed by atomic absorption spectroscopy after tissues had been digested with concentrated nitric acid. Parentheses show birds known to be paired.

*Birds known to have an egg. Fulmars 3 and 4 were prospecting birds not yet ready to breed. All other subjects were thought to be breeding but this could not be confirmed due to inaccessibility of the nest site.

|| This bird had a shelled egg in the oviduct.

In June–July 1976 21 seabirds representing six species were collected, under licence, at a major breeding colony on St Kilda, a remote island group 80 km west of the Outer Hebrides, Scotland. All the animals were apparently healthy and unaffected by the metal levels they carried (Table 1). In all species, residues were higher in the kidney than in the liver, as in cadmium-contaminated humans¹. Cadmium residues were considerably higher in the pelagic species (shearwaters, puffins, and petrels) than in the razorbill, which feeds in the region of the continental shelf throughout the year^{9,10}. Since the pelagic species on St Kilda are unlikely to be exposed to industrial cadmium, it seemed possible

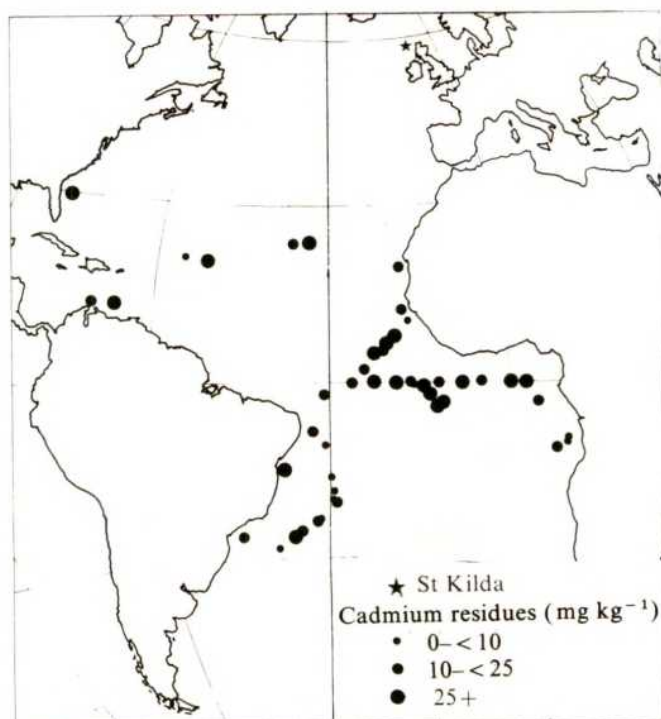
that there might be important sources of cadmium in the open ocean.

Leatherland *et al.*¹¹ examined a range of plankton organisms and their predators, although very small samples were involved. They found appreciable levels of cadmium in some species, the maximum being 13 mg per kg dry wt in the decapods *Systellaspis debilis* (four examined) and *Ophophorus* sp. (two examined). Plankton samples are usually of a variably mixed species composition and it is not easy to isolate a single species from a wide range of localities to check for geographical variations in cadmium content.

Sea-skaters of the genus *Halobates* (Gerridae: Heteroptera) are pelagic marine insects. They live at the sea surface and feed on zooplankton trapped at the air–sea interface. Relatively high cadmium contents have been detected in *Halobates* from various parts of the Pacific Ocean, and these may reflect correspondingly high cadmium levels in the waters where the samples were collected¹². It seems unlikely that the high cadmium levels detected in the sea-skaters resulted from atmospheric pollution, as no cadmium was detectable (< 5 mg per kg dry wt) in another gerrid, *Rheumatobates aestuarius*, collected from coastal mangrove lagoons in the Gulf of California, close to the area where *Halobates* was found at sea with very high cadmium levels (up to 200 mg per kg dry wt)¹². Since these insects are widely distributed in the oceans and single-species samples can be obtained over a large area, their cadmium contents can indicate the distribution of this heavy metal in the surface layers of the ocean.

Samples of *Halobates micans* were obtained from tropical areas of the Atlantic Ocean. These were rinsed of all foreign matter with acidified distilled water, digested in concentrated nitric acid and then analysed for cadmium using atomic absorption spectrophotometry. The distributions of residues in the samples were not uniform (Fig. 1) but there was no pattern suggesting pollution. The mean concentration in 111 samples was 22.7 ± 3.1 mg per kg dry wt, ranging from 0 to 309 mg per kg (in a sample from the Gulf of Guinea). In many instances several samples were collected at one

Fig. 1 Cadmium content of *Halobates micans* collected from 47 sites along survey routes in the Atlantic; the species is confined to warmer waters. In cases when more than one sample was collected from the same site the highest value has been plotted. St Kilda was the collecting locality for the seabirds analysed for cadmium (Table 1). Sources of *Halobates* samples and acknowledgments are given by Cheng¹².



site and in Fig. 1 maximum values are depicted; the mean for these was 30.5 ± 6.7 mg per kg ($n = 47$).

The concentration of cadmium in the ocean varies little from the depths to within 0.5 km of the surface layer, but declines rapidly near the surface, conceivably as a result of incorporation into living organisms¹³. Some of the areas where the higher cadmium residues occur, for example, off the coast of West Africa, have little industrial activity, but have upwelling ocean currents of cold water which bring to the surface nutrients, and possibly cadmium too. Areas of ocean upwelling are known to be important wintering locations for pelagic seabirds, because they are rich feeding grounds, and it is likely that the birds accumulate at least some of their residues in these places. The Manx shearwater spends the contranuptial season off the coast of South America, while the storm petrel winters off South Africa and Leach's petrel very widely in the tropics; puffins ringed on the western coast of Britain have been recovered south of the Straits of Gibraltar but the precise wintering grounds remain to be defined¹⁴. We cite the *Halobates* data as an indication of the widespread occurrence of cadmium in tropical waters. It is doubtful whether many seabirds take large quantities of *Halobates* though the phoenix petrel *Pterodroma alba* and blue-grey noddy tern *Procelsterna cerulea* have been proved to eat them in the Pacific^{15,16}. We might, therefore, expect these insects to be among the sources from which such birds as storm petrels could obtain cadmium.

We conclude that the high cadmium concentrations found in seabirds originate from natural sources, rather than from industrial activity, and that the birds, having evolved in an ecosystem containing cadmium, have developed, through natural selection, mechanisms which enable them to tolerate this metal. One such mechanism, now being investigated, involves production of a metal-binding protein which has been identified in the fulmar—it has many of the properties of metallothionein.

We thank P. Freestone and L. A. Sheppard for help with the heavy metal analyses.

K. R. BULL
R. K. MURTON
D. OSBORN
P. WARD

*Institute of Terrestrial Ecology,
Monks Wood Experimental Station,
Abbots Ripton, Huntingdon, UK*

*Scripps Institution of Oceanography,
University of California,
San Diego, California 92037*

LANA CHENG

Received 8 March; accepted 9 August 1977.

1. Friberg, L., Piscator, M., Nordberg, G. F. & Kjellström, T. *Cadmium in the Environment* (CRC Press, Cleveland, Ohio, 1971).
2. Loftis, B. & Murton, R. K. *J. Reprod. Fert.* **13**, 155–164 (1967).
3. Richardson, M. E., Fox, M. R. S. & Fry, B. E. Jr. *J. Nutr.* **104**, 323–338 (1974).
4. Pritzl, M. C., Lie, Y. H., Kienholz, E. W. & Whiteman, C. E. *Poult. Sci.* **53**, 2026–2029 (1974).
5. Cohen, M. in *Problems of the Contamination of Man and his Environment by Mercury and Cadmium* 543–570 (Commission of the European Communities, Luxembourg, 1973).
6. Anderlini, V. C., Connors, P. G., Risebrough, R. W. & Martin, J. H. in *Proc. Symp. Conserv. Probl. Antarctica* (ed. Parker, B. C.) 49–62 (Virginia Polytechnic Institute and State University, Blacksburg, 1972).
7. Parslow, J. L. F., Jefferies, D. J. & French, M. C. *Bird Study* **19**, 18–33 (1972).
8. Bourne, W. R. P. in *Marine Pollution* (ed. Johnston, R.) 403–502 (Academic, London, 1976).
9. Mead, C. J. *Bird Study* **21**, 15–86 (1974).
10. Lloyd, C. *Bird Study* **21**, 182–216 (1974).
11. Leatherland, T. M., Burton, J. D., Culkin, F., McCartney, M. J. & Morris, R. J. *Deep-Sea Res.* **20**, 679–685 (1973).
12. Cheng, L., Alexander, G. V. & Franco, P. J. *Water Air Soil Pollut.* **6**, 33–38 (1976).
13. Boyle, E. A., Scallier, F. & Edmond, J. M. *Nature* **263**, 42–44 (1976).
14. Cramp, S., Bourne, W. R. P. & Saunders, D. *The Seabirds of Britain and Ireland* (Collins, London, 1974).
15. Ashmole, N. P. & Ashmole, M. J. *Bull. Peabody Mus. nat. Hist.* **24**, 1–131 (1967).
16. Cheng, L. *Mar. Fish. Rev.* **36**, 1–7 (1974).

Pectolytic anaerobic bacteria cause symptoms of cavity spot in carrots

CAVITY spot, a disease which causes considerable damage to the mature storage roots of carrots in several countries including the United Kingdom and the United States¹, has been reported to be caused by calcium deficiency, either actual or induced by excessive potassium levels in the soil². We have examined diseased carrots in

East Scotland since 1966 and have been unable to confirm the relationship with calcium levels. Evidence reported here indicates that pectolytic bacteria of the anaerobic genus *Clostridium* are the cause.

In the light of observations that the incidence of cavity spot was greatest in wet seasons on poorly drained land, and that more roots had lesions in waterlogged than in freely drained pots, the effect of restricting aeration to roots was investigated. Carrots were grown in plastic pots containing UC sand-peat compost (a mixture of three parts peat to one part coarse sand, formulated at the University of California) and when the crown diameters were greater than 20 mm, the compost surface was sealed with paraffin wax (melting point 55 °C). Roots of similar size were also lifted from the field, washed carefully and transplanted to similar pots. The pots were placed in trays containing 25 mm of water and kept in a controlled environment cabinet at 20 °C with an 18-h daylength of 12,000 lx. Analyses of gas composition in the compost (gas chromatogram, Pye model 106, MS 5A column, length 152.4 mm, oven temperature 100 °C) indicated that anoxic conditions developed within 48 h. After 5 or 6 d the wax was removed and the pots were returned to the glasshouse bench. Characteristic lesions were observed 3 weeks later. The proportion of roots affected was greatly increased when soil from a field outbreak was added to the roots before sealing. For example, the percentage of roots with lesions in an experiment in which all pots were sealed and supplemented with field soil or autoclaved field soil, or not supplemented, were 72, 28, and 24% respectively ($P < 0.001$ on contingency analysis).

Because there was evidence of a heat-labile factor in field soil and because no fungi or bacteria which were pathogenic on re-inoculation had been isolated by conventional techniques, isolations and subsequent inoculations were carried out under hydrogen in McIntosh and Fildes anaerobic jars fitted with palladium catalysts. Using a reducing medium with a pectate layer³, we isolated pectolytic bacteria at 25 °C from lesions formed on roots in sealed pots supplemented with field soil. Isolates were repeatedly sub-cultured from single colonies on streaked pectate plates and when grown in liquid basal medium anaerobically and inoculated to carrots, lesions identical to those caused by the soil supplement developed in sealed but not in open pots. The bacteria were recovered from these lesions by anaerobic culture.

Similar bacteria were obtained from several fields where cavity spot had occurred by inoculating carrot disks with about 1 g soil, and they were also recovered from some natural lesions incubated, like the disks, in anaerobic conditions. The colonies were cream coloured and the bacteria grew rapidly in anaerobic conditions at 25 °C producing a plaque in the pectate layer of up to 20 mm diameter in 2 d. Pectolysis on media and on carrot disks was still evident at 10 °C and no growth occurred on agar or on carrot disks in air. The bacteria were gram negative rods, 2.8–5.2 × 0.6–0.8 nm, which produced sub-terminal ovoid endospores, 1.4–2.4 × 0.8–1.0 nm. Gelatin was not liquified within 7 d at 25 °C. The organism was identified as a member of the genus *Clostridium*⁴, although results of further tests did not agree with those of any described species.

Cavity spot often occurs on poorly drained, compacted soils and it is most common after heavy rainfall in midsummer when the soil biomass and the high root population of carrots grown for canning may deplete oxygen more rapidly than it can be replaced by diffusion from the atmosphere. In these conditions it is suggested that the anaerobic bacteria described here may initiate cavity spot lesions. Pectolytic *Clostridium* spp. have been implicated in the rotting of potatoes in storage but they have not been associated previously with a field disease syndrome.

We thank Mr R. A. Fox and Dr M. C. Pérombelon for helpful suggestions and Dr G. Hobbs for examining isolates of the bacterium.

D. A. PERRY
J. G. HARRISON

*Scottish Horticultural Research Institute,
Invergowrie,
Dundee, UK*

Received 4 July; accepted 16 August 1977.

1. Guba, E. F., Young, R. E. & U. T. *Plant Dis. Repr* **45**, 102-105 (1961).
2. Maynard, D. N., Gersten, B., Young, R. E. & Vernell, H. F. *Proc. Am. Soc. hort. Sci.* **83**, 506-510 (1961).
3. Lund, B. J. *appl. Bact.* **35**, 609-614 (1972).
4. Buchanan, R. E. & Gibbons, N. E. *Bergey's Manual of Determinative Bacteriology*, 8th edn (Williams and Wilkins, Baltimore, 1974).

Carcinogenic activity of hexachlorobenzene in hamsters

HEXACHLOROBENZENE (HCB), has been widely used as a fungicide, is a contaminant of various pesticides including DACTHAL and pentachloronitrobenzene, and is also a by-product in the manufacture of many chlorinated hydrocarbons (in the United States probably more than 2×10^6 pounds of HCB are produced annually¹). In Turkey, HCB was the causative agent in a severe outbreak of porphyria cutanea tarda symptomata involving several thousand people between 1955 and 1959 (refs 2-4). In spite of the ban of HCB in 1959, symptoms of disease due to this toxic porphyria have persisted for a long time^{5,6}. The aim of present studies was to determine the chronic toxicity of HCB after prolonged oral administration and we report here that HCB is carcinogenic in hamsters.

Six-week-old Syrian golden hamsters from the Eppler colony were used. They were fed *ad libitum* for life a diet containing 50, 100 and 200 parts per million (p.p.m.) HCB. The HCB was more than 99.5% pure (BDH, England). Dosage regime and experimental design are shown in Table 1. At 50 weeks of age, 71% of the treated animals were alive, a survival rate

Table 1 Dosage regime for HCB in hamsters

Group	No. of animals		Concentration of HCB in diet (p.p.m.)	Duration of treatment	Calculated daily intake (mg kg ⁻¹ d ⁻¹)
	F	M			
Control	40	40	0	Lifespan	0
Low	30	30	50	Lifespan	4
Medium	30	30	100	Lifespan	8
High	60	59	200	Lifespan	16

comparable with that of the controls. After 70 weeks, a shorter lifespan occurred in both females and males treated at the highest dose level of HCB; compared with the controls, males treated at 200 p.p.m. HCB showed a marked weight reduction. Table 2 gives cumulative data on the tumour incidence at each dose level. The percentage of tumour-bearing animals (TBA) varied, with controls showing a 10% incidence and hamsters treated at 200 p.p.m. HCB, a 92% average incidence. There was, therefore, an obvious difference relative to treatment. The average number of tumours per hamster and the percentage of hamsters with more than one tumour also showed a dose-response relationship.

All 15 thyroid tumours found were alveolar adenomas and were present only in treated animals. There was a significant increase ($P < 0.05$) in occurrence of these tumours in males treated at 200 p.p.m. HCB, when compared with male controls.

No hepatomas were seen in the control group. In the HCB-treated animals, the incidence increased from 46% in the group treated at 50 p.p.m. to 85% in hamsters receiving 200 p.p.m. HCB. No hepatoma metastases were found. The first hepatoma (with multiple nodules, the largest in diameter being 7 mm) occurred in one female hamster that died after 18 weeks of treatment.

Liver haemangioendotheliomas were also observed only in the HCB-treated group. The highest and significant incidence occurred in the group treated at 200 p.p.m. HCB, consisting of 11.6% of females and 35% of the males. Three of the haemangioendotheliomas in these animals metastasised. A few haemangioendotheliomas were observed in the spleen.

Among other tumours, an increased incidence of adrenal neoplasms occurred; however, no clear dose-response relationship was shown, nor was there a significant difference between the groups.

The results indicate the carcinogenicity of HCB. In the case of other known carcinogens, such as dimethylnitrosamine, vinyl chloride, 2-acetylaminofluorene and 2-naphthylamine, there is a wide spectrum of carcinogenic activity in different tissues and species, including the hamster. In our present experiment, doses of HCB ranging from 50 to 200 p.p.m. resulted in a significant induction of hepatomas, haemangioendotheliomas and thyroid adenomas. Further, an increased number of tumour-bearing animals and of tumours per animal was noted, as were a shortened lifespan and a reduced latency period for onset of liver tumours. These effects may indicate that HCB behaves like certain carcinogens shown to have multipotential activity.

A purely quantitative approach indicates that an intake of 4-16 mg kg⁻¹ d⁻¹ of HCB in our hamsters was within the range of the estimated quantities accidentally consumed by the Turkish people for several months at a time, resulting in severe toxic porphyria⁴.

In a study conducted in Louisiana, in 86 persons who resided in an HCB-contaminated area, the mean HCB concentration in plasma samples was 0.0036 p.p.m. (ref. 7). In yet another investigation, farm workers occupationally exposed to HCB-contaminated DACTHAL had blood HCB concentrations averaging 0.040 p.p.m. but there was no evidence of porphyria in this group⁸. It must, however, be stressed that with Burns' two cross-sectional epidemiological studies^{7,8}, it would have been very difficult to demonstrate the carcinogenicity of HCB.

The extrapolation of the present experimental results to man is not possible without considering all available data on the biological effects of HCB. In this sense, we believe there is urgent need to obtain epidemiological information to evaluate the relevance of the experimental studies.

This work was supported by a US Public Health Service

Table 2 Tumour incidence in hamsters given HCB

Group	Effective no.	TBA no.	%	No. of tumours per hamster		More than one tumour		Thyroid		Hepatoma		Haemangioendotheliomas Liver		Spleen		Other	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	39 F	5	12.8	5	0.13	0	0	0	0	0	0	0	0	1	2.5	4	10.2
	40 M	3	7.5	3	0.08	0	0	0	0	0	0	0	0	0	0	3	7.5
HCB50	30 F	16	53.3	21	0.70	4	13.3	2	6.6	14	46.6	0	0	0	0	5	16.6
	30 M	18	60.0	27	0.90	8	26.6	0	0	14	46.6	1	3.3	1	3.3	11	36.6
HCB100	30 F	18	60.0	32	1.06	11	36.6	1	3.3	17	56.6	2	6.6	3	10.0	9	30.0
	30 M	27	90.0	45	1.50	14	46.6	1	3.3	26	86.6	6	20.0	3	10.0	9	30.0
HCB200	60 F	52	86.6	73	1.21	15	25.0	3	5.0	51	85.0	7	11.6	4	6.6	8	13.3
	57 M	56	98.2	87	1.52	27	47.3	8	14.0	49	85.9	20	35.0	4	7.0	6	10.5

contract. We thank Dr B. Terracini for helpful comments and Ms M. Susman for editorial assistance.

J. R. P. CABRAL*
P. SHUBIK
T. MOLLNER
F. RAITANO

*Eppley Institute for Research in Cancer,
University of Nebraska Medical Center,
42nd Street and Dewey Avenue,
Omaha, Nebraska 68105*

Received 28 June; accepted 23 August 1977.

*Present address: MRC Toxicology Unit, Woodmansterne Road, Carshalton, Surrey, UK.

1. *Assessing Potential Ocean Pollutants*, 188 (National Academy of Sciences, Washington, DC, 1975).
2. Schmid, R. *New Engl. J. Med.* **263**, 397–398 (1960).
3. Ockner, R. K. & Schmid, R. *Nature* **189**, 499 (1961).
4. Cam, C. & Nigogosyan, G. *J. Am. med. Assoc.* **183**, 88–91 (1963).
5. Dogramaci, I. *Adv. Pediatr.* **13**, 11–63 (1964).
6. Peters, H. A. *et al.* *Am. J. med. Sci.* **251**, 104–112 (1966).
7. Burns, J. E. & Miller, F. M. *Arch. environ. Hlth* **30**, 44–48 (1975).
8. Burns, J. E., Miller, F. M., Gomes, E. D. & Albert, R. A. *Arch. environ. Hlth* **29**, 192–194 (1974).

Effect of aspirin administration on retinoic acid toxicity in mice

THIS study was designed to provide an indirect assessment of the possible role of prostaglandins in retinoid intoxication. If the symptoms of retinoid toxicity are mediated by prostaglandins, an inhibitor of prostaglandin synthesis should ameliorate the symptoms of sublethal intoxication and perhaps even increase the lifespan of mice treated with lethal doses of retinoids. The observations reported here indicate that aspirin can antagonise the toxic effects of retinoic acid.

Sporn *et al.*^{1,2} have presented information on the biological activity of a number of retinoids (vitamin A analogues). Retinoids seem to influence differentiation of epithelial cells in several tissues to the extent that in certain test systems, they prevent or reverse malignant transformation of epithelial cells exposed to known carcinogens¹. This activity holds promise for the 'chemoprevention'² of cancer in man. Since chemoprevention will involve use of a drug by individuals who have a greater than average chance of developing cancer but who may be healthy otherwise, the acceptability of retinoids may depend on their toxicity. Excessive doses of retinol or all-*trans*-retinoic acid (RA) produce a hypervitaminosis A syndrome in man and experimental animals^{3–6}. Hixson and Denine⁶ suggested that this syndrome might be more appropriately referred to as retinoid intoxication, since many of the retinoids exhibit similar side effects, including headache, vomiting, pruritus, skeletal pain, and bone resorption^{3–5,7}.

The mechanism of retinoid toxicity has been open to question^{1,2}, but the syndrome suggests a possible mediator. Headache^{8,9}, vomiting⁹, pruritus⁸, skeletal pain^{10,11} and bone resorption^{12–14} are common symptoms of retinoid intoxication and prostaglandin activity. Hypothetically, the symptoms of retinoid intoxication may be mediated by prostaglandin production. As a preliminary test of this hypothesis, we have treated mice with a combination of aspirin and toxic doses of RA. Vane⁸ demonstrated that aspirin inhibits prostaglandin production, and subsequent studies have confirmed his conclusions^{15,16}. Since bone thinning (resorption) and occurrence of fractures provide a dose-dependent indication of retinoid intoxication in mice⁶, we have used this endpoint and the incidence of mortality to demonstrate that aspirin protects mice from retinoid intoxication.

To produce retinoid intoxication in mice, RA (30 mg kg⁻¹) was administered intraperitoneally (i.p.) daily for 21 d

(Q1D×21). This dose approximated the LD₅₀ obtained previously with this schedule in our laboratories^{6,17}. RA was suspended in an aqueous solution of 8% Cremophor EL (Sigma) and 10% propylene glycol; these suspensions were protected from light. Aspirin (ASA) was suspended in a mixture of physiological saline and 0.3% hydroxypropyl cellulose. Sixty adult Swiss mice weighing approximately 24 g (range: 21–27 g) were distributed into three experimental groups of 20 mice each (10 male, 10 female). Drugs were administered according to individual body weight as follows: RA only, 30 mg kg⁻¹ i.p., Q1D×21; ASA only, 150 mg kg⁻¹ by mouth (p.o.), Q1D×21; RA 30 mg kg⁻¹ i.p. plus ASA 150 mg kg⁻¹ p.o., Q1D×21. Mice were weighed twice weekly and X-rayed on treatment days 1, 8, 15, and on the day after the last treatment. X-ray films were evaluated weekly to determine the number of fractured bones per mouse. During the treatment period, including the day after last treatment, deaths per total mice (group) were: 10/20 (RA), 1/20 (ASA), and 0/20 (RA+ASA). This experiment was repeated: 60 mice weighing approximately 26 g (range: 22–30 g) were distributed and treated as before, except that these mice were not X-rayed on treatment day 1. In this experiment, deaths per total mice (group) were: 7/20 (RA), 0/20 (ASA), and 3/20 (RA+ASA).

The frequency of deaths and the frequency of fractures among survivors on days 8, 15, and one day after last treatment were arranged as a contingency table for each experiment. Since no fractures occurred in ASA-treated mice, they were excluded. Chi-square analysis¹⁸ indicated that the data from the two experiments were sufficiently homogeneous to justify pooling them. The pooled data (Table 1) were then tested for statistical significance¹⁸. The difference in mortality between the RA group (17/40) and the RA+ASA group (3/40) was significant ($P < 0.001$) on the day after last treatment. On day 15, the number of mice with three or more fractures was significantly greater in the RA group than in the combination group ($P < 0.05$); no other significant differences were observed and there seemed to be no sex differences. Differences were apparent between the general condition of mice in the RA group and the RA+ASA group on the day after last treatment. Loss of body weight, for example, is characteristic of sublethal retinoid intoxication.

Data presented in Table 1 suggest that ASA treatment either delayed or antagonised the development of sublethal retinoid toxicity. Although no differences were detected in the number of fractures after three weeks of drug delivery, the reduced mortality in the RA+ASA-treated mice strengthens the suggestion of benefit from ASA treatment. Agents other than ASA are more potent inhibitors of prostaglandin synthesis¹⁵; and if prostaglandins do play a part in retinoid intoxication, the protection indicated by these results can probably be improved with optimal treatment.

Although more direct support of our hypothesis is required, a question worthy of early consideration is how prostaglandins might fit into a mechanistic scheme for retinoid intoxication. Mallia *et al.*¹⁹ have concluded that retinol toxicity occurs in rats when the binding capacity of plasma retinol-binding protein is exceeded. Plasma concentrations of free retinol and retinyl esters then become elevated. These results have also been demonstrated in humans²⁰. Similarly, saturation of intracellular or extracellular proteins that bind retinoids would result in higher concentrations of free retinoids. Free retinol^{19,20} and free retinoids^{21,22} destabilise lysosomal membranes. The subsequent release of lysosomal enzymes, particularly phospholipase A, induces prostaglandin synthesis²³. Destabilisation of lysosomal membranes and release of lysosomal enzymes have been invoked to explain the local elevation of prostaglandin concentrations following

Table 1 Effects of retinoic acid alone and in combination with aspirin on Swiss mice

Day	Group	0	No. of fractures per survivor			No. of deaths per total
			1	2	3+	
8	RA	22/40	13/40	5/40	0/40	0/40
	RA + ASA	16/40	20/40	3/40	1/40	0/40
15	RA	7/36	10/36	8/36	11/36*	4/40
	RA + ASA	9/38	15/38	10/38	4/38*	2/40
22 (Day after last treatment)	RA	5/23	4/23	3/23	11/23	17/40†
	RA + ASA	5/37	7/37	13/37	12/37	3/40†

* The number of animals with three or more fractures is significantly lower in the RA + ASA group ($P < 0.05$).

† The number of deaths is significantly lower in the RA + ASA group ($P < 0.001$).

application of RA to guinea pig skin²⁴. Our observations suggest that systemic administration of RA has a similar effect. Rational control of retinoid intoxication will have an important influence on the development of retinoids for human use.

This work was supported by a contract from the Lung Cancer Segment, Division of Cancer Cause and Prevention, National Cancer Institute, NIH. We thank the staff of the Preclinical Pharmacology Section, Southern Research Institute, for technical assistance.

S. D. HARRISON, JR
E. J. HIXON
J. A. BURDESHAW
E. P. DENINE

Southern Research Institute,
2000 Ninth Avenue South,
Birmingham, Alabama 35205

Received 1 June; accepted 1 August 1977.

- Sporn, M. B., Dunlop, N. M., Newton, D. L. & Henderson, W. R. *Nature* **263**, 110–113 (1976).
- Sporn, M. B., Dunlop, N. M., Newton, D. L. & Smith, J. M. *Fedn Proc.* **35**, 1332–1338 (1976).
- Bollag, W. *Chemotherapy* **21**, 236–247 (1975).
- Mandel, H. G. in *The Pharmacological Basis of Therapeutics*, 5th edn (eds Goodman, L. S. & Gilman, L.) 1570–1578 (Macmillan, New York, 1975).
- Leelaprute, D., Boonpucknavig, V., Bhamarapravati, N. & Weerapradist, W. *Archs Path.* **96**, 5–9 (1973).
- Hixson, E. J. & Denine, E. P. *Toxicol. appl. Pharmac.* (in the press).
- Scrimshaw, N. S. in *Textbook of Medicine*, 14th edn, II (eds Beeson, P. B. & McDermott, W.) 1368–1375 (Saunders, Philadelphia, 1975).
- Vane, J. R. *Nature new Biol.* **231**, 232–235 (1971).
- Smith, E. R. & Mason, M. M. *Prostaglandins* **7**, 247–268 (1974).
- Minton, J. P., Bronn, D. G. & Kibbey, W. E. *New Engl. J. Med.* **294**, 340 (1976).
- Sacks, P. V. *Lancet* **ii**, 1385 (1974).
- Raisz, L. G., Sandberg, A. L., Goodson, J. M., Simmons, H. A. & Mergenhagen, S. E. *Science* **185**, 789–791 (1974).
- Dowsett, M. *et al. Nature* **263**, 72–74 (1976).
- Galasko, C. S. B. & Bennett, A. *Nature* **263**, 508–510 (1976).
- Flower, R. J. *Pharmac. Rev.* **26**, 33–67 (1974).
- Levine, L., Pong, S. S., Robinson, D. & Kantrowitz, F. J. *invest. Dermatol.* **67**, 665–666 (1976).
- Hixson, E. J. *Proc. Am. Ass. Cancer Res.* **18**, 47 (1977).
- Zar, J. H. *Biostatistical Analysis*, 47–53 (Prentice-Hall, Englewood Cliffs, N. J., 1974).
- Mallia, K. A., Smith, J. E. & Goodman, D. S. *J. Lipid Res.* **16**, 180–188 (1975).
- Smith, F. R. & Goodman, D. S. *New Engl. J. Med.* **294**, 805–808 (1976).
- Wang, C., Straight, S. & Hill, D. L. *Biochem. Pharmac.* **25**, 471–475 (1976).
- Fell, H. B., Dingle, J. T. & Webb, M. *Biochem. J.* **83**, 63–69 (1962).
- Flower, R. J. & Blackwell, G. J. *Biochem. Pharmac.* **25**, 285–291 (1976).
- Ziboh, V. A., Price, B. & Fulton, J. J. *invest. Dermatol.* **65**, 370–374 (1975).

L-Dopa methyl ester as a new antitumour agent

L-DOPA (L-3,4-dihydroxyphenylalanine) is extensively used in man for the treatment of Parkinson's disease, and it can also inhibit selectively the growth of pigmented human melanoma cells *in vitro*¹. Demonstration of *in vivo* antitumour activity, however, has been lacking except in the special case of hormonally responsive mammary carcinoma² where the antitumour effect is thought to be mediated indirectly. Since *in vitro* cytotoxicity was not observed until concentrations of 600–1,200 $\mu\text{g ml}^{-1}$ were attained the use of the more soluble methyl ester derivative was proposed as a method for delivery of cytotoxic doses. *In vivo* antitumour activity of L-dopa methyl ester is reported here in the following tumour systems: L1210

lymphocytic leukaemia; P388 lymphocytic leukaemia and B-16 melanoma.

The results of *in vivo* antitumour experiments are summarised in Table 1. L-Dopa itself, because of insolubility, could not be administered to mice at doses greater than 100 mg kg^{-1} and did not show therapeutic activity at this dose. The methyl ester at the maximally tolerated dose of 600 mg kg^{-1} , however, exhibits activity in each of the test systems and is relatively

Table 1 *In vivo* antitumour activity of L-dopa methyl ester against L1210 and P388 murine leukaemias and B-16 melanoma

Drug	Dose (mg kg^{-1})	% Increase lifespan (ILS)*		
		L1210	P388	B-16
L-dopa methyl ester	500	+33	+46	+22
	600	+33	+27	+34(+50)†
	200	+22	+10	0
Ro4-4602	100	+10†	+0	0
Ro4-4602 and L-dopa methyl ester	200+800			+25
	200+1,000	+44	+45	+30
	200+1,250	+55	+36	
MK 486 and L-dopa methyl ester	100+1,000	+55	+70	

L-Dopa and Ro4-4602 were gifts from the Hoffman LaRoche Company, Nutley, New Jersey; MK 486 was from the Merck, Sharpe and Dohme Co., West Point, Pennsylvania; L-dopa methyl ester was from the Sigma Chemical Company, St Louis, Missouri. Antitumour activity of each compound was determined by measuring the median ILS of mice given intraperitoneal (i.p.) inoculations of either 1×10^6 P388, 1×10^6 L1210 lymphocytic leukaemia cells or 0.5 ml of a 1:10 homogenate of B-16 melanoma tumour. The assay procedures followed the standard National Cancer Institute protocols³ for these tumour systems, with treatment beginning on the first day after tumour inoculation and continuing daily for 7 d with L1210 and P388 and 12 d for B-16 tumour. Agents were given i.p. in saline. Ro4-4602 was given 1 h before treatment with ester. C57Bl \times DBA/2 F_1 male mice (Jackson Laboratory, Bar Harbor, Maine) were used throughout. Percentage ILS was calculated according to the equation: % ILS = $100 \times (t/c - 1)$ where t is the median survival time (in d) of the treatment group and c is the median survival time of the control group.

*Values are significant at $P < 0.001$ unless otherwise noted.

†NS, Not significant.

‡Administered daily for 24 d.

active against the B-16 melanoma, which is considered to be highly refractory to conventional agents. The combination of L-dopa methyl ester with the inhibitors of dopa decarboxylase benserazide (Ro4-4602) and carbidopa (MK 486) (refs 3, 4) results in a marked increase in both the antitumour effect and the maximally tolerated dose.

Table 2 summarises the effects of L-dopa methyl ester on thymidine, uridine and leucine incorporation in the S-91 Cloudman melanoma and L929 mouse fibroblast *in vitro*. There is a selective and profound inhibition of DNA synthesis as measured

during drug exposure, with relatively greater inhibition in the melanoma cells. Uridine and leucine incorporation are largely unaffected. The greater inhibition observed in the pigmented line as well as the activity observed against B-16 melanoma may be related in part to the role of L-dopa as a biochemical intermediate in the formation of the pigment, melanin¹.

Although the precise cellular target of L-dopa methyl ester is not known, the pharmacological factors important to *in vivo* activity may be inferred. The acute toxic effects of L-dopa are mediated by its conversion to dopamine by the enzyme L-aromatic amino acid decarboxylase. At the high doses of L-dopa administered, a hypercatecholamine-type state was observed. The animals became agitated and tremulous and usually died with 1–2 h after administration. These acute toxic effects of L-dopa are probably mediated by its conversion to dopamine by the enzyme, L-aromatic amino acid decarboxylase (dopa decarboxylase). The striking increase in both the therapeutic

through inhibition of DNA synthesis. Extensive experience with both L-dopa and dopa decarboxylase inhibitors in human disease should permit rapid evaluation of their potential clinical significance.

I thank Dr E. Frei III and Dr T. B. Fitzpatrick for advice and encouragement.

MICHAEL M. WICK

Sidney Farber Cancer Institute
and Harvard Medical School,
44 Binney Street,
Boston, Massachusetts 02115

Received 13 May; accepted 1 August 1977.

- 1 Wick, M. M., Byers, L. & Frei, E., III *Science* **197**, 468 (1977).
- 2 Shohat, B., Lott, E. & Bornstein, B. *Experientia* **31**, 110 (1975).
- 3 Burkhard, W. P., Gey, K. F. & Pletscher, A. *Arch. Biochem. biophys.* **107**, 187 (1964).
- 4 Lotti, V. J. & Porter, C. C. *J. Pharmac. exp. Ther.* **172**, 406 (1970).
- 5 Bartholini, G. & Pletscher, A. *J. Pharmac. exp. Ther.* **161**, 14 (1968).
- 6 Geran, R. A., Greenberg, N. H., MacDonald, M. M., Schumacker, A. & Abbot, B. J. *Cancer Chemother. Rep.* **3**, 7 (1972).
- 7 Agrup, G. *et al. Acta derm-ven.*, *Stockh.* **57**, 113 (1977).
- 8 Livingston, D. M., Ferguson, C., Gollogly, R. & Lazarus, H. *Cell* **7**, 41 (1976).

Table 2 Inhibition of macromolecule biosynthesis by L-dopa and L-dopa methyl ester in S-91 Cloudman melanoma and mouse fibroblast L929 *in vitro*

Cell line	L-Dopa Concentration ($\mu\text{g ml}^{-1}$)	DNA	RNA	Protein
S-91	600	49 \pm 4	15 \pm 6	10 \pm 5
	1,200	70 \pm 5	30 \pm 5	16 \pm 8
L929	600	41 \pm 4	21 \pm 5	34 \pm 8
	1,200	49 \pm 5	22 \pm 6	32 \pm 5
L-dopa methyl ester				
S-91	600	96 \pm 1	44 \pm 12	27 \pm 7
	1,200	98 \pm 1	65 \pm 5	47 \pm 10
L929	600	74 \pm 2	44 \pm 5	52 \pm 8
	1,200	84 \pm 3	67 \pm 11	76 \pm 8

Values represent % inhibition and are mean \pm s.e.m. for triplicate determinations. The origin and maintenance of cell lines has been described in detail previously. Cells were grown in McCoy's 5A medium supplemented with 15% foetal calf serum, 100 units ml^{-1} of penicillin and 100 $\mu\text{g ml}^{-1}$ of streptomycin¹. Cells were plated in Linbro multi-well tissue culture trays and after 48 h they were in the log phase of growth. Medium was then aspirated, the monolayer washed and 1 ml of serum-free medium containing 2 $\mu\text{Ci ml}^{-1}$ of either ³H-thymidine (specific activity 2 Ci mmol^{-1}), ³H-5-uridine (specific activity 25 Ci mmol^{-1}), or ³H-leucine (specific activity 41 Ci mmol^{-1}) (New England Nuclear, Boston, Massachusetts) was added. After 60 min at 37 °C medium was removed, cells were washed once with saline and 1 ml of 10% trichloroacetic acid was added. The precipitate was washed three times with 0.9% sodium chloride and 0.5 ml of 1.0 M KOH added. Precipitate containing RNA, DNA and protein was then digested at 37 °C for 4 h and an aliquot added to scintillation vials containing Aquasol (New England Nuclear).

effect and maximally tolerated dose observed by pretreatment with inhibitors of this enzyme, may be related to the resulting elevated plasma levels of L-dopa and decreased levels of dopamine⁵. Alternatively, since Ro4-4602, an analogue of L-dopa, exhibited significant antitumour activity in L1210 and P388 systems, ($P < 0.001$), the increase in activity of this combination may be partially the result of an additive antitumour effect.

An intriguing possibility concerning the mechanism of action of L-dopa methyl ester and Ro4-4602 may be proposed. It is well known that L-dopa can combine with cysteine to yield 5-cysteinyl-dopa, a metabolite found in large amounts in the urine of patients with metastatic melanoma⁷. Livingston *et al.* have demonstrated⁸ that a large number of lymphoid and myeloid tumour lines, including L1210 and P388, are unable to grow in tissue culture in the absence of pre-formed L-cysteine⁸. It is possible, therefore, that L-dopa methyl ester and its analogue Ro4-4602 function as sulphhydryl scavengers, depriving tumours of essential growth factors.

L-Dopa methyl ester is a novel and potent antitumour agent in experimental tumour systems. It seems to act primarily

Dopamine agonists induce recovery from surgically-induced septal rage

ALTHOUGH recovery of function following brain damage has long been known to occur, the mechanisms involved are not completely understood¹. Of relevance to this problem are reports of pharmacological facilitation of recovery from brain damage in humans² and from experimentally-induced nervous system lesions in animals^{3–9}. We have previously reported that L-dopa injections produce a dramatic and apparently permanent abolition of the hyper-reactivity or rage syndrome that results from surgical damage to the septal nuclei of the rat forebrain¹⁰. Independent confirmation of this finding has also appeared¹¹. We report here that other dopamine agonists share this ability to attenuate or abolish the septal rage syndrome (SRS).

Individually housed male Long-Evans hooded rats (280–320 g) were anaesthetised with sodium thiopental and subjected to bilateral radio frequency lesions of the septal nuclei, using standard surgical and stereotaxic techniques. These lesions destroyed the medial and lateral septal nuclei and damaged the adjacent caudate nuclei, nucleus accumbens septi, and stria terminalis.

After recovery from the anaesthesia, approximately 60% of the lesioned animals exhibited a clear and persistent SRS, consisting of explosive reactivity to visual, tactual and auditory stimulation, coupled with pronounced biting, attack, fleeing, muscle tension and muricide¹². These hyper-irritable animals were then returned to their home cages; and, on the next day, rated for SRS hyperirritability¹³ in special observation chambers. Each animal was then given an intraperitoneal injection of one of the following: L-dopa (100, 60, 40, 30 or 10 mg per kg body weight), apomorphine (20, 15, 10 or 5 mg per kg), pibedil (300 or 200 mg per kg), amphetamine (4 or 2 mg per kg), methohexital (40 mg per kg), or saline. Each animal was returned to its home cage, and then placed in the observation chamber and rated for hyperirritability at 0.5, 1, 1.5, 2, 3, 4, 24, 48, 72, 96, and 120 h post-injection. All ratings of drug-injected animals were done blind. The methohexital treated animals were additionally rated at 0.25 and 0.75 h post-injection, and the apomorphine animals were also rated at 5 and 6 h. A few animals from some groups were also rated at times beyond 120 h (up to 1 month after injection). In this particular preparation, competing stereotypic behaviours were not observed for any of the doses of the catecholaminergic agonists used. Statistical analyses of changes in hyperirritability were performed as appropriate using the Friedman non-parametric analysis of variance (χ^2), the Wilcoxon matched-pairs signed-ranks test (T), and the Mann-Whitney rank test for independent samples (U).

Saline-injected animals (Fig. 1f) exhibited a constant level of irritability for the first 24 h, followed by gradual diminution over

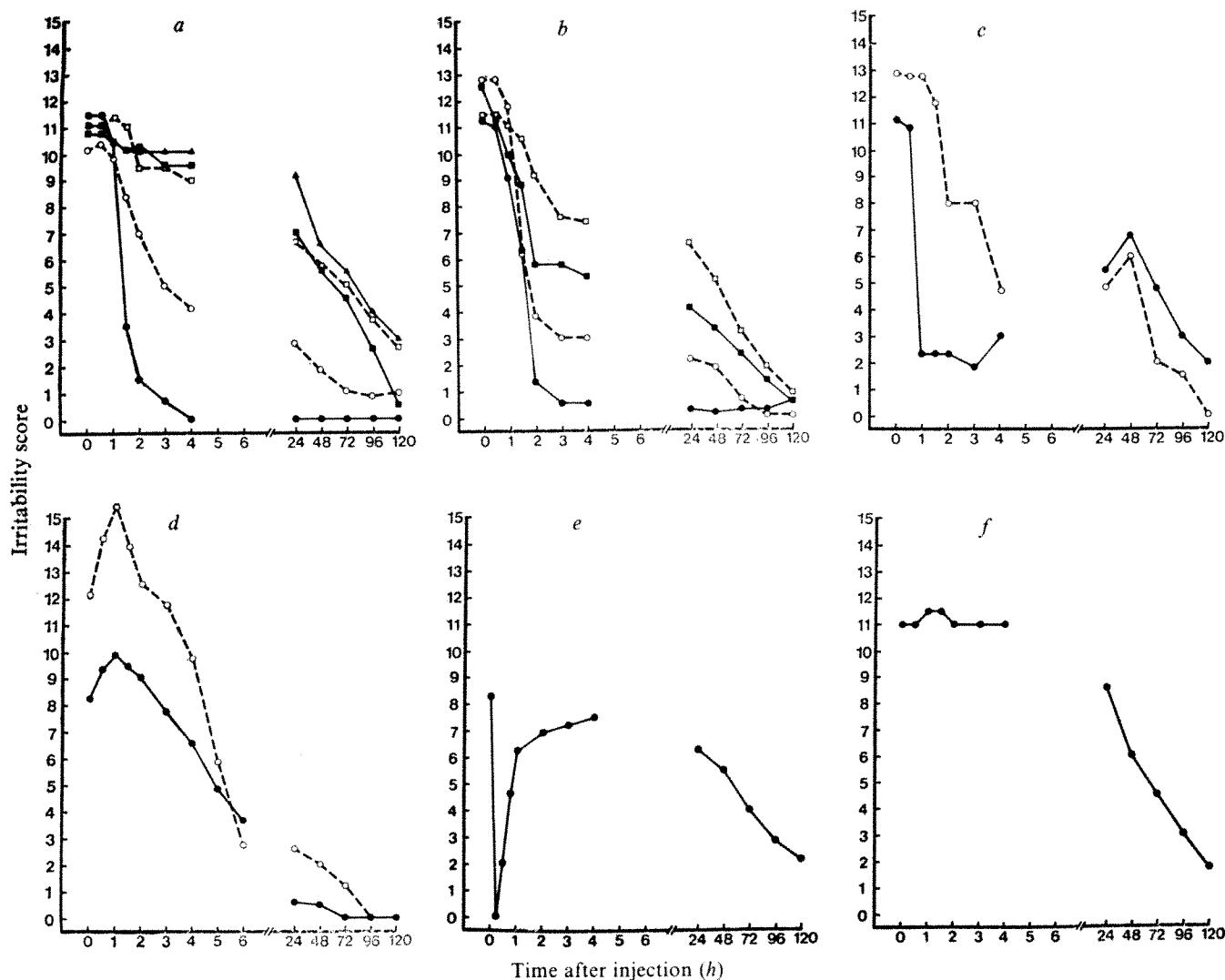


Fig. 1 Post-injection changes in SRS brain lesion symptoms ('irritability') following single administrations of *a*, L-dopa: ●, 100 mg per kg body weight, $n = 8$; ○, 60 mg per kg, $n = 5$; ■, 40 mg per kg, $n = 3$; □, 30 mg per kg, $n = 4$; ▲, 10 mg per kg, $n = 4$. *b*, Apomorphine: ●, 20 mg per kg, $n = 12$; ○, 15 mg per kg, $n = 5$; ■, 10 mg per kg, $n = 6$; □, 5 mg per kg, $n = 5$. *c*, Piribedil: ●, 300 mg per kg, $n = 7$; ○, 200 mg per kg, $n = 3$. *d*, Amphetamine: ●, 4 mg per kg, $n = 10$; ○, 2 mg per kg, $n = 6$. *e*, Methohexital, 40 mg per kg, $n = 14$. *f*, Saline, $n = 10$.

the ensuing 5 d ($\chi^2 = 47.8$, $P < 0.001$), similar to non-injected animals. In contrast, animals given L-dopa (100 mg per kg) showed a dramatic and prompt return (Fig. 1*a*) to pre-operative levels of irritability (for the 0–4 h post-injection period, $\chi^2 = 43.8$, $P < 0.001$). Latency for a measurable effect was 90 min ($T = 0$, $P < 0.01$), and by 120 min after injection, the animals were indistinguishable from non-lesioned controls (the difference between the 100 mg per kg L-dopa animals and the saline controls also reaching significance by 120 min after injection; $U = 1.5$, $P < 0.001$). The L-dopa animals showed normal righting, placing and balance reflexes, and gave no appearance of being sedated; they continued to eat and drink, and remained normally responsive to environmental stimuli. The L-dopa abolition of lesion-induced SRS symptoms was also noteworthy for its permanence; none of the 100 mg per kg treated animals showed return of hyperaffective behaviour when examined up to 1 month post-injection. At 60 mg per kg L-dopa, a less marked, but still prompt and clear, attenuation of lesion-induced symptoms occurred ($\chi^2 = 19.8$, $P < 0.01$).

Animals given apomorphine also showed a drug-induced SRS amelioration (Fig. 1*b*). Apomorphine at 20 mg per kg produced complete and permanent abolition of lesion-induced symptoms within 120 min ($\chi^2 = 35.4$, $P < 0.001$); lower doses produced less effect on the time course of recovery (Fig. 1*b*). As with L-dopa, the animals were not merely sedated, and the drug-induced behavioural change was permanent.

Piribedil and DL-amphetamine produced differing effects on the time course of SRS recovery (Fig. 1*c, d*). Piribedil produced the same initial SRS amelioration during the first 4 h as L-dopa and apomorphine (χ^2 for 200 mg per kg piribedil = 15.1, $P < 0.01$; χ^2 for 300 mg per kg piribedil = 26.7, $P < 0.001$). But, this was followed by subsequent SRS increase at 24 and 48 h (most noticeable in the 300 mg per kg animals, which showed the most SRS attenuation during the first 4 h ($\chi^2 = 9.3$, $P < 0.05$)). The irritability of the piribedil-injected rats then attenuated over the next 4 d (χ^2 for 200 mg per kg = 8.5, $P < 0.01$; χ^2 for 300 mg per kg = 13.0, $P < 0.01$) at the same rate observed in untreated controls. Amphetamine produced an initial SRS increase during the first 90 min (only the 2 mg per kg animals being significant; $\chi^2 = 9.25$, $P < 0.01$), followed by a rapid and permanent SRS abolition (χ^2 for 2 mg per kg = 44.4, $P < 0.001$; χ^2 for 4 mg per kg = 43.6, $P < 0.001$).

In marked contrast to the L-dopa, apomorphine, piribedil or amphetamine effects, the short-acting barbiturate methohexital produced immediate sedation ($T = 0$, $P < 0.001$), followed by rapid return to full, lesion-induced SRS irritability ($\chi^2 = 29.1$, $P < 0.001$), which then attenuated over the next 5 d ($\chi^2 = 27.8$, $P < 0.001$) at a rate similar to control animals (Fig. 1*e*). Essentially, methohexital produced a brief and profound sedation, but no effect on the time course of recovery from the lesion. Thus, the action of L-dopa and apomorphine cannot be ascribed to the effect of handling during transient tranquillisation.

Apomorphine seems relatively selective in its dopamine receptor stimulating properties^{14,15}, so activation of dopamine receptors seems sufficient to induce neuronal processes leading to recovery from septal damage. The initial SRS exacerbation under amphetamine may reflect a noradrenergic effect (preliminary data from SRS animals given imipramine, which also seems to intensify the SRS symptoms, seem confirmatory); the biphasic response to piribedil may reflect an action on presynaptic release of dopamine at brain sites distinct from the sites wherein apomorphine and L-dopa exert their effects¹⁶.

Our data contrast with reports of pharmacological diminution of SRS symptoms by neuroleptic agents, barbiturates, physostigmine, chlorthalidoxepoxide and non-narcotic analgesics¹⁷⁻²⁰. In these cases, the effects were transient and most closely resemble the present methohexital effect (albeit with less brief and profound behavioural inhibition). There are also reports of sedative and/or excitatory effects of L-dopa and related compounds on normal or brain-damaged animals, but these effects, too, are transient and reversible. The only previous report of permanent SRS abolition is that of Dominguez and Longo⁹, using *p*-chlorophenylalanine (pCPA). These data have been interpreted to implicate a serotonergic role in SRS recovery⁹, but we suggest that they may support a catecholaminergic (possibly dopaminergic) role in SRS recovery, since the time course of the action of pCPA action on the SRS is more consistent with an action on catecholaminergic systems²¹⁻²³.

The time course that we find for dopamine agonist-induced SRS recovery is only a few hours. Most neurological or behavioural processes invoked as explanations for recovery from brain damage have time courses measured usually in weeks, for example neural reorganisation, vicarious function, denervation supersensitivity, axonal sprouting and regrowth, and behavioural substitution¹. Changes in blood-brain barrier permeability²⁴ and diaschitic alterations in brain function^{2,25} may have shorter time courses, however. We are unable to find any alteration in blood-brain barrier permeability to noradrenaline in septally lesioned animals displaying SRS symptoms (uptake of intravenous ³H-noradrenaline into brain tissue was studied in six SRS, six septally-lesioned non-irritable, and six sham-operated rats 24 h after surgery; no differences between groups were found). The possibility of a diaschitic process remains—dopaminergically induced SRS recovery may involve the return of function in transiently dysfunctional regions either adjacent to or neuronally connected to the lesioned area. Possible regions of transient dysfunction might include the fibres and nuclei of the dopamine-rich mesolimbic forebrain system^{26,27}, for example, the nucleus accumbens adjacent to the septal nuclei, and the olfactory tubercle and anterior amygdala, reciprocally connected to the septum²⁸. The mechanism by which dopaminergic stimulation might hasten recovery of function in these areas remains to be determined.

This research was supported by a Sigma Xi Grant-in-Aid of Research (R.F.M.), by funds from the Psychobiology Doctoral Program of the City University of New York (R.F.M., E.L.G.), by Research Foundation for Mental Hygiene grant 303-E275H (M.P.), by USPHS grant MH-13579 (M.G.), and by USPHS grant NS-09649 (E.L.G.). We thank Dr Morton Levitt, New York State Psychiatric Institute, for carrying out the biochemical studies on ³H-noradrenaline uptake.

ROCCO F. MAROTTA
NANCY LOGAN

Department of Psychology,
Hunter College of the City University of New York,
New York, New York 10021

MICHAEL POTEAL
MURRAY GLUSMAN

Department of Behavioral Physiology,
New York State Psychiatric Institute,
New York, New York 10032

ELIOT L. GARDNER*

Departments of Neurology and Pharmacology,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received 19 May; accepted 1 August 1977.

*To whom correspondence should be addressed.

- ¹ Stein, D. G., Rosen, J. J. & Butters, N. (eds) *Plasticity and Recovery of Function in the Central Nervous System* (Academic, New York, 1974).
- ² Luria, A., Naydin, V., Tsvetkova, L. & Vinarskaya, E. in *Handbook of Clinical Neurology* (eds Vinken, R. J. & Bruyn, G. W.) 13 (North Holland, Amsterdam, 1968).
- ³ Wolf, A. J. *neurol. Dis.* **92**, 614-622 (1940).
- ⁴ Watson, C. & Kennard, M. J. *Neurophysiol.* **8**, 221-231 (1945).
- ⁵ Matting, H. & Acheson, G. J. *Neurophysiol.* **9**, 379-386 (1946).
- ⁶ Braun, J. J., Meyer, P. M. & Meyer, D. R. J. *comp. Physiol. Psychol.* **61**, 79-82 (1966).
- ⁷ Cole, D., Sullins, W. & Isaac, W. *Psychopharmacologia* **11**, 311-316 (1967).
- ⁸ Berger, B., Wise, C. & Stein, L. *Science* **172**, 281-284 (1971).
- ⁹ Dominguez, M. & Longo, V. *Physiol. Behav.* **5**, 607-610 (1970).
- ¹⁰ Marotta, R. F., Potegal, M., Gardner, E. L. & Glusman, M. M. *Am. Psychol.* **28**, 100-101 (1973).
- ¹¹ Gage, F. H. & Olton, D. S. *Behav. Biol.* **17**, 213-218 (1976).
- ¹² Brady, J. B. & Nauta, W. J. H. J. *comp. Physiol. Psychol.* **46**, 339-346 (1953).
- ¹³ King, F. J. *neurol. Dis.* **126**, 57-63 (1958).
- ¹⁴ Ungersiedt, U. *Acta physiol. scand. Suppl.* **367**, 69-93 (1971).
- ¹⁵ Costall, B. & Naylor, R. J. *Eur. J. Pharmacol.* **24**, 8-24 (1973).
- ¹⁶ Cools, A. R. & Van Rossum, J. M. *Psychopharmacologia* **45**, 243-254 (1976).
- ¹⁷ Raitt, J., Nelson, J. & Lye, A. Br. J. *Pharmacol.* **17**, 473-478 (1961).
- ¹⁸ Cytawa, J. & Kutulas, G. *Psychopharmacologia* **27**, 389-392 (1972).
- ¹⁹ Stark, P. & Henderson, J. *Neuropharmacologia* **11**, 839-841 (1972).
- ²⁰ Loizzo, A. & Massotti, M. *Pharmacol. Biochem. Behav.* **1**, 367-370 (1973).
- ²¹ Welch, B. L. & Welch, A. S. J. *Pharm. Pharmacol.* **20**, 244-246 (1968).
- ²² Brody, J. *Psychopharmacologia* **17**, 14-33 (1970).
- ²³ Gal, E. M. in *Serotonin and Behavior* (eds Barchas, J. D. & Usdin, E.) (Academic, New York, 1973).
- ²⁴ Glusman, M. *Rev. Publ. Ass. Res. neurol. Dis.* **52**, 52-92 (1974).
- ²⁵ Rosner, B. S. in *Plasticity and Recovery of Function in the Central Nervous System* (eds Stein, D. G., Rosen, J. J. & Butters, N.) (Academic, New York, 1974).
- ²⁶ Brownstein, M., Saavedra, J. M. & Palkovits, M. *Brain Res.* **79**, 431-436 (1974).
- ²⁷ Lindvall, D. & Bjorklund, A. *Acta physiol. scand. Suppl.* **412**, 1-48 (1974).
- ²⁸ Powell, P. S., Cowan, W. M. & Raisman, G. J. *Anat.* **99**, 791-813 (1965).

Viable chimaeras produced from normal and parthenogenetic mouse embryos

PARTHENOGENESIS occurs spontaneously in about 10% of ovulated eggs of inbred strain LT/Sv mice^{1,2} and is experimentally inducible in other strains by various physical and chemical agents (see ref. 3 for review). The development of most parthenotes seems normal up to the expanded blastocyst stage⁴. They implant in the uterus, but, for reasons unknown, are resorbed within a few days. Although they rarely survive to 8 d of gestation when they develop somites, heart muscle, amnion, and neuroepithelium, Kaufman *et al.*⁵ obtained two embryos with 25 somites by transferring parthenogenetic blastocysts to the uteri of ovariectomised females treated with exogenous hormones. Even though parthenogenetic embryos do not survive to birth, their cells contain genetic information that permits prolonged survival. If two-cell parthenotes are cultured to the blastocyst stage and then grafted to extrauterine sites such as the testis or kidney, they may survive as teratomas composed of several types of tissues and undifferentiated embryonal cells (refs 1, 6 and L.C.S. and D.S.V., unpublished). Furthermore, strain LT/Sv spontaneous ovarian teratomas contain numerous differentiated tissues composed of parthenogenetically derived cells. The questions remains—why do parthenotes not survive at the organismic level *in utero*? Eicher and Hoppe⁷ used experimental chimaeras composed of normal and abnormal embryos to transmit a recessive X-linked lethal mutation, and we considered the possibility that parthenogenetic embryonic cells might also be rescued if combined with normal embryonic cells. Here we present evidence of production of at least two viable chimaeras between normal and parthenogenetic embryos.

Parthenogenetic eight-cell embryos were obtained from F₁ hybrid females produced by mating females of the LT/Sv strain to males of the incipient recombinant inbred strain LTXBJ (L.C.S. and D.S.V., unpublished). (The progenitors of LTXBJ were strains LT/Sv and C57BL/6J. Strain LTXBJ had been inbred for 11 generations of brother × sister matings when the experiments were initiated.) Nearly all of the females of the LTXBJ recombinant strain have bilateral ovarian teratomas at 3 months of age. These teratomas are derived from parthenogenetically activated ovarian eggs. About 30% of their ovulated eggs undergo spontaneous parthenogenetic development. (LT/Sv × LTXBJ)F₁ hybrid females have the same high incidence of spontaneous parthenogenesis as LTXBJ females.

Inbred strain LT/Sv is homozygous for the alleles *a*, *B⁺*, *C*, and *Gpi-1^a* at the agouti, brown, albino, and glucose-phosphate-isomerase-1 loci, respectively. LTXBJ is homozygous for the

alleles *a*, *B*, *C*, and *Gpi-1^b*. Although parthenogenetic embryos from the LT/Sv strain are diploid (P. C. Hoppe, personal communication), it is not known whether they result from initiation of mitosis in primary oocytes or secondary oocytes (lack of the second meiotic division), or diploidisation of haploid ootids. Regardless of their origin the parthenogenetic embryos from the (LT/Sv × LTXBJ)_F₁ females would appear *a/a C/C*. Depending on the nature of their origin, however, their genotypes for the brown and glucose-phosphate-isomerase-1 loci could be homozygous (for either allele) or heterozygous. Interpreting the origin of parthenogenetic cells in chimaeras was further complicated because we combined two parthenogenetic embryos per single normal embryo to form each chimaera. The two parthenotes could have different genotypes.

The normal eight-cell embryos were derived from eggs of albino 129/Sv (homozygous *A^w*, *B*, *c*, *Gpi-1^a*) females fertilised by sperm from A/HeJ (homozygous *a*, *b*, *c*, *Gpi-1^a*) males. Thus, these *F*₁ embryos were genotypically *A^w/a*, *B/b*, *c/c*, *Gpi-1^a/Gpi-1^a*.

Experimental chimaeras were produced by aggregating eight-cell embryos *in vitro*⁸. After removal of the zona pellucida with Pronase, one normal and two parthenogenetic morulae were combined in Whitten's medium⁹ and cultured overnight. The aggregated 'triplets' were then introduced into the uteri of (SJL × C57BL/10)_F₁ pseudopregnant females. To date, 55 'triplet' embryos have been transferred into six pseudopregnant females and two litters have been delivered.

On 30 January 1977 a litter of five mice was delivered by a female that had received eight 'triple' embryos. Of the four live young, one was a male about half the size of his three female littermates. This male was white with small patches of pigmented hair on the back and head that could only have been derived from the parthenogenetic embryos. Some of the pigmented hair was agouti and some non-agouti indicating that the mouse was chimaeric with respect to hair follicle cells. Both irises had pigmented and non-pigmented areas (Fig. 1). No nipples were evident. At 26 d old the chimaeric male weighed 13 g, and his littermates weighed 16 g each.

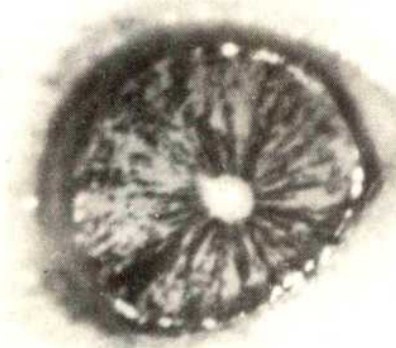


Fig. 1 Eye of chimaera mouse. The non-pigmented areas were derived from a normally fertilised egg, the pigmented areas from one or two parthenogenetically activated eggs.

At 36 d old, blood samples were collected from the male and his four littermates and analysed for GPI (Fig. 2). All of the non-chimaeric females showed only the GPI-1A form of glucose phosphate isomerase. The red blood cells of the chimaeric male apparently included cells derived from the parthenogenetic embryos as shown by a GPI-1B band (Fig. 2) that could only

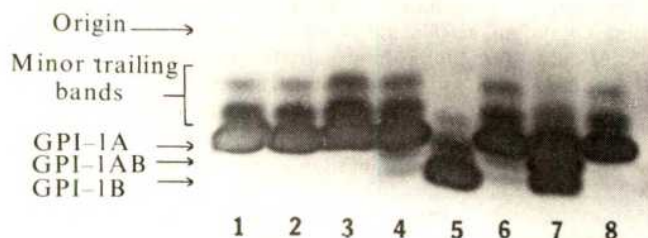


Fig. 2 Electrophoresis of red blood cell lysates on cellulose acetate gels stained for glucose phosphate isomerase using method of Eichler and Washburn¹⁰. In slots 1–3 and 8 are lysates from the four normal female sibs (GPI-1A) of the chimaeric male. Slot 5 includes lysate from a C57BL/6J male (GPI-1B) and slot 7 from a (C57BL/6J × DBA/2J)_F₁ male (GPI-1AB). Slot 4 (repeated in slot 6) contains lysate from the chimaeric male. Of interest are the two bands present in slots 4 and 6 that have migrated at a faster rate than the major band in the position of GPI-1A. These two minor bands line up such that one is in the same position as the GPI-1AB (hybrid) band and the other in the position of the GPI-1B band. It seemed that the slower of the two bands (position of GPI-1AB hybrid band) stained more intensely than the faster band (position of GPI-1B), as is the case in lysates from a *Gpi-1^a/Gpi-1^b* individual, suggesting that at least one parthenogenetically-derived embryo in the chimaeric male mouse was of the *Gpi-1^a/Gpi-1^b* genotype.

result if cells of parthenogenetic origin were present. In addition, a GPI-1AB band was evident. Its intensity seemed greater than the GPI-1B, as is the normal case of *Gpi-1^a/Gpi-1^b* cells, suggesting that at least one of the parthenogenetic embryos was of the *Gpi-1^a/Gpi-1^b* genotype. The presence of a GPI-1AB band would imply that at least one of the parthenogenetic embryos resulted from mitosis of a primary oocyte or lack of a second meiotic division in a secondary oocyte (with crossing over between the centromere and the *Gpi-1* locus), but not diploidisation of haploid ootids. It is also possible that a polar body nucleus fused with its egg nucleus.

At 38 d of age the male gonads were examined through an incision in the abdominal skin and musculature. Both seemed to be normal testes. The right testis was removed and prepared for histological examination. There was no histological evidence that cells of parthenogenetic origin were present in the testis. To date the chimaeric male has sired 27 albino offspring, none of which shows evidence of parthenogenetic cells.

On 27 March 1977, a second litter of three mice was delivered. This litter was obtained by transferring 9 'triple' embryos each composed of two LT/Sv parthenotes with one albino 129 embryo. The female of this litter was much smaller than its two male littermates. Both her irises were pigmented showing the presence of parthenogenetic cells.

The evidence presented here shows that cells of parthenogenetic origin can survive and participate in normal organ formation. Since both parents of the normal embryo were albino, all of the pigment cells in the coat and irises of both chimaeras must have been derived from parthenogenetic embryos. Since the agouti pattern is determined by the genotype of the hair follicle cells, the presence of agouti hairs in the male chimaera indicates that some of these cells originated from the parthenogenetic member. Finally, the presence in the male chimaera of LT/Sv strain-specific GPI shows that red blood cells were derived from parthenogenetic embryos of the chimaera.

Although our preliminary results indicate that parthenogenetic cells are capable of differentiating normally in combination with normal cells, it is still not clear why parthenotes cannot survive to term *in utero* in strain LT/Sv mice.

We thank W. K. Whitten and S. Carter for useful discussions

and for demonstrating the method of making chimaeras. We thank S. Reynolds and D. Dorr for technical assistance. This research was supported by grant CA 02662 from the USNIH, and GM 20919 from the National Institute of General Medical Sciences.

Note added in proof: It has recently been found that ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division¹¹.

LEROY C. STEVENS
DON S. VARNUM
EVA M. EICHER

The Jackson Laboratory,
Bar Harbor, Maine 04609

Received 18 May; accepted 8 August 1977.

- ¹ Stevens, L. C. & Varnum, D. S. *Dev. Biol.* **21**, 364–382 (1974).
- ² Stevens, L. C. *33rd Symp. Soc. dev. Biol.* (ed. Markert, C. L.) 93–106 (1975).
- ³ Tarkowski, A. K. in *The Developmental Biology of Reproduction* (eds Markert, C. L. & Papaconstantinou) 107–129 (Academic, New York, 1975).
- ⁴ Van Blerkom, J. & Runner, M. N. *J. exp. Zool.* **196**, 113–123 (1976).
- ⁵ Kaufman, M. T., Barton, S. C., & Surani, M. A. *Nature* **265**, 53–55 (1977).
- ⁶ Ilse, S. A., McBurney, M. W., Bramwell, S. R., Deussen, Z. A. & Graham, C. F. *J. Embryol. exp. Morph.* **34**, 387–406 (1975).
- ⁷ Eicher, E. M. & Hoppe, P. C. *J. exp. Zool.* **183**, 181–184 (1973).
- ⁸ Mintz, B. in *Methods in Mammalian Embryology* (ed. Daniel, J. C.) 186–214 (W. H. Freeman, San Francisco, 1971).
- ⁹ Whitten, W. K. *Adv. Biosci.* **6**, 129–139 (1971).
- ¹⁰ Eicher, E. M. & Washburn, L. W. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ¹¹ Eppig, J. J., Kozak, L. P., Eicher, E. M. & Stevens, L. C. *Nature* **269**, 517–518 (1977).

Ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division

SPONTANEOUS ovarian teratomas are found in about 50% of strain LT/Sv mice by the time they are 90 d old¹. These teratomas result from parthenogenetic cleavage of ovarian oocytes. Some parthenotes reach a developmental stage equivalent to 7 d embryo before they become disorganised and further develop into a teratoma. Since some cleaved ovarian oocytes are accompanied by polar bodies, but others seem to lack them, it was uncertain whether the teratomas derive from oocytes that complete the first meiotic division or from oocytes that cleave mitotically without previous meiotic division.

A strain of mice having an even higher frequency of ovarian teratomas than LT/Sv was produced by making a recombinant inbred line from strains LT/Sv and C57BL/6J (L.C.S. and D.S. Varnum, unpublished). Nearly all of the females of this recombinant line, called LTXBJ, have bilateral ovarian teratomas by 90 d of age. Fortuitously, among the C57BL/6J genes retained in the recombinant strain is the *Gpi-1^b* allele at the glucosephosphate isomerase (*Gpi-1*) locus on chromosome 7. Since strain LT/Sv is homozygous for the *Gpi-1^a* allele at this locus, (LT/Sv × LTXBJ)_{F1} hybrids are heterozygous at *Gpi-1* and express the A, hybrid AB and B allozymes in a ratio of 1:2:1 (Fig. 1a).

Electrophoresis of 23 teratomas from _{F1} female mice revealed a homozygous A or B allozyme banding pattern in 21 cases (Fig. 1b). The other two teratomas showed the 1:2:1 heterozygous banding pattern, indicating that they were heterozygous *Gpi-1^a/Gpi-1^b*.

We concluded from these results that the teratomas found in the (LT/Sv × LTXBJ)_{F1} female mice originated from parthenogenetically cleaved oocytes which had completed the first meiotic division (Fig. 2). In 21 of 23 cases, this division segregated the *Gpi-1^a* and *Gpi-1^b* alleles so that the parthenogenetic embryo and subsequently the teratomas were homozygous for either of those alleles at the *Gpi-1* locus. In the other two cases, where the heterozygous banding pattern was found, the most probable conclusion is that crossing-over has occurred between the centromere and the *Gpi-1* locus, so that the *Gpi-1^a* and *Gpi-1^b* alleles

were located on two chromatids attached to the same centromere.

Other studies on the origin of ovarian teratomas in women who were heterozygous for alleles producing allozymes of glucose-6-phosphate dehydrogenase and phosphoglucomutase, have shown that these teratomas were homozygous for the alleles in most cases, and thus that they arose from oocytes that had completed the first division of meiosis².

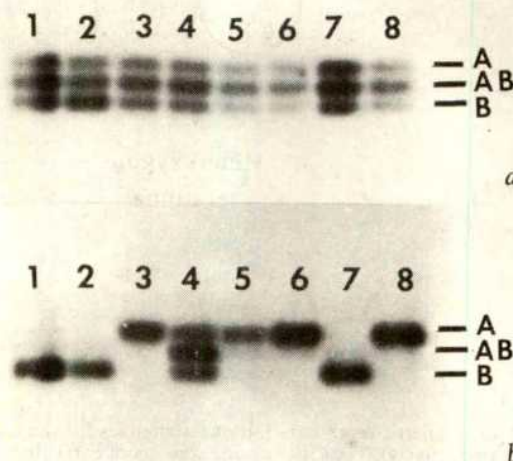


Fig. 1 *a*, Electrophoresis of supernatants prepared from ovaries (slots 1–6, and 8) and spleen (slot 7) from (LT/Sv × LTXBJ)_{F1} mice. Male LTXBJ mice were mated with female LT/Sv to produce the hybrids. At 8–10 weeks of age, the _{F1} females were autopsied and teratomas were removed while taking care to contaminate the tumour with as little ovarian tissue as possible. Excessively bloody tumours were not used. Pieces of tissue were homogenised with ground glass microhomogenisers in 0.05 ml 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and 1 mM β-mercaptoethanol. The homogenate was centrifuged for 20 min at 16,000g and the supernatant solution used as the enzyme source. Electrophoresis was carried out on Titan-III Zip Zone cellulose acetate plates (Helena Laboratories, Beaumont, Texas) with 0.043 M Tris, 0.046 M glycine buffer, pH 8.6, for 1.75 h at 180 V. Glucose phosphate isomerase (EC 5.3.1.9.) was detected with a 1% agar overlay containing 50 mM Tris-HCl, pH 8.0, 2.5 mM MgCl₂, 2.5 mM fructose-6-phosphate, 1 mM NADP, 0.1 mg ml⁻¹ phenazine methosulphate, 0.5 mg ml⁻¹ nitroblue tetrazolium and 5 IU ml⁻¹ glucose-6-phosphate dehydrogenase. The GPI-1B form in slot 2 appears darker than the GPI-1A, suggesting that this piece of ovary was contaminated with some teratoma tissue which was not obvious when the sample was taken. A piece of _{F1} tissue was tested from each animal from which a teratoma was assayed to assure heterozygosity. *b*, Electrophoresis of supernatants prepared from teratomas from (LT/Sv × LTXBJ)_{F1} female mice. Samples 1–3 and 5–8 show either the GPI-1A or GPI-1B allozyme pattern. Sample 4 is one of the two teratomas that showed a hybrid allozyme pattern. Samples 7 and 8 were taken from contralateral ovaries of the same animal (slot 7, Fig. 1a) and are homozygous for alternative allozymes. In some teratomas, the hybrid AB allozyme and the other homozygous allelic product were detected, but in these cases the amount of the AB form greatly exceeded the minor homozygous form, indicating that the tumour was probably contaminated with normal ovarian tissue.

Preliminary chromosomal analysis of LT teratoma has indicated that most of the cells are diploid and a small number are polyploid (L.C.S. and D.S. Varnum, unpublished). Also, the parthenotes recovered from the oviduct of superovulated LT/Sv mice are also diploid, although at least one polar body was usually found (P. C. Hoppe, personal communication). Therefore, diploidisation of the egg karyotype must have occurred after the first division. The mechanism of this diploidisation is unknown but it is possible, in principle, that karyokinesis of the second

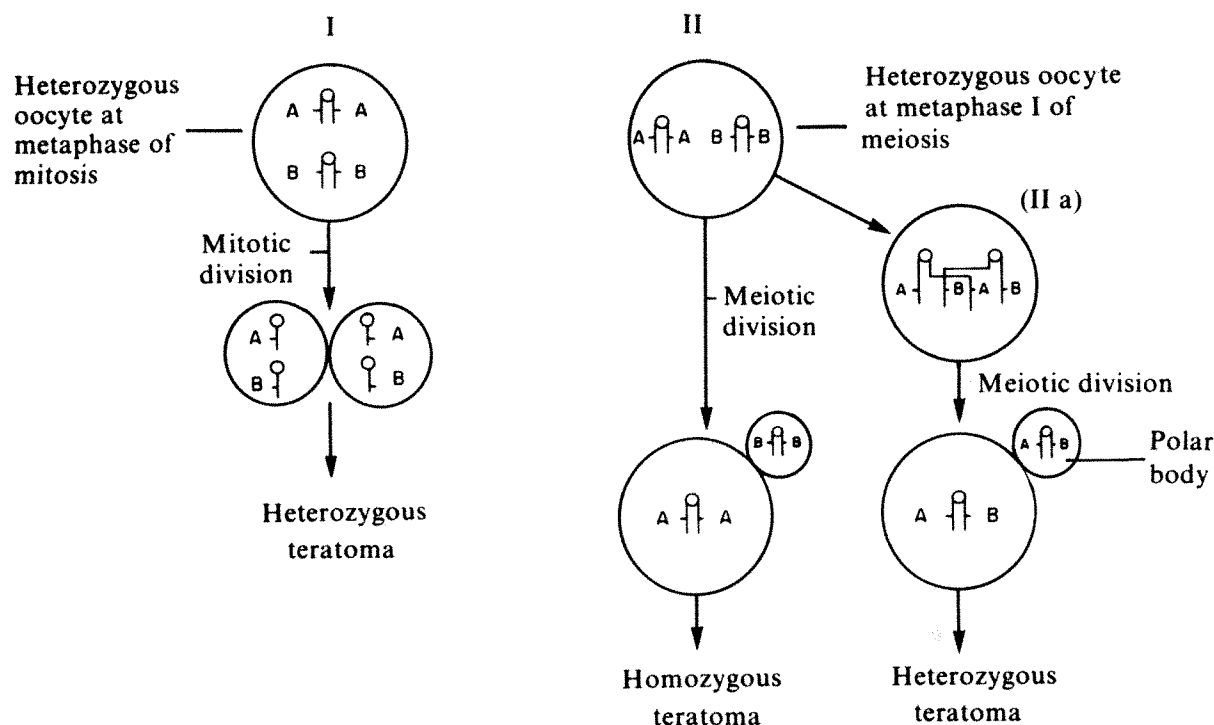


Fig. 2 Diagrammatic representation of alternatives for the distribution of alleles at the *Gpi-1* locus (A and B refer to the *Gpi-1^a* and *Gpi-1^b* alleles, respectively). The GPI enzyme consists of two subunits which associate randomly. Consequently, homozygous *Gpi-1^a* or *Gpi-1^b* cells contain enzyme with two A or B subunits, respectively. Heterozygous *Gpi-1^{ab}* cells contain three forms of the enzyme: one with two A subunits, one with an A and a B subunit, and one with two B subunits in a 1 : 2 : 1 ratio. If teratomas originate from oocytes which cleave mitotically without previous meiotic division (alternative I), then all teratomas would show the heterozygous banding pattern for GPI. If, however, the first meiotic division occurs before parthenogenetic cleavage, then (as the data presented here indicates) most teratomas would be homozygous for either the GPI-1A or GPI-1B allozymes (alternative II). In a minority of cases, heterozygous teratomas would be found resulting from a chromatid exchange between the *Gpi-1* locus and the centromere (IIA).

meiotic division occurs without cytokinesis, thus suppressing the formation of polar body II; or that there is a fusion of the second polar body with the ovum. Our preliminary results do not allow us to distinguish between these possible alternatives.

This research was supported by NSF (grant PCM 76-03047 to J.J.E.) and the NIH (grant HD 08431 to L.P.K. GM 20919 to E.M.E. and CA 02662 to L.C.S.). The Jackson Laboratory is fully accredited by the American Association for accreditation of Laboratory Animal Care.

JOHN J. EPPIG
LESLIE P. KOZAK
EVA M. EICHER
LEROY C. STEVENS

The Jackson Laboratory,
Bar Harbor, Maine 04609

Received 5 July; accepted 8 August 1977.

1 Stevens, L. C. & Varnum, D. S. *Devl Biol.* 21, 364-382 (1974).
2 Linder, D. *Proc. natn. Acad. Sci. U.S.A.* 63, 699-704 (1969).

Extreme sensitivity of some intestinal crypt cells to X and γ irradiation

THE destructive effects of radiation have been studied for 80 yr. Most techniques involve looking at the surviving cells, which tend to be the more resistant cells of the tissue. On the assumption that the results are representative of all cells in the tissue, many conclusions have been drawn. On the other hand, Cheng and Leblond have used tritiated thymidine ($^3\text{HTdR}$) to kill cells synthesising DNA in the crypts of the small intestine¹. Two surprising features of their experiments have provoked little comment. First, very low doses (40-50 μCi per mouse) of $^3\text{HTdR}$

caused measurable cell killing and second, the killing (evident from the presence of labelled apoptotic-like² phagosomes¹) was not random throughout the crypt but occurred selectively at the crypt base where relatively few cells are in S (refs 3 and 4) and where the stem cells are presumably located^{1,3-5}. I report here that the presence of hypersensitive cells at the base of the crypts can be demonstrated after whole-body X or γ irradiation, and to describe the time sequence for the production and loss of these killed cells together with their dose-response relationship.

Figure 1 compares the changes in the number of apoptotic cells in sections of the whole crypt and in sections of the lower third of the crypt after whole-body irradiation. Peak values are obtained within 3-6 h, with 2-3 of the total apoptotic bodies occurring in the lower third. Because the upper third contains few apoptotic bodies after 3 or 6 h this represents a twofold difference in the yield in the base and mid-crypt region. The difference in sensitivity may be much greater for certain cell positions because the base region contains the mature Paneth cells which do not contribute extensively to the apoptotic yield. The control level of apoptoses fluctuates slightly with the time of day but is not affected by handling of the animals (saline injections) or sham irradiation (Fig. 1).

Apoptotic bodies in the crypt base can be ingested and gradually absorbed by neighbouring Paneth cells, stem cells or amplifying committed cells^{1,3-5}. In the last case they will be carried rapidly into the mid-crypt region and then out of the crypt on to the villus. For higher doses the apoptotic yield may remain at elevated levels for longer times because cell cycle progression, crypt transit and cell movement may be retarded when many cells are killed; furthermore, other more radiation-resistant cells may take longer to appear apoptotic. In the case of ingestion by Paneth or stem cells, the apoptotic bodies will remain in the basal region for a longer time, either until absorbed to a residual body no longer detectable in the light microscope (Paneth cells) or until passed by stem cell division into the cytoplasm of a new committed cell

(because stem cells cycle more slowly than the committed cells^{1,3-7}). For these reasons sampling was restricted to either 3 or 6 h after irradiation and in many cases the average of both 3 and 6-h samples was used.

Figure 2 shows the dose dependence for the yield of apoptotic cells in the lower third of the crypt 3 h after irradiation. There was a fivefold increase in yield after 1–2 rad and a tenfold increase after 5 rad. Beyond 20 rad the yield increased more slowly. Because the points are somewhat scattered it is not clear whether the yield was constant beyond 20 rad or continued to increase gradually. The highest values were obtained after a dose of 900 rad and represent a 30–40-fold increase. Beyond 900 rad, crypt survival seems to be related exponentially to dose⁸⁻¹⁰, with a D_0 value of 100–200 rad⁸⁻¹⁰. Thus a few cells (perhaps one to three) survived with an intact reproductive potential after 900 rad, that is 98–99% of the 250 crypt cells were sterilised. But only a very small fraction of these sterilised cells appeared apoptotic.

Figure 3 shows the same data in greater detail with the crypt base and total crypt apoptotic yields using the average of both the 3- and 6-h samples. The points show less scatter and illustrate that

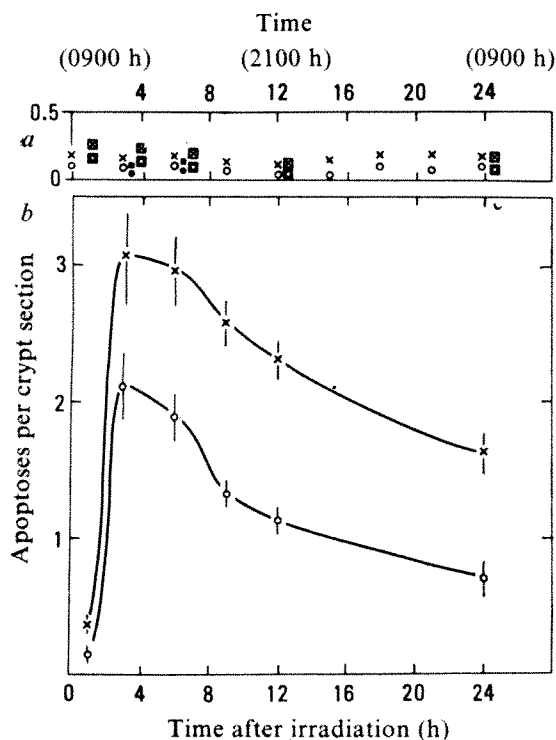


Fig. 1 Apoptotic yield in unirradiated mice (a) and after 63 rad whole-body ¹³⁷Cs γ irradiation (520 rad min⁻¹)(b). Each point shows the mean and standard error of at least four individual mouse values. Scoring was done at $\times 500$ magnification on haematoxylin and eosin 5–7- μ m paraffin sections. Forty crypt sections were scored from each mouse. Crypts were scored only if they were well sectioned longitudinally with the Paneth region, a clear lumen and at least 17 cells up one side of the crypt (the mean number of cells up one side was about 22). The crypts were then divided into three regions, each of approximately 1/3 the total number of cells. It is assumed that the number of apoptotic cells was directly proportional to the number of cells killed over the range of radiation doses studied. Because apoptotic cells tend to be centripetally positioned and disperse slightly a good longitudinal crypt section yields about 60% of the apoptoses per crypt (as revealed by comparing sections and whole squashed crypts). Thus the total number of hypersensitive cells per crypt would have been about 4.5 (based on the value at 40 rad). Groups of untreated animals were killed at various times of the day (\times , total; \circ , base), at various times after an intraperitoneal injection of physiological saline (0.2 ml) (cross in square, total; circle in square, base), and 3 and 6 h after sham irradiation (\blacksquare , total; \bullet , base). In none of these cases did the total apoptotic yield exceed 0.25 per crypt section (overall mean 0.17 total and 0.08 crypt base). There was thus an approximately 20-fold increase in total apoptoses within 3 h of irradiation. The proportion of apoptotic cells is even larger when the crypt base is considered.

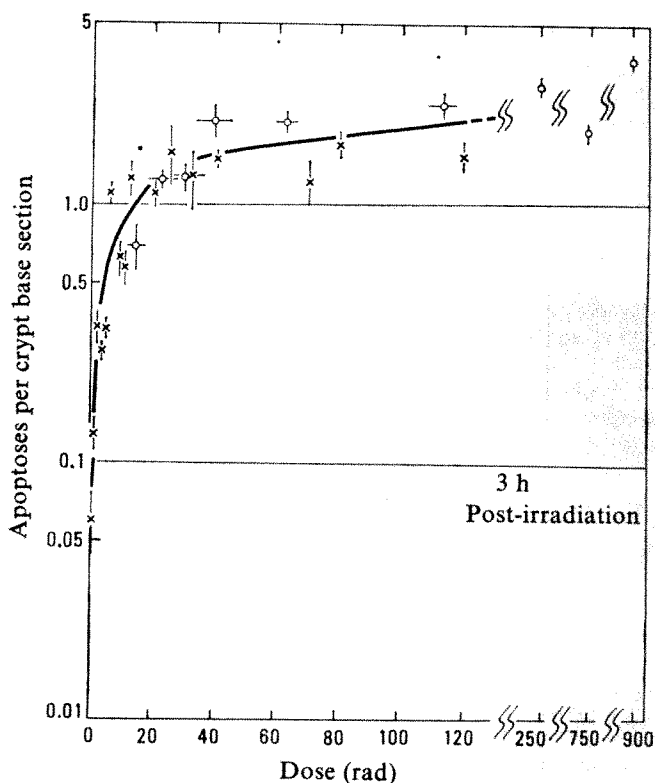


Fig. 2 Dose-response for apoptotic cells in the lower third of the crypt 3 h after irradiation. Apoptotic yield is plotted on a logarithmic scale against dose on a linear scale. Each point shows the mean, with its standard error, of at least four mice. \circ , Values for ¹³⁷Cs γ rays (520 rad min⁻¹); \times , 290 or 300 kVp X rays (30 rad min⁻¹). The errors on the doses were estimated to be about $\pm 5\%$ for all doses except the lowest γ -ray doses where it was not more than $\pm 20\%$.

radiation hypersensitivity was confined largely to cells in the base of the crypt. These results clearly illustrate the presence of some extremely sensitive cells in the lowest region of the crypt—in the region immediately above the Paneth cells and below most of the rapidly proliferating cells, that is, within the presumptive stem cell region^{1,5-7}. The number of hypersensitive cells per control crypt section is uncertain. If it is assumed that most of the hypersensitive cells are killed by 40 rad, which is just on the resistant portion of the dose-response curve shown in Fig. 3, the number of apoptoses observed after 40 rad would be close to the total number of hypersensitive cells present in a crypt section. The data (from Fig. 3) can thus be expressed as survivors rather than killed cells and a survival curve can be generated (Fig. 4). The resulting points are scattered about a line with a 50% effective dose of 8 rad and a D_0 of about 10 rad. This places these crypt cells among the most sensitive mammalian cells. Figure 4 shows that the most sensitive type of oocytes (stage 1) have a similar sensitivity¹² while the most sensitive spermatogonia (preleptotene)¹³ and the sensitive cortical thymic lymphocytes¹⁴ are more resistant than the crypt cells. An extreme sensitivity can also be seen in some early mouse embryo cells after doses in range 5–25 R (refs 15–17). This survival curve is speculative and the actual slope (D_0) depends somewhat on the value used for the number of hypersensitive cells per crypt, but whatever way the data are expressed, within the stem cell region at the crypt base there are clearly a few extremely radiosensitive cells. Preliminary observations indicate the presence of similar hypersensitive cells in the growing hair follicle matrix and colonic crypts.

The precise function of the radiosensitive cells in the crypt is uncertain. They do not represent any known histologically distinctive cell type (at the level of either light or electron microscopy). Their presence suggests at least two radiobiologically distinct crypt cell populations. If they are stem cells they must represent a subpopulation of the total complement of potential

stem cells (about 80 per crypt⁹) because the crypts are not destroyed when these hypersensitive cells are killed. The number of functional stem cells per crypt is uncertain but is probably about 20, that is about six times the number of hypersensitive cells. The subpopulation of hypersensitive cells might be at some extremely sensitive stage of the cell cycle peculiar to stem cells. The normal sensitivity changes through the cell cycle are relatively small and the sensitive phases (G_2 and M)¹⁸ do not exhibit sensitivities such as those shown here. It is not clear whether these apoptotic bodies are formed from interphase cells or from abortive divisions. In the latter case their appearance within 3h could suggest a hypersensitive point somewhere in G_2 .

One rad of radiation of this quality produces sufficient ionising tracks for at least one track to cross some part of about 50% of the cell nuclei (assuming an average diameter of about $4\mu\text{m}$). The average track length per rad per cell nucleus is about $2\mu\text{m}$. The average energy deposition per nucleus is 2KeV rad^{-1} . The data in Fig. 3 suggest that after 1 rad one sensitive cell in ten will have at least one track traversing its critical volume which might be about $1.4\mu\text{m}$ in diameter (that is about $1/20$ of the nuclear volume) and that one track crossing this volume is sufficient to kill that cell.

The mechanism by which these sensitive cells are killed is unknown, but DNA damage can be detected at similar doses (up to 5 rad)¹⁹. It has been suggested that stem cells would benefit from a mechanism by which newly synthesised DNA strands (containing replication errors) were selectively distributed to the committed cells and thus eliminated, ensuring the genetic stability of the stem cell DNA²⁰. In these conditions damage to the stem cell template DNA might be intolerable and the cells might die. The extreme sensitivity seen here would suggest that any DNA damage would result in death; furthermore that other cells differing in the way they normally handle their DNA may assume the role of regenerative stem cells which would be a potentially hazardous process because possibly imperfect DNA would be 'immortalised'. This assumes that the vital stem cells lack any means of repairing genetic errors. An alternative explanation implies the

Fig. 3 Dose-response for apoptotic cells in the lower third (base, \times and \circ) and the total crypt (\bullet). The individual data points are the mean of 3- and 6-h post-irradiation groups for X rays (\times) and γ rays (\circ). The insert shows the first nine data points for the base (0-12 rad).

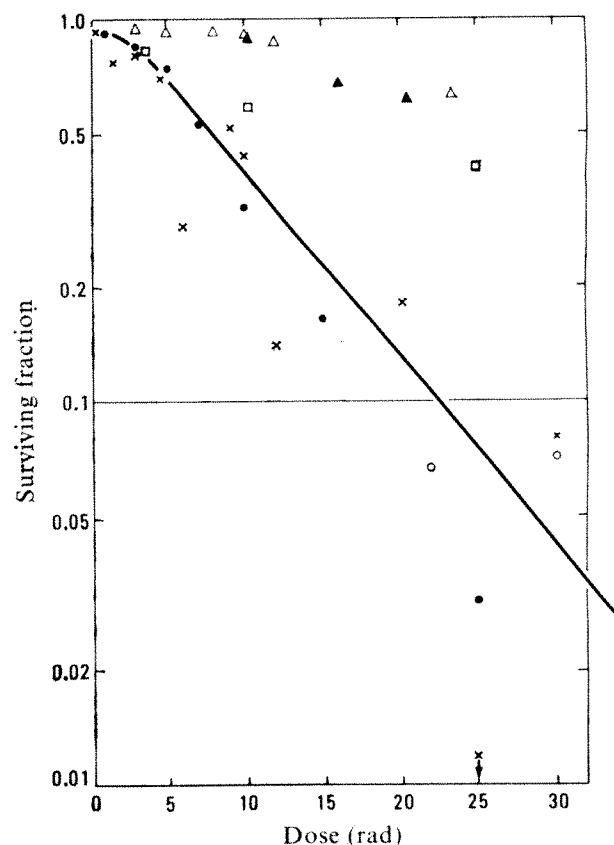
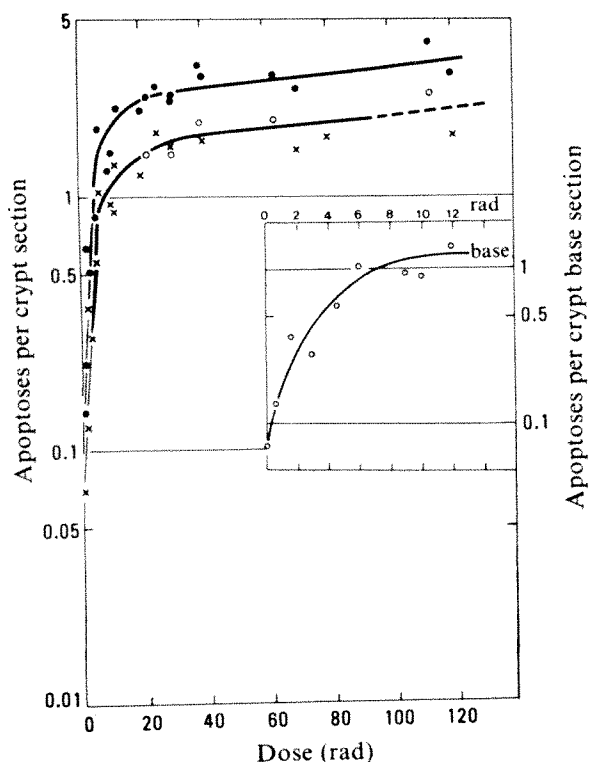


Fig. 4 Survival curve for apoptotic sensitive crypt cells (\times , X rays, \circ , γ rays). Surviving fraction is plotted on a logarithmic scale against radiation dose on a linear scale. The points were obtained by assuming that there was an average of 2.7 apoptotic sensitive cells per crypt section (value reached at 40 rad, see Fig. 3). Data points for stage I oocytes¹² (\bullet), preleptotene spermatogonia¹³ (Δ), type B spermatogonia (\blacktriangle) and cortical thymic lymphocytes¹⁴ (\square) have also been plotted. The line has been fitted by eye and has a D_{01} of approximately 10 rad.

reverse, that the stem cells possess an extremely efficient means of restoring the DNA integrity; the stem cells may possess a mechanism for detecting template errors and (1) transferring G_1 induced errors by means of sister chromatid exchanges and mitosis to the committed daughter which subsequently dies, or (2) eliminating G_2 -induced errors by elevating the newer strands to stem cell template status and discarding the damaged DNA to a cell that subsequently dies. Any of these possibilities could explain the apparent indifference of the crypt as a whole to this type of death.

The significance of this phenomenon in public health (maximum permissible doses)—doses within the range studied here are delivered in several diagnostic radiological procedures—and radiotherapy, depends on the function of the hypersensitive cells and the mechanism by which they are killed.

This work was supported by grants from the MRC and the Cancer Research Campaign. I thank Jeffrey Searle for suggesting apoptosis; John Cairns and Don Major for helpful discussions; Irene Wylie for technical assistance and Caroline West for microscopy.

CHRISTOPHER S. POTTEN

Paterson Laboratories,
Christie Hospital and Holt Radium Institute,
Manchester, UK

Received 31 May; accepted 2 August 1977.

- Cheng, H. & Leblond, C. P. *Am. J. Anat.* **141**, 537-562 (1974).
- Kerr, J. F. R., Wylie, A. H. & Currie, A. R. *Br. J. Cancer* **26**, 239-257 (1972).
- Potten, C. S., Kovacs, L. & Hamilton, E. *Cell Tissue Kinet.* **7**, 271-283 (1974).
- Al-Dewachi, H. S., Wright, N. A., Appleton, D. R. & Watson, A. J. *Virchows Arch. B Cell Path.* **18**, 225-243 (1975).
- Potten, C. S. *Bull. Cancer* **62**, 419-430 (1975).
- Leblond, C. P. & Cheng, H. in *Stem Cells of Renewing Cell Populations* (ed. Cairnie, A. B., Lala, P. K. & Osmond, D. G.) 17-31 (Academic, New York, 1976).

7. Potten, C. S. in *Stem Cells of Renewing Cell Populations* (ed. Cairnie, A. B., Lala, P. K. & Osmond, D. G.) 79-84 (Academic, New York, 1976).
8. Hendry, J. H. & Potten, C. S. *Int. J. radiat. Biol.* **25**, 583-588 (1974).
9. Potten, C. S. & Hendry, J. H. *Int. J. radiat. Biol.* **27**, 413-424 (1975).
10. Withers, H. R. & Elkind, M. M. *Int. J. radiat. Biol.* **17**, 261-267 (1970).
11. Alper, T. *et al. Br. J. Radiol.* **35**, 722-723 (1962).
12. Oakberg, E. F. *Proc. Soc. exp. Biol. Med.* **109**, 763-767 (1962).
13. Oakberg, E. F. & Clark, E. J. *Cell comp. Physiol.* **58**, Suppl. 1, 173-182 (1961).
14. Trowell, O. A. *Int. J. radiat. Biol.* **4**, 163-173 (1961).
15. Ohzu, E. *Radiat. Res.* **26**, 107-113 (1965).
16. Rugh, R. & Grupp, E. *J. exp. Zool.* **141**, 571-587 (1959).
17. Snow, M. H. L. *J. Embryol. exp. Morph.* **79**, 601-615 (1973).
18. Sinclair, W. K. & Morton, R. A. *Radiat. Res.* **29**, 450-474 (1966).
19. Steffensen, D. M. *Nature* **201**, 205-206 (1964).
20. Cairns, J. *Nature* **255**, 197-200 (1975).
21. Bianchi, M., Baarli, J. & Sullivan, A. H. *IAEA-SM-179/6* 349-357 (International Atomic Energy Agency, Vienna, 1974).

Cellular serine proteinase induces chemotaxis by complement activation

INFILTRATION of inflamed or injured tissues by polymorphonuclear leukocytes is a fundamental pathophysiological response. Undoubtedly, there are numerous mechanisms by which leukocytes are attracted to an area of damage. One possibility is that cellular injury could release or activate a proteolytic enzyme which could generate chemotactic factors. Extracts of parenchymatous tissues¹ and cells² are chemotactic when they are incubated with complement sufficient serum. Macrophages³ and leukocytes⁴ contain a proteinase which generates chemotactic peptides, and recently human polymorphonuclear leukocytes have been shown to secrete an enzyme which activates complement⁵. Lazarus and Barrett extracted and characterised a serine proteinase which induced polymorphonuclear leukocyte infiltration of the skin when injected intradermally⁶. Subsequently, this proteinase, which is both cytotoxic and phlogistic, has been purified to homogeneity from whole human skin⁷, human epidermis⁸, human lymphocytes⁹ and cultures of newborn mouse epithelium (G.S.L., unpublished). This report demonstrates that this proteinase is significantly more active than trypsin, plasmin or Pronase in generating polymorphonuclear leukocyte accumulation and that chemotactic activity is largely dependent on complement activation.

The procedure for purification of the enzyme has been described previously⁷; this was used with several modifications. Frozen human skin, obtained from amputated limbs, was minced and added to 10 volumes 50 mM phosphate buffer, pH 7.4, containing 1.0 M potassium chloride. The mixture was frozen and thawed five times and further extracted by agitation at 4 °C for 18 h. After centrifugation at 50,000g, the supernatant was subjected to 85% saturation with ammonium sulphate and the proteinase was extracted from the precipitate with a minimum volume of extraction buffer. The extract was concentrated and placed on a Sephadex G-75 superfine column and the eluted fractions containing the smallest molecular weight proteinase (molecular weight approximately 28,000) were combined and further purified by chromatography on a soybean trypsin inhibitor-Sepharose affinity column.

This purification method yielded a single protein band on polyacrylamide gel electrophoresis and SDS-polyacrylamide gel electrophoresis. Injection of the 250-fold purified enzyme into rabbits produced an antibody which gives a single line of identity in Ouchterlony double-diffusion plates against purified enzyme

and crude starting material. The enzyme was inhibited by diisopropyl fluorophosphate (DFP), soybean trypsin inhibitor, α_2 -macroglobulin and α_1 -antitrypsin. Further characterisation has demonstrated that it was not elastase, cathepsin G or plasminogen activator.

Polymorphonuclear leukocyte chemotaxis was assayed by the method of Snyderman *et al.*¹⁰. Sample (2 ml) was injected into the peritoneal cavity of a group of normal or C5-deficient male mice (Jackson Laboratories, Bar Harbor, Maine). After 12 h, the animals were killed by inhalation of carbon dioxide, and the peritoneal cavity was vigorously washed with 9 ml Dulbecco's Modified Eagle's Media with 10% foetal calf serum (GIBCO), containing 90 IU herapin (Upjohn). The peritoneal wash was counted for total number of white cells and for percentage polymorphonuclear leukocytes (PMN) by standard techniques. Results were expressed as absolute number of PMN. All assays were carried out in triplicate on three occasions, and the materials tested included saline controls, proteose peptone, a complement-independent chemotactic agent (9%, DIFCO Laboratories), and proteinase with and without inhibitors. Proteolytic activity of all materials was assayed by measuring production of trichloroacetic acid-soluble peptides from tritium-labelled casein⁸.

Polymorphonuclear leukocyte accumulation was directly proportional to enzyme concentration in the range 1-50 μ g enzyme protein. Table 1 compares the ability of the purified human proteinase to elicit polymorphonuclear leukocyte accumulation with other common proteolytic enzymes operative at neutral pH. Our enzyme (20 μ g) induced a brisk accumulation of polymorphonuclear leukocytes. Pre-incubation of enzyme with diisopropyl fluorophosphate (1 mM final concentration, 1 h, 25 °C) followed by exhaustive dialysis against physiological saline, almost completely inhibited polymorphonuclear leukocyte accumulation. These data suggest that enzymatic activity is necessary for polymorphonuclear leukocyte accumulation. By contrast, injection of 200 μ g plasmin, Pronase or trypsin attracted one-third the number of polymorphonuclear leukocytes into the peritoneum. This was not simply a function of increased proteolysis by our enzyme; expression of polymorphonuclear accumulation per amount of casein-degrading activity, revealed that our purified skin proteinase was 3-5 logs more effective in attracting polymorphonuclear leukocytes on a proteolytic activity basis than the other proteinases tested.

We next attempted to elucidate the mechanism by which our purified human proteinase induced such a significant polymorphonuclear leukocyte accumulation. Equal amounts of the purified proteinase were injected into normal and C5-deficient mice (Fig. 1). The enzyme evoked the expected dramatic response in normal mice, which was almost completely inhibited by DFP. By contrast, the enzyme was a quarter as effective in evoking polymorphonuclear leukocyte infiltration in C5-deficient mice. Furthermore, the minimal chemotactic response in C5-deficient mice could be reduced to control levels by pre-incubation of the enzyme with DFP. These data show that the enzyme evokes its chemotactic response principally through the complement cascade and probably requires the production of C5 fragmentation for maximal activity. The data also suggest that the proteinase is capable of inducing limited chemotaxis by a C5-independent mechanism.

Table 1 Relative chemotactic activity of proteolytic enzymes

	μ g Injected	Proteolytic activity (c.p.m. TCA-soluble protein)	PMN per mouse $\times 10^{-4}$	Chemotactic index PMN/c.p.m. proteolytic activity
Human skin proteinase	20	1.2×10^3	370	3,060
Plasmin	200	3.5×10^6	90	2.6
Pronase	200	2.8×10^7	126	0.05
Trypsin	200	6.2×10^7	128	0.02

Proteinases were assayed for proteolytic activity before injection and polymorphonuclear leukocyte (PMN) accumulation was measured 12 h after injection. Chemotactic index expresses PMN accumulation per unit of proteolytic activity.

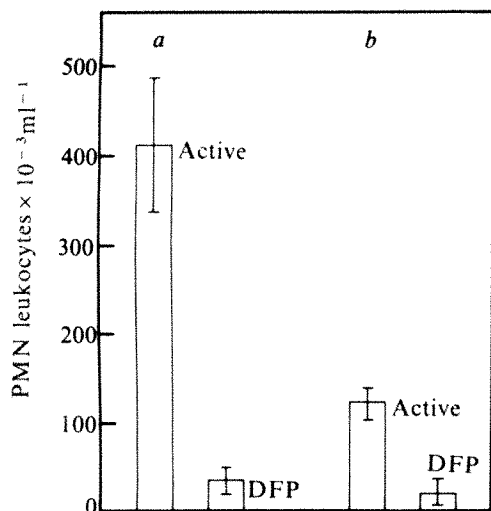


Fig. 1 Purified human proteins (20 μg) and a comparable amount of enzyme inhibited with DFP, were injected into normal (a) and C5-deficient mice (b). Polymorphonuclear (PMN) leukocyte accumulation was determined 12 h after injection.

Our data suggest that cells contain a proteinase operative at neutral pH which is extraordinarily effective in activating complement and inducing polymorphonuclear leukocyte accumulation. The presence of this enzyme in whole skin⁷, human epidermis⁸, human lymphocytes⁹ and mouse epithelial cells in culture (G.S.L., unpublished), could suggest that cellular injury might activate this proteinase, which through the mediation of complement induces polymorphonuclear leukocyte accumulation. Such a mechanism might explain how cellular injury induces polymorphonuclear leukocyte accumulation.

This investigation was supported by the National Institute of Arthritis, Metabolic and Digestive Diseases (grant 7 ROI AM17370-03 and 5T32 AM07093-02) and the National Institute of Dental Research (grant 2 NID E03738-04). We thank Dr Irma Gigli, New York University Medical Center, for assistance and discussions. Dr Patrick McKee, Duke University Medical Center, kindly provided us with pure human plasmin. R.S. is an Investigator of the Howard Hughes Medical Institute. G.S.L. is a Senior Investigator of the Arthritis Foundation.

CHARLOTTE A. THOMAS

FRED J. YOST, JR

RALPH SNYDERMAN

VICTOR B. HATCHER*

GERALD S. LAZARUS

Department of Medicine,
Divisions of Dermatology and Rheumatology,
Duke University Medical Center,
Durham, North Carolina 27710

Received 2 June; accepted 1 August 1977.

*Present address: Departments of Biochemistry and Medicine, Montefiore Hospital of the Albert Einstein College of Medicine Bronx, NY.

¹ Hill, J. H. & Ward, P. A. *J. exp. Med.* **130**, 505–58 (1969).

² Brier, A. M., Snyderman, R., Mergenhagen, S. E. & Notkins, A. L. *Science* **170**, 1104–06 (1970).

³ Snyderman, R., Shin, H. S. & Dannenberg, A. M. *J. Immun.* **109**, 896–898 (1972).

⁴ Goldstein, I. M. & Weissman, G. J. *Immun.* **113**, 1583–88 (1974).

⁵ Wright, D. G. & Gallin, J. I. *Inflammation* **1**, 23–29 (1975).

⁶ Lazarus, G. S. & Barrett, A. J. *Biochim. biophys. Acta* **350**, 1–12 (1974).

⁷ Hatcher, V. B., Lazarus, G. S., Levine, N., Burk, P. G. & Yost, F. J. *Biochim. biophys. Acta* **483**, 160–171 (1977).

⁸ Levine, N., Hatcher, V. B. & Lazarus, G. S. *Biochim. biophys. Acta* **452**, 458–67 (1976).

⁹ Hatcher, V. B., Oberman, M. S., Lazarus, G. S. & Grayzel, A. I. *Fedn Proc.* **36**, 892 (1977).

¹⁰ Snyderman, R., Phillips, J. K. & Mergenhagen, S. E. *J. exp. Med.* **134**, 1131–43 (1971).

The ECF-A tetrapeptides and histamine selectively enhance human eosinophil complement receptors

VARIOUS products of the anaphylactic reaction such as the ECF-A tetrapeptides¹ (Val-Gly-Ser-Glu and Ala-Gly-Ser-Glu)² and histamine^{3,4} preferentially attract the human eosinophil in chemotaxis *in vitro*. We have designed experiments to show whether these chemical mediators have a direct effect on the eosinophil cell membrane, as assessed by their capacity to alter receptors for either IgG or complement (C). We have established here that these two ECF-A peptides and histamine both enhance markedly the expression of eosinophil receptors for C3 but not for IgG. Neutrophil and monocyte receptors seemed to be unaltered and a number of other chemical mediators of hypersensitivity had no effect on these membrane markers when tested at comparable concentrations. Our demonstration of eosinophil membrane receptor enhancement by chemotactic factors may have more general biological significance in terms of how surface recognition mechanisms by phagocytic cells are regulated.

Receptors were measured by the 'rosette technique' using sheep red blood cells (RBC) sensitised with either IgG or C3b (Fig. 1). Human eosinophils, neutrophils⁵ and monocytes⁶ were prepared from venous blood from patients with eosinophilia of various aetiology and adjusted to a concentration of $4 \times 10^6 \text{ ml}^{-1}$ in

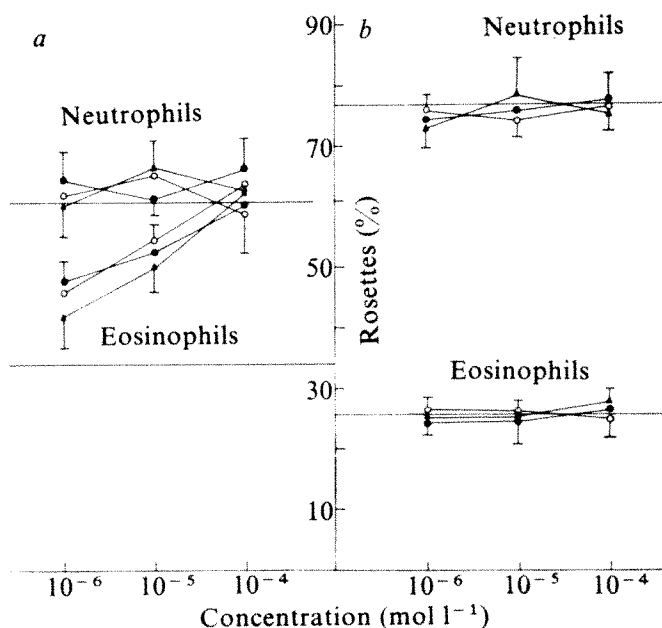


Fig. 1 The effect of increasing concentrations of the valyl- (●) and alanyl- (○) ECF-A peptides and histamine (▲) on a, EAC3b and b, EAG rosettes by human eosinophils and neutrophils. The lines represent the percentage rosettes of untreated eosinophils and neutrophils. Each point represents the mean of five experiments \pm 1 s.d. Dextrose-gelatin-veronal buffer (DGVB²⁺ pH 7.4) was used for washing sheep RBC during sensitisation and coating with various complement components. This was prepared by mixing equal volumes of isotonic veronal-buffered saline (containing 0.0015 M Ca²⁺, 0.0005 M Mg²⁺ and 0.1% gelatin-veronal buffer (GVB²⁺) with 5% dextrose in water containing the same concentration of Ca²⁺ and Mg²⁺ (ref. 10). The IgG and IgM fractions of rabbit antisera to sheep red cells were prepared by Sephadex G-200 gel filtration. Sheep cells were sensitised with either the IgG fraction for preparing EAG_{rab} (EAG) or IgM (EA_M^{rab}) for EAC1423b rosettes. Functionally pure human complement components were added sequentially to EA_M^{rab} to prepare C3b-coated cells as described¹¹. The amounts were as follows: 400 effective molecules of C1, 400 of C4, 50 of C2 and 2,500 of C3. This amount of C4 was insufficient to give EAC14 rosettes with neutrophils, eosinophils or monocytes¹¹. The valyl- ((HCl)-Val-Gly-Ser-Glu, molecular weight 427) and alanylpeptides ((HCl)-Ala-Gly-Ser-Glu, molecular weight 497) were a gift from Dr R. Camble (ICI Limited, Pharmaceuticals Division, Alderley Park, Macclesfield); histamine acid phosphate (BDH Chemicals Limited, Poole).

medium 199 (pH 7.4). Equal volumes of leukocyte suspensions and various concentrations of the pharmacological agents under study, or control medium alone, were mixed and incubated in a shaking water-bath at 37 °C for varying intervals. The cells were then washed twice in medium 199 and the numbers adjusted to $2 \times 10^6 \text{ ml}^{-1}$ in the same medium. An aliquot (0.1 ml) of IgG-(EAG) or complement-coated (EAC3b) red cells containing $1 \times 10^8 \text{ ml}^{-1}$ was then added to 0.1 ml of the cell suspension. The mixtures were centrifuged at 100g for 10 min at 4 °C and the pellets incubated at 0 °C (for EAG rosettes) or 37 °C (for EAC3b rosettes) for 30 min as described. The pellets were gently resuspended and smears were prepared in duplicate. The slides were dried in air, fixed in methanol and stained. Cells with three or more adherent erythrocytes were counted as rosettes. Two hundred cells were counted on each slide and the number of rosettes expressed as a percentage of the total number counted.

With no treatment the percentages of neutrophil, IgG or complement receptors were usually more than twice that of the eosinophil (Fig. 1). With increasing concentrations of the valyl- or alanyl-peptide, or histamine there was a dose-dependent enhancement of the numbers of eosinophils, but not of neutrophils, which formed rosettes with EAC3b. There was no increase in the numbers of eosinophils or neutrophils forming rosettes with EAG. When other pharmacological mediators, which included bradykinin and the prostaglandins PGE_1 , E_2 and $\text{F}_{2\alpha}$, were incubated at comparable concentrations (10^{-4} – $10^{-6} \text{ mol l}^{-1}$) to that of the ECF-A peptides or histamine, there was no appreciable increase in the numbers of eosinophil- or neutrophil-EAC3b rosettes (Table 1). An increase of 25% was observed with 5-hydroxytryptamine, but only at the highest concentration ($10^{-4} \text{ mol l}^{-1}$). The enhancement with the ECF-A peptides or histamine, with this dose was almost three times this value, thus achieving virtually the same percentage of rosetting eosinophils as neutrophils (Figs 1 and 2). The monocyte was also tested with the valyl- and alanyl-peptide and histamine but, unlike the eosinophil, there was no apparent alteration in the numbers of rosetting cells (Table 1). The enhancement of EAC3b eosinophil rosettes by the ECF-A peptides or histamine also increased with incubation time (Fig. 2). With the peptides, however, most of the increase in rosette formation was apparent at 40 min. In contrast, most of the histamine-induced rosette enhancement took place between 40 and 80 min.

These experiments suggest that the ECF-A peptides and histamine not only attract eosinophils selectively in chemotaxis but also have a direct effect on the eosinophil membrane as shown by the apparent increase in the numbers of complement receptors. It is not known whether this enhancement is caused by the generation of new receptors or whether receptors, previously 'hidden', are revealed as a result of membrane changes. The inability of these pharmacological agents to increase neutrophil and monocyte complement receptors emphasises further the unique, intimate relationship between the human eosinophil and

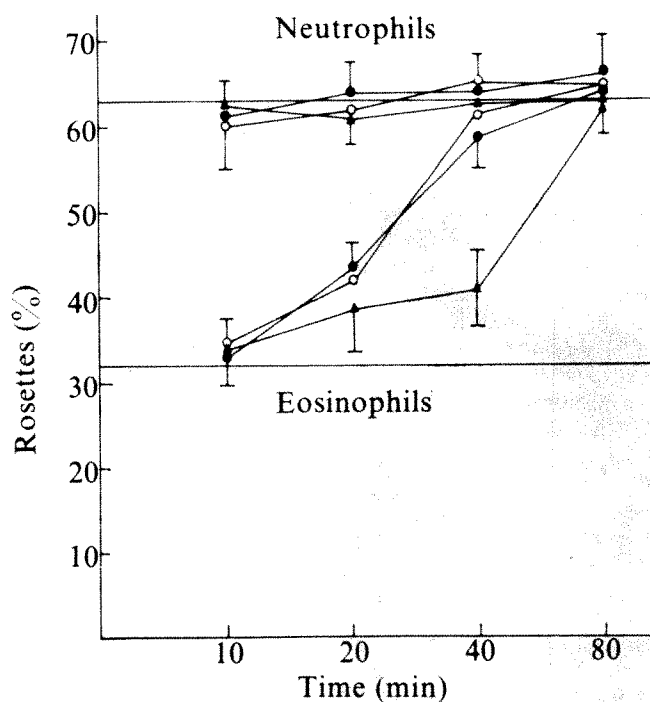


Fig. 2 The effect of time on enhancement of EAC3b rosettes by eosinophils and neutrophils following incubation with the valyl-(●) and alanyl-(○) ECF-A peptides and histamine (▲). Each mediator was tested at a concentration of $10^{-4} \text{ mol l}^{-1}$. The lines represent the percentage rosettes of untreated eosinophils and neutrophils. Each point represents the mean of five experiments $\pm 1 \text{ s.d.}$

these anaphylaxis-associated agents. We also explored the possibility that neutrophil and monocyte complement receptor enhancement may be revealed when the amount of C3 on the indicator cell was limited. In experiments in which the quantity of C3 used was adjusted to give approximately half the number of rosetting cells (that is, 1,000 effective molecules) however, there was no apparent increase in rosette formation following incubation with these agents. Similar negative results were found with neutrophils and monocytes when the amounts of IgG were decreased to give lower numbers of EAG rosettes. The reason why complement, and not IgG, receptors were altered by the ECF-A peptides and histamine is unknown. It was previously shown that IgG and C3b receptors act together to aid macrophage phagocytosis⁷. A similar mechanism may exist for eosinophils; optimal receptor ratio being determined by the presence of pharmacological mediators such as ECF-A and histamine.

The role of the eosinophil in immediate-type hypersensitivity is thought to be that of a homeostatic cell affecting mediator release,

Table 1 Percentage increase in the numbers of rosetting eosinophils, neutrophils and monocytes with EAC3b cells following incubation with increasing concentrations of various chemical mediators of hypersensitivity

	$10^{-6} \text{ (mol l}^{-1}\text{)}$			$10^{-5} \text{ (mol l}^{-1}\text{)}$			$10^{-4} \text{ (mol l}^{-1}\text{)}$		
	Eosinophils	Neutrophils	Monocytes	Eosinophils	Neutrophils	Monocytes	Eosinophils	Neutrophils	Monocytes
Valyl-peptide	43.3	5.6	(6.8)	55.6	0.5	(0.3)	77.8	8.1	(4.5)
Alanyl-peptide	37.0	1.0	5.3	60.6	8.1	(1.8)	87.8	(3.1)	(7.0)
Histamine	25.1	(1.1)	(3.3)	48.8	7.8	(3.3)	83.4	1.3	3.0
Bradykinin	0.5	7.8	—	0.5	(4.9)	—	3.0	2.3	—
PGE_1	5.6	4.2	—	(2.0)	(8.5)	—	14.7	1.3	—
PGE_2	(0.9)	(3.9)	—	6.5	2.9	—	6.8	(7.7)	—
$\text{PGF}_{2\alpha}$	5.6	(3.1)	—	13.3	1.3	—	(3.3)	2.1	—
5-HT	3.0	(5.2)	—	17.8	(3.4)	—	25.0	6.7	—

The figures represent the mean of five experiments, with the exception of bradykinin (three experiments). Decreases in rosette formation are shown in parentheses. Prostaglandins E_1 , E_2 and $\text{F}_{2\alpha}$ were supplied by Dr John Pike, Upjohn Company, Kalamazoo, USA; bradykinin triacetate and 5-hydroxytryptamine were obtained from Sigma, Kingston-upon-Thames, UK.

mediator inactivation and mediator replenishment by mast cells⁸. Their function in helminth disease may be that of a cytotoxic cell requiring the participation of IgG, in an analogous fashion to lymphocyte-antibody-dependent cell-mediated cytotoxicity⁹. Although complement does not seem to be required in this system, it is nevertheless possible that adherence by way of the C3 receptor may amplify the parasitocidal properties of eosinophils. Our observations may therefore, be of relevance in helminth disease where the IgE-mediated release of chemical mediators is well recognised. In any event, our finding of a new biological activity for both the ECF-A peptides and histamine may provide further insight into both the essential biochemical differences between human eosinophils and other blood leukocytes, and the way in which chemical mediators modulate recognition mechanisms by cell membranes.

A.R.E.A. is a British Council Fellow.

A. R. E. ANWAR

A. B. KAY

Department of Pathology,
University Medical School,
University of Edinburgh,
Teviot Place,
Edinburgh, UK

Received 28 June; accepted 9 August 1977.

1. Kay, A. B. & Austen, K. F. *J. Immunol.* **107**, 899-902 (1971).
2. Goetzl, E. J. & Austen, K. F. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4123-4127 (1975).
3. Clark, R. A. F., Gullin, J. I. & Kaplan, A. P. *J. exp. Med.* **142**, 1462-1476 (1975).
4. Turnbull, L. W. & Kay, A. B. *Immunology* **31**, 797-802 (1976).
5. Day, R. P. *Immunology* **18**, 995-959 (1970).
6. Böyum, A. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77-89 (1968).
7. Lay, W. H. & Nussenzweig, V. *J. exp. Med.* **128**, 991-1007 (1968).
8. Kay, A. B. *Br. J. Haemat.* **33**, 313-318 (1976).
9. Butterworth, A. E. *et al. Nature* **256**, 727-729 (1975).
10. Nelson, R. A., Jensen, J., Gigh, I. & Tamura, N. *Immunochimistry* **3**, 111-135 (1965).
11. Anwar, A. R. E. & Kay, A. B. *J. Immunol.* (in the press).

Molecular basis for acquired haemoglobin H disease

HAEMOGLOBIN H (Hb H; β_4) disease results from a reduced rate of synthesis of the α chains of human adult haemoglobin (Hb A; $\alpha_2\beta_2$). In the absence of sufficient α chains, excess β chains form β_4 tetramers which are unstable, precipitate and cause a shortened red cell survival¹. Hb H disease is one of the α -thalassaemia syndromes. These disorders usually result from interactions of three α -thalassaemia (α thal) genes; α thal 1, α thal 2 and Hb Constant Spring. In many human populations the α -chain loci are duplicated, that is, there are two per haploid genome². The gene α thal 1 results from a deletion of both of the pair of haploid α -chain genes^{3,4}, α thal 2 from a deletion of one of the pair⁵, and Hb Constant Spring from a chain termination mutation affecting one of the linked loci which markedly reduces the output of α chains and produces a phenotype almost identical to α thal 2 (ref. 6). Haemoglobin H disease results from the inheritance of α thal 1 together with either α thal 2 or Hb Constant Spring. There have been occasional reports of an acquired form of Hb H disease occurring in association with leukaemia or related myeloproliferative disorders⁷⁻¹². We have studied the blood and bone marrow of a patient with acquired Hb H disease and we report here that it has a completely different molecular basis from the genetic form.

The patient (WD), an 81-yr-old British male, had a bizarre myeloproliferative disease which terminated in acute myeloblastic leukaemia. Full haematological and biochemical data will be presented elsewhere. His blood picture showed two cell populations; most of the cells were poorly haemoglobinised but about 5% seemed normal. Chromosomal analysis of the

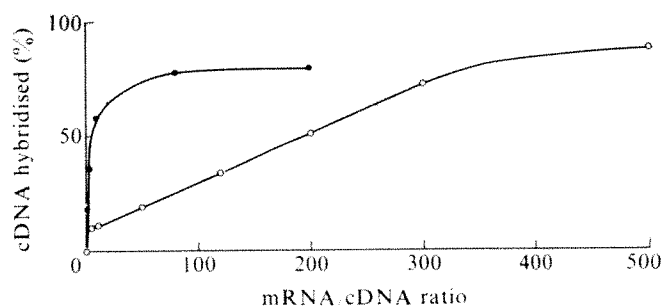


Fig. 1 Hybridisation of human globin cDNA α and cDNA β to mRNA from the peripheral blood of WD. cDNA α and cDNA β were prepared by the hybridisation of 7 μ g of $\beta^0/\delta^0\beta^0$ -thalassaemia mRNA to 190 ng of 3 H-cDNA $\alpha\beta$ (prepared as previously described¹⁹ by RNA-dependent DNA polymerase purified from avian myeloblastosis virus, a gift from Dr Beard, Life Sciences Research Laboratories) in 50 μ l of hybridisation buffer (0.5 M NaCl, 25 mM HEPES, 10 mM EDTA pH 6.8, 50% formamide and 500 μ g ml⁻¹ of *Escherichia coli* RNA) for 3 h at 43 °C. The non-hybridised component (cDNA β enriched) was separated from the hybridised component (cDNA α enriched) by hydroxylapatite fractionation at 60 °C. The cDNA α probe hybridised to mRNA $\alpha\beta$ to 90% completion and contained approximately 10% cDNA β as determined by hybridisation to mRNA lacking α -globin sequences. The cDNA β probe hybridised to 80% completion and contained 12% cDNA α as determined by hybridisation to mRNA lacking β -globin sequences. 9S mRNA from the peripheral blood of WD was prepared by phenol-chloroform extraction followed by sucrose gradient sedimentation. 0.1 ng of cDNA α (○) or cDNA β (●) were mixed with increasing amounts of mRNA in 2 μ l of hybridisation buffer in a sealed siliconised glass capillary tube and incubated at 43 °C for 100 h. The amount of cDNA hybridised was determined after incubation with S₁ nuclease¹⁹. The ratio of mRNA β to mRNA α is equal to the mRNA/cDNA ratio at which 50% of the cDNA α is hybridised divided by the ratio at which 50% of the cDNA β is hybridised. The cDNA concentration is corrected for the maximum amount of hybridisable cDNA. 50% of cDNA α was hybridised at ratio of approximately 200:1, and 50% of cDNA β at 3:1, giving a mRNA β /mRNA α ratio of 65:1.

peripheral blood and marrow was normal. Approximately 95% of the red cells generated typical Hb H inclusions and haemoglobin analysis showed 50-65% Hb H. The genetic form of Hb H disease was excluded because WD had been shown to have a normal blood picture 10 yr previously and examination of his children showed no evidence of thalassaemia or defective α -chain synthesis. The ratio of α - to β -chain synthesis in the peripheral blood was 0.07 as measured by 3 H-leucine incorporation^{13,14}. In the genetic form of Hb H disease this ratio ranges from 0.3 to 0.62 with a mean of 0.5^{15,16}.

mRNA prepared from the peripheral blood was translated in the wheat germ cell free protein synthesis system and the product analysed by CM-cellulose chromatography¹⁷. The mRNA directed the synthesis of β chains but no α -chain synthesis could be detected. In a similar study using mRNA from five patients with the genetic form of Hb H disease the α/β -chain production ratio ranged from 0.05 to 0.25 (mean 0.16)¹⁷. To determine whether the absence of α -chain synthesis was due to the absence of mRNA α or due to an abnormal mRNA α , mRNA was hybridised to purified complementary cDNAs as described previously¹⁸ (Fig. 1). At low mRNA/cDNA ratios the cDNA α probe hybridised to approximately 10%, which represents the hybridisation of contaminating cDNA β sequences. The cDNA α probe continued to hybridise at a slow linear rate up to the maximum level of 90% hybridisation at very high mRNA/cDNA ratios, indicating that a small amount of intact mRNA α sequences were present in the mRNA. The mRNA β /mRNA α ratio was calculated to be 65:1 and hence the amount of mRNA α relative to mRNA β was 1.5%.

To determine whether the virtual absence of mRNA α resulted from a transcriptional defect or a deletion of the α -globin genes in the malignant cell line, the hybridisation

kinetics of cDNA_α in cDNA excess with DNA from the erythroid bone marrow of WD were compared with those of normal DNA^{18,19}. Figure 2 shows that the cDNA_α probe gave a similar hybridisation curve with both DNA species indicating that α-globin sequences were present in the DNA to the same extent as normal DNA. But, the cDNA_α probe is less than half the length of the α-globin gene and thus a gene deletion beyond the region of DNA complementary to the cDNA_α probe would not be observed. To check that a gene deletion in the region of DNA complementary to the cDNA_α probe could be detected in our experimental conditions, cDNA_α was hybridised in cDNA excess to DNA prepared from the liver of an infant with the Hb Bart's hydrops syndrome^{3,4} and to a mixture of equal amounts of hydrops DNA and normal DNA (Fig. 2). The cDNA_α hybridised to the mixture to half the extent seen with normal DNA and hybridised to the hydrops DNA to a lesser extent than the mixed DNA, showing only a very small rise above the background value. The plateau value of the amount of cDNA hybridised to DNA in cDNA excess was used to calculate the number of globin genes hybridising to the cDNA_α. Table 1 lists the results for the hybridisation curves shown in Fig. 2, together with results of other cDNA excess hybridisations (not shown) conducted as control experiments. These results indicate that there is no major deletion of the α-chain genes in the DNA of the abnormal cell line of WD. Furthermore, they confirm previous observations^{3,4} that the α-globin gene sequences are largely deleted in the Hb Bart's hydrops syndrome, that is, the homozygous state for α thal 1. The observed small amount of hybridisation of hydrops DNA (0.2 genes) to the cDNA_α probe can be accounted for by contamination of the cDNA_α probe with about 10% cDNA_β.

The deficit of α-chain synthesis in this patient with acquired Hb H disease was much more marked than that which is found in the genetic form of the disease. Indeed, there was probably no α-chain synthesis in the neoplastic red cell line. Thus the pattern of haemoglobin synthesis was very similar to that observed in the Hb Bart's hydrops syndrome²¹ except that in this case

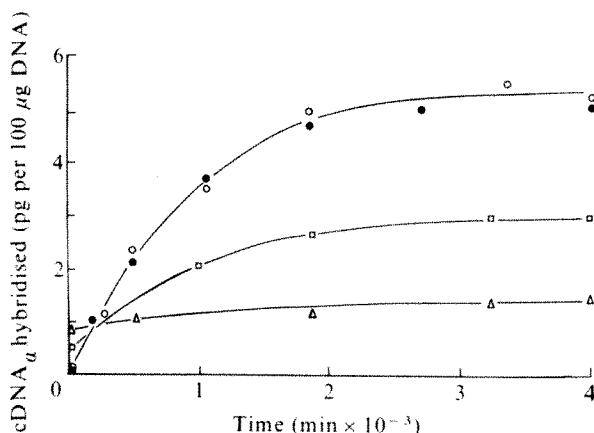


Fig. 2 Hybridisation of human globin cDNA_α in cDNA excess to DNA from the bone marrow of WD, DNA from normal human spleen and DNA from Hb Bart's hydrops foetalis liver. DNA was prepared from cell nuclei by hydroxylapatite chromatography²¹ and sonicated to 200–300 nucleotides in length. cDNA_α 20 pg was mixed with 100 μg of DNA in 20 μl of 0.12 M sodium phosphate, pH 6.8 and sealed in a siliconised glass capillary tube. The mixtures were denatured for 10 min at 100 °C and annealed at 66 °C. At the appropriate times, the amount of cDNA hybridised was determined after incubation with S₁ nuclease¹⁹. The percentage of cDNA hybridised was converted to pg of cDNA hybridised using the formula described by Bishop and Freeman²⁰. The cDNA concentration is corrected for the maximum amount of hybridisable cDNA. The figure shows the hybridisation of cDNA_α in cDNA excess to DNA from the bone marrow of WD (●), normal DNA (○), hydrops DNA (△) and a mixture (■) of equal amounts of normal DNA and hydrops DNA.

Table 1 No. of globin genes per haploid genome as determined by cDNA excess hybridisations

cDNA	DNA	cDNA hybridised (pg)	Gene complexity	Gene no.
cDNA _α	WD, blood	5.0	90,000	1.5
cDNA _α	WD, bone marrow	5.6	101,000	1.6
cDNA _α	Normal	5.4	97,000	1.6
cDNA _α	Normal/hydrops	2.4	43,000	0.8
cDNA _α	Hydrops	0.5	9,000	0.2
cDNA _β	WD, Blood	7.2	130,000	1.9
cDNA _β	WD, Bone marrow	7.6	137,000	2.0
cDNA _β	Normal	8.1	146,000	2.1

cDNA excess hybridisations were performed as described in Fig. 2 legend. The gene complexity is calculated from the plateau value pg of cDNA hybridised per 100 μg DNA using the formula described by Bishop and Freeman²⁰. The gene number is equal to the gene complexity divided by the cDNA complexity (60,000 for cDNA_α, 70,000 for cDNA_β.)

β-chains were being produced instead of γ chains. Furthermore, unlike the Hb Bart's hydrops syndrome, the α-globin genes were intact in the neoplastic cell line in which α-chain synthesis was abolished. These findings suggest that there must have been a defect in α-chain gene transcription affecting both sets of haploid α-chain genes. One possible explanation is that there are specific repressors of the α-chain loci which are normally active only in embryonic life before α-chain synthesis commences, and that these repressors had been activated in the leukaemic cell line. This seems to be the most likely type of mechanism which could produce a *cis-trans* effect as evidenced by the suppression of both pairs of α-chain genes. There are examples of reversion to foetal erythropoiesis in leukaemia²² although regression to an earlier stage has not been reported. The lack of synthesis of embryonic ζ and ε chains in the abnormal cell line is incompatible with there having been a complete reversion to embryonic haemoglobin synthesis; the genetic mechanism which inhibited α-gene transcription was highly specific for the α-chain loci.

We thank Drs A. Pollock and M. J. Lewis, General Hospital, Birmingham, for allowing us to study this patient, and Professor Paul Polani and Dr M. G. Daker of Guy's Hospital for chromosome analysis. The work was supported by grants from the MRC and CRC.

J. OLD
J. LONGLEY
W. G. WOOD
J. B. CLEGG
D. J. WEATHERALL

Nuffield Department of Clinical Medicine,
Radcliffe Infirmary,
Oxford, UK

Received 30 June; accepted 8 August 1977.

- Weatherall, D. J. & Clegg, J. B. *The Thalassemia Syndromes*, 2nd edn (Blackwell, Oxford, 1972).
- Weatherall, D. J. & Clegg, J. B. *Ann. Rev. Genet.* **10**, 157–78 (1977).
- Ottolenghi, S. *et al. Nature* **251**, 389–392 (1974).
- Taylor, J. M. *et al. Nature* **251**, 392–393 (1974).
- Kan, Y. W. *et al. Nature* **255**, 255–256 (1975).
- Weatherall, D. J. & Clegg, J. B. *Phil. Trans. R. Soc.* **271**, 411–455 (1975).
- Bergen, W. R. & Sturgeon, P. *Proc. 7th Int. Cong. Hematology*, 488 (Grune and Stratton, New York, 1960).
- White, J. C. *et al. Br. J. Haemat.* **7**, 171–177 (1960).
- Beaven, G. H., Stevens, B. L., Dance, N. & White, J. C. *Nature* **199**, 1297–1298 (1963).
- Rosenweig, A. I., Heywood, J. D., Motulsky, A. G. & Finch, C. A. *Acta haemat.* **39**, 91–101 (1968).
- Hamilton, R. W., Schwartz, E., Atwater, J. & Erslev, A. J. *New Engl. J. Med.* **285**, 1217–1221 (1971).
- Pagnier, J., Labie, D. & Schapira, G. *Biochimie* **55**, 1245–1252 (1973).
- Weatherall, D. J., Clegg, J. B. & Naughton, M. A. *Nature* **208**, 1061–1065 (1965).
- Weatherall, D. J., Clegg, J. B., Na-Nakorn, S. & Wasi, P. *Br. J. Haemat.* **16**, 251–267 (1969).
- Clegg, J. B. & Weatherall, D. J. *Nature* **215**, 1241–1243 (1967).
- Schwartz, E., Kan, Y. W. & Nathan, D. G. *Ann. N. Y. Acad. Sci.* **165**, 288–294 (1969).
- Pritchard, J., Longley, J., Clegg, J. B. & Weatherall, D. J. *Br. J. Haemat.* **32**, 473–486 (1976).
- Ottolenghi, S. *et al. Cell* **9**, 71–80 (1976).
- Old, J. *et al. Cell* **8**, 13–19 (1976).
- Bishop, J. O. & Freeman, K. B. *Cold Spring Harb. Symp. quant. Biol.* **38**, 707–716 (1974).
- Weatherall, D. J., Clegg, J. B. & Wong, H. B. *Br. J. Haemat.* **18**, 357–367 (1970).
- Weatherall, D. J. *et al. Nature* **257**, 710–712 (1975).

Polymerisation of haemoglobin SA hybrid tetramers

ERYTHROCYTE sickling and gelation of concentrated solutions of deoxyhaemoglobin (Hb) S ($\alpha_2\beta_2^{6Val}$) results from helical polymerisation of the tetramers, with a spatial orientation approximated by recent ultrastructural and optical studies. Earlier observations of the gelling behaviour of Hb S or Hb C Harlem ($\alpha_2\beta_2^{6Val, 73Asn}$) mixed with Hb A or other haemoglobins gave indirect evidence concerning intertetrameric contact sites, and we proposed that only one $\beta 6$ valine-determined site was active per tetramer, the other β chain providing different polymer contacts^{1,2}. Our arguments required that asymmetrical hybrids (for example $\alpha_2\beta^A\beta^S$) occurred in Hb mixtures; their long-suspected presence has recently been established³⁻⁵, but the dissociation equilibria by which these hybrids form (Fig. 1) hinders their isolation and direct testing of their ability to polymerise. To circumvent this difficulty we have prepared Hb SA hybrids cross-linked intratetramERICALLY to prevent dissociation, and have found them capable of polymer and gel formation quite similar to that of Hb S.

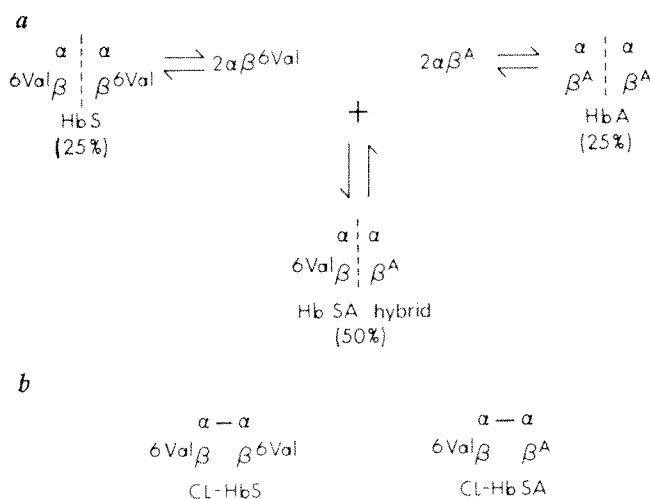


Fig. 1 Comparison of hybrid tetramer formation with cross-linked haemoglobins. *a*, Formation and distribution at equilibrium of hybrid and non-hybrid tetramers in a 1:1 mixture of Hb S and Hb A. *b*, Cross-linked Hb S and Hb SA hybrids as prepared in the present experiments; the covalent cross-link between the α -chain amino termini prevents dissociation into $\alpha\beta$ dimers, stabilising the SA hybrid.

Haemoglobin S and mixtures of equimolar amounts of Hb S and Hb A were each reacted with the bifunctional cross-linking reagent, difluorodinitrophenylsulphone, as described by Macleod and Hill⁶, and the cross-linked tetramers were separated from unreacted haemoglobin by chromatography on Sephadex G-100 in 1 M $MgCl_2$. Isoelectric focusing of the cross-linked tetramer fraction of reacted Hb S alone showed only one major haemoglobin band, whereas the corresponding tetramer fraction from the Hb S-Hb A reaction mixture showed three distinct bands which were stable in the liganded (CO) state. One of these three bands corresponded in position to the cross-linked (CL-) Hb S, another to CL-Hb A; midway between those two was a more prominent band, not seen with the fractions from Hb S or Hb A alone, which represented the hybrid CL-Hb SA ($\alpha_2\beta^S\beta^A$). Preparative isoelectric focusing permitted complete separation of the bands of interest (Macleod and Hill had obtained three subfractions by ion-exchange chromatography of the tetramer fraction of their Hb SA reaction mixture, and they identified the middle fraction as a true S-A hybrid⁴. We observed (as they did) incomplete separation of the three fractions by their method; we therefore used isoelectric focusing

on a preparative scale, which achieved complete separation of the three fractions with the corresponding charge differences. Migrating anodal to the well defined bands on each gel was a diffuse zone of Hb which probably represented a variety of other cross-linked reaction products⁶). Although the corresponding subfractions were shown by Macleod and Hill to be heterogeneous, including tetramers with unidentified cross-links between $\alpha\beta$ dimers together with the major product cross-linked between the α -chain amino termini, our present experiments required only the isolation of a stable non-dissociating $\alpha_2\beta^S\beta^A$ fraction free from $\alpha_2\beta_2^S$. (Some CL-Hb S was obtained from the major band of focused gels for which the starting material was Hb S alone rather than the Hb S-Hb A mixture. As noted, this major band coincided with the cathodal band on gels of the S-A mixtures. After the gelling experiments the CL-Hb S and CL-Hb SA hybrids were re-examined by isoelectric focusing each alone and both mixed together. Although all the steps in handling resulted in some charge-heterogeneity in each sample, none of the CL-Hb S bands co-focused with the CL-Hb SA bands, confirming the absence of contamination of CL-Hb SA by any detectable form of $\alpha_2\beta_2^S$.) The gel bands were sliced and the Hb fractions, eluted by the technique of Suzuki *et al.*⁷, and stored in the CO-form in liquid N_2 . When sufficient material was accumulated, the CL-Hb S and CL-Hb SA were converted from the CO-form to the cyanmet form (to permit rapid conversion by dithionite into deoxy Hb) and minimum gelling concentrations (MGCs) were determined in duplicate, as previously described¹ with the following minor modifications: 300- μ l aliquots of Hb solution were equilibrated with N_2 in 5 ml Erlenmeyer flasks, and the cyanmethaemoglobin was converted to the deoxy form by anaerobic addition of sodium dithionite (2 mol per mol total haem). Both the CL-Hb S and CL-Hb SA gelled, liquified on chilling the flask in an ice bath and gelled on subsequent re-warming to room temperature, as occurs with native Hb S. The MGC values and other details of the experimental conditions are shown in Table 1. Following gelation the chilled duplicate samples were combined and transferred anaerobically to centrifuge tubes under oil, allowed to gel again by equilibration at room temperature and centrifuged at 25 °C for 120 min at 122,000*g* (in a Beckman L3-50 centrifuge using an SW65K rotor) to sediment the solid phase. The supernatant concentrations, representing polymer solubility⁸, were 25.6 g Hb dl⁻¹ for CL-Hb S and 28.7 g Hb dl⁻¹ for CL-Hb SA. The supernatants were decanted and portions of the packed solid phases were fixed in deoxygenated solutions of 3% glutaraldehyde. After osmication and dehydration, the pellets were embedded in Epon 812. Examination of ultrathin sections by electron microscopy revealed 'polymer' rods in the solid phases of both CL-Hb S and CL-Hb SA which were irregularly distributed in all directions, measured 165-175 Å in diameter, and were indistinguishable from those seen by us and others in sections of deoxygenated sickle (SS) cells.

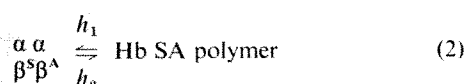
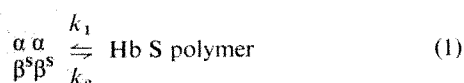
These findings show that the hybrid tetramers $\alpha_2\beta^S\beta^A$ are capable of gelation and polymerisation which is similar or identical to that of Hb S. Conformational changes in the cross-linked Hb, as indicated by its abnormal respiratory functions (high oxygen affinity and loss of cooperativity in oxygen binding)⁶ must account for the higher MGC value for CL-Hb S as compared with native Hb S. The CL-Hb S may, nevertheless, serve as a control for comparison with the gelling behaviour of the CL-Hb SA. Having established that only one

Table 1 MGC values of cross-linked Hb SA hybrids compared with cross-linked and native Hb S

Native Hb S ($\alpha_2\beta_2^S$)	22.2
CL-Hb S	27.3
CL-Hb SA ($\alpha_2\beta^S\beta^A$)	30.8

Haemoglobin dialysed against 0.15 M potassium phosphate, pH 7.35, deoxygenated with N_2 gas at 25 °C, sodium dithionite added; final pH of Hb 6.9-7.0; mean MGC values expressed in g Hb dl⁻¹.

β^S chain per tetramer is required for polymerisation, we predict that Hb SA hybrids would have a smaller tendency to polymerise than Hb S tetramers. Compared with Hb S, which should be capable of fitting into the helical polymer in either of two symmetrical orientations (with either β^S chain providing the intermolecular binding site determined by the β^6 valine substitution), the SA hybrids would be expected to exhibit some steric hindrance for entry into the polymer, resulting in $k_1 > h_1$ in the following equilibria



There is reason to believe that the non-S β chain (or non-'active' β^S chain) of a tetramer provides other contacts in the polymer involving the regions of β^73 (E 17) and β^121 (GH4), but not the N-terminal region^{9,10} so that the forces maintaining the Hb S and Hb SA polymers, reflected in k_2 and h_2 , may be similar. In this case, the differences we have observed in both the MGC and polymer solubility values for CL-Hb S and CL-Hb SA reflect the expected differences in k_1 and h_1 described above.

By mixing haemoglobins in the deoxy state or by covalently cross-linking Hb A or Hb F tetramers (by the methods of Macleod and Hill) to prevent hybrid formation in Hb mixtures, we have previously shown that the well known inhibitory effect of Hb F on gelation of Hb S requires formation of $\alpha_2\beta^S\gamma$ hybrid tetramers; in contrast, the presence or absence of hybrid formation in mixtures of Hb S with Hb A had no net effect on the minimum gelling concentration¹¹. Similar results and conclusions were subsequently described by Goldberg *et al.*¹² who centrifuged the gels as an assay of polymer solubility. The differences in MGC and polymer solubility values of CL-Hb S and CL-Hb SA observed in our study are qualitatively consistent with our proposed explanation for the equivalent gelling tendencies of mixtures of Hb S and Hb A in which hybrid formation was permitted or prevented¹¹.

It is not yet clear whether or not Hb tetramers containing no β^S chains, such as Hb A or Hb F, are capable of entering the polymer. Our previous results suggested that these non-S tetramers facilitated gelling in a less specific manner¹¹ but the available evidence concerning this issue is quite indirect, and partially conflicting¹². Our results provide direct evidence that Hb SA hybrids can enter the polymer in place of Hb S tetramers, but with somewhat less efficiency than Hb S.

This work was supported by grants from the US NIH and from the American Heart Association.

ROBERT M. BOOKCHIN
TANIA BALAZS
RONALD L. NAGEL
ISABEL TELLEZ

Division of Hematology,
Department of Medicine,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received 20 June; accepted 8 August 1977.

1. Bookchin, R. M. & Nagel, R. L. *J. molec. Biol.* **60**, 263-270 (1971).
2. Bookchin, R. M. & Nagel, R. L. in *Sickle Cell Disease, Diagnosis, Management, Education and Research* (eds Abramson, Bertles, J. F. & Wethers, D. L.) 140-154 (Mosby, St Louis, 1973).
3. Bunn, H. F. in *Hemoglobin and Red Cell Structure and Function* (ed. Brewer, G. J.) 41-53 (Plenum, New York, 1972).
4. Macleod, R. M. & Hill, R. J. *J. biol. Chem.* **248**, 100-103 (1973).
5. Park, C. M. *Ann. N.Y. Acad. Sci.* **209**, 237-257 (1973).
6. Macleod, R. M. & Hill, R. J. *J. biol. Chem.* **245**, 4875-4879 (1970).
7. Suzuki, T., Benesch, R. E., Yung, S. & Benesch, R. *Analyt. Biochem.* **55**, 249-254 (1973).
8. Magdoff-Fairchild, B., Poillon, W. N., Li, T. & Bertles, J. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 990-994 (1976).

9. Bookchin, R. M. & Nagel, R. L. *Semin. Hematol.* **11**, 577-595 (1974).
10. Nagel, R. L. & Bookchin, R. M. in *Sickle Cell Anemia and Other Hemoglobinopathies* (ed. Levere, R. D.) 51-65 (Academic, New York, 1975).
11. Bookchin, R. M., Nagel, R. L. & Balazs, T. *Nature* **256**, 667-668 (1975).
12. Goldberg, M. A., Husson, M. A. & Bunn, H. F. *J. biol. Chem.* **252**, 3414-3421 (1977).

The mechanism by which actinomycin D inhibits protein synthesis in animal cells

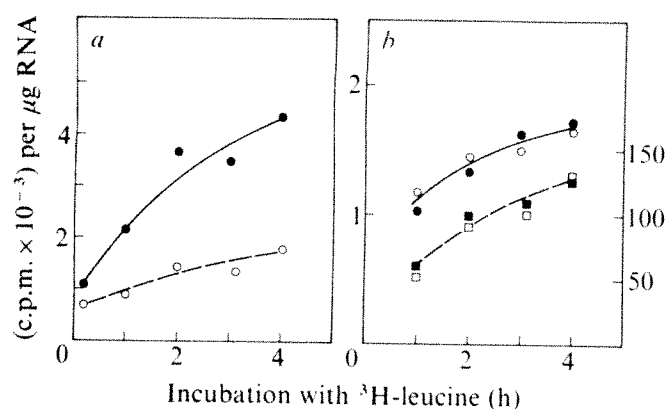
In the course of studies on the regulation of protein synthesis during the activation of human lymphocytes by phytohaemagglutinin (PHA), it became necessary to determine whether an effect which occurred following treatment with actinomycin D (AMD) was secondary to the action of the drug on transcription, or resulted from a direct effect on translation. Using enucleated lymphocytes, we have shown that AMD has no effect on protein synthesis in the absence of the cell nucleus and conclude that AMD interferes with protein synthesis by its effect on transcription of RNA.

AMD is a potent inhibitor of RNA synthesis in eukaryotic cells¹ and is widely used by molecular biologists. Protein synthesis diminishes in cells treated with AMD (refs 2, 3) and this was originally attributed to decay of messenger RNA (mRNA), but more recent findings have made that view untenable⁴⁻⁷. An additional effect of the drug, at the level of initiation of protein synthesis, has therefore been proposed, although the mechanism of this putative action has not been elucidated^{4,5}. In particular, it remains uncertain whether AMD inhibits protein synthesis by preventing the synthesis of some RNA component, other than mRNA, or by a direct action on the initiation of translation. Because of this uncertainty, the interpretation of much published data is subject to doubt.

To clarify this question, lymphocytes were enucleated by treatment with cytochalasin B followed by centrifugation in a density gradient as described by Wigler and Weinstein⁸. Lymphocyte cytoplasts produced in this way continued to incorporate ³H-leucine into acid-insoluble material for several hours (Fig. 1a). Since this incorporation was inhibited by puromycin (Fig. 1a), the action of which requires peptide bond formation⁹, we conclude that protein synthesis was occurring in cytoplasts by the normal mechanism. Cycloheximide, which inhibits protein synthesis by a different mechanism¹⁰, was also effective (results not shown). Cytoplasts prepared from lymphocytes previously stimulated by PHA for 16 h showed a ninefold higher rate of incorporation of amino acids than those from resting cells, reflecting the difference in protein synthetic activity commonly seen between resting and growing lymphocytes (Fig. 1b).

Addition of AMD to cytoplasts from either resting or growing lymphocytes had no effect on protein synthesis (Fig. 1b). Treatment of intact lymphocytes, either resting or growing, with AMD caused a diminution of protein synthesis, with progressive reduction in the incorporation of amino acids (Fig. 2a). Treatment of intact cells with cytochalasin B, in a manner similar to that used for producing cytoplasts, did not itself interfere with protein synthesis, nor did it prevent the inhibition of protein synthesis by AMD (Fig. 2b).

Inhibition of protein synthesis by AMD in resting and growing lymphocytes thus only occurs in nucleated cells. If a direct effect of AMD on translation occurred, it should be detectable in cytoplasts as well. Since it was not, we conclude that AMD inhibits protein synthesis in lymphocytes as a consequence of its well known action on nuclear RNA synthesis, and not by any direct action on translation. If the only action of AMD were functionally to enucleate the cell with respect to RNA synthesis, it follows that its effect on protein synthesis should be equivalent to mechanical enucleation. This hypothesis was



The upper layer of cytoplasts was aspirated, diluted with MEM and sedimented at 1,000g for 15 min. Cytoplasts were resuspended in MEM containing 1/20th the usual concentration of amino acids plus 2% autologous plasma, and incubated (37 °C) with ^3H -leucine (Schwarz-Mann, 50 Ci mmol^{-1} , 10 $\mu\text{Ci ml}^{-1}$). At the times indicated, duplicate 1.0-ml samples were applied to GF/C filters moistened with phosphate-buffered saline (PBS) containing unlabelled leucine and washed first with PBS-leucine under gentle suction, then with cold 5% trichloroacetic acid-leucine. After drying, radioactivity remaining on the filter was measured by liquid scintillation counting. The RNA content of the suspensions was measured by precipitation with cold 2% perchloric acid (PCA) (10 min), washing with cold 2% PCA, digestion in 10% PCA at room temperature for 17 h, removal of insoluble material by centrifugation and measurement of A_{260} , using $E_{1\%}^{1\text{cm}} = 320$. Results were expressed as c.p.m. μg^{-1} RNA. No correction was made for radioactivity in leucyl-tRNA, as previous experiments showed the contribution from this source to be negligible. *a*, Effect of puromycin on amino acid incorporation: ●, untreated cytoplasts from resting lymphocytes; ○, 100 $\mu\text{g ml}^{-1}$ puromycin added 5 min before addition of ^3H -leucine. *b*, Effect of actinomycin D on protein synthesis by cytoplasts: ●, resting-cell cytoplasts, no AMD; ○, resting-cell cytoplasts, 1 mg μl^{-1} AMD added with ^3H -leucine. ■, Cytoplasts from lymphocytes incubated 16 h with PHA (5 $\mu\text{g ml}^{-1}$), no AMD; □, growing-cell cytoplasts, 1 $\mu\text{g ml}^{-1}$ AMD added.

supported by a comparison between data from several experiments with cytoplasts and with AMD-treated intact cells (Fig. 2c). The two sets of data showed no significant difference in the kinetics which characterise the progressive fall in protein synthesis.

It has been reported previously that at least 50% of newly-synthesised mRNA (cytoplasmic RNA⁺ poly(A)) in resting lymphocytes is very short lived, with a half life of the order of 20 min, whereas the remainder of newly synthesised mRNA is highly stable, with a half life in excess of 20 h (ref. 11). Using this information, and assuming a minimum half life for stable mRNA of 20 h, it may be calculated that the steady-state level of labile mRNA in non-growing lymphocytes is on the order of 2% of the total, the remainder being highly stable. It is

likely, therefore, that AMD interferes with translation by abolishing the synthesis of some RNA species other than mRNA⁺ poly(A), since the small contribution of labile mRNA to the steady-state total would not account for the effect of the drug.

Lymphocyte protein synthesis is virtually unaffected by concentrations of AMD which specifically inhibit rRNA synthesis¹², and ribosomal 5S RNA synthesis is not abolished by such low concentrations of AMD, but its appearance in the cytoplasm is prevented¹³. Since this deficiency is not associated with diminished protein synthesis, it is evident that interruption in the supply of new 5S RNA to the cytoplasm is not the means by which higher concentrations of AMD inhibit protein synthesis. Craig³ dismissed reduced tRNA synthesis as a possible cause

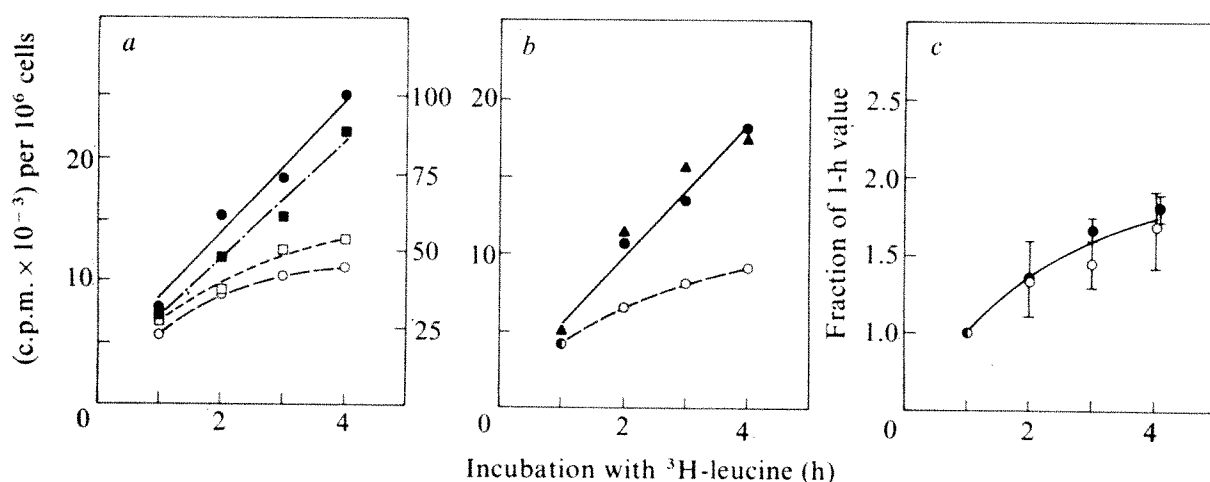


Fig. 2 Effect of actinomycin D on protein synthesis by intact lymphocytes. Lymphocytes were suspended at $2 \times 10^6 \text{ ml}^{-1}$ in MEM, 1/20th amino acid concentration, 2% autologous plasma and incubated with ^3H -leucine (10 $\mu\text{Ci ml}^{-1}$). Cell collection and analysis were as in Fig. 1. *a*, Effect of AMD on protein synthesis in resting and growing lymphocytes: ●, resting lymphocytes, no AMD; ○, resting lymphocytes, 1 $\mu\text{g ml}^{-1}$ AMD added at zero time; ■, PHA, 16 h before labelling, no AMD; □, PHA, 16 h, AMD, 1 $\mu\text{g ml}^{-1}$, added at zero time. *b*, Effect of cytochalasin pretreatment on protein synthesis by intact lymphocytes. Resting cells were incubated for 1 h with 10 $\mu\text{g ml}^{-1}$ cytochalasin B in MEM, 10% autologous plasma, then centrifuged and resuspended in labelling medium. ●, No CB treatment; ▲, CB-treated cells; ○, CB-treated cells, 1 $\mu\text{g ml}^{-1}$ AMD added with ^3H -leucine. *c*, Comparison of kinetics of amino acid incorporation in resting-cell cytoplasts with those of intact resting cells treated with AMD. Results from three experiments with intact cells and four experiments with cytoplasts were normalised by expressing results as fraction of 1-h value. Mean and ± 2 s.e.m. are given. ○, Cytoplasts; ●, AMD-treated resting cells.

for inhibition of protein synthesis in AMD-treated L cells because 8 h of exposure to $0.25 \mu\text{g ml}^{-1}$ AMD caused a 50% reduction in protein synthesis. This concentration of the drug was thought to be too low to inhibit tRNA synthesis. No actual data were given to demonstrate unimpeded tRNA synthesis, however, and in our view, tRNA has not been eliminated as the critical molecule whose synthesis, when interrupted by AMD, causes cessation of protein synthesis. This thesis is presently being examined, together with the more general proposition that availability of tRNA may have a regulatory role in governing the rate of protein synthesis in resting lymphocytes.

HERBERT L. COOPER
RICHARD BRAVERMAN

Section on Cellular and Molecular Physiology,
Laboratory of Pathophysiology,
National Cancer Institute,
Bethesda, Maryland 20014

Received 26 May; accepted 8 August 1977.

1. Reich, E., Franklin, R. M., Shatkin, A. J. & Tatum, E. L. *Proc. natn. Acad. Sci. U.S.A.* **48**, 1238–1245 (1962).
2. Penman, S., Scherrer, K., Becker, Y. & Darnell, J. E. *Proc. natn. Acad. Sci. U.S.A.* **49**, 654–662 (1963).
3. Craig, N., Kelley, D. E. & Perry, R. P. *Biochim. biophys. Acta* **246**, 493–498 (1971).
4. Singer, R. H. & Penman, S. *Nature* **240**, 100–102 (1972).
5. Craig, N. J. *cell. Physiol.* **82**, 133–150 (1973).
6. Greenberg, J. R. *Nature* **240**, 102–104 (1972).
7. Murphy, W. & Attardi, G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 115–119 (1973).
8. Wigler, M. H. & Weinstein, I. B. *Biochem. biophys. Res. Commun.* **63**, 669–674 (1975).
9. Nathans, D. *Fedn Proc.* **23**, 984–989 (1964).
10. McKeehan, W. & Hardisty, B. *Biochem. biophys. Res. Commun.* **36**, 625–630 (1969).
11. Berger, S. L. & Cooper, H. L. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3873–3877 (1975).
12. Kay, J. E., Leventhal, B. G. & Cooper, H. L. *Expl Cell Res.* **54**, 94–100 (1969).
13. Perry, R. P. & Kelley, D. E. *J. cell. Physiol.* **72**, 235–246 (1968).
14. Cooper, H. L. *Meth. Enzymol.* **32**, 633–636 (1974).
15. Burton, K. *Meth. Enzymol.* **12b**, 163–166 (1968).

Regulation of maternal mRNA translation in developing embryos of the surf clam *Spisula solidissima*

MESSANGER RNA not bound to ribosomes (non-polysomal mRNA) has been found in the cytoplasm of sea urchin^{1–3} and *Spisula solidissima* eggs⁴. The mRNA synthesised and stored during oogenesis (maternal mRNA) is utilised when protein synthesis is activated by fertilisation. Translation of maternal mRNA allows sea urchin embryos to proceed through several cellular divisions even when synthesis of embryonic mRNA is blocked by actinomycin D^{5–6}. But, embryonic mRNA is synthesised soon after fertilisation and already translated at the early cleavage stage⁷. Qualitative and quantitative changes in the pattern of histone synthesis during sea urchin embryogenesis have suggested that the synthesis of these proteins is temporally regulated in the embryo and that different mRNA species are translated at successive stages of embryo development⁸. We report here that part of the mRNA is found unassociated with ribosomes in eight-cell embryos of *S. solidissima*. Some of the translation products of this non-polysomal mRNA are different from those of polysomal mRNA, but identical to translation products of maternal mRNA isolated from eggs. This suggests that part of the non-polysomal mRNA of embryos consists of maternal mRNA species which are inefficiently translated after fertilisation.

Cytoplasmic extracts of *S. solidissima* embryos were fractionated by centrifugation on sucrose gradients (Fig. 1). The RNA isolated from the polysome pellet and from gradient fractions was translated in the wheat germ cell-free system⁹. Polysomal RNA and the RNA extracted from a cytoplasmic fraction sedimenting between 20S and 60S (non-polysomal mRNA) significantly stimulated protein synthesis and were

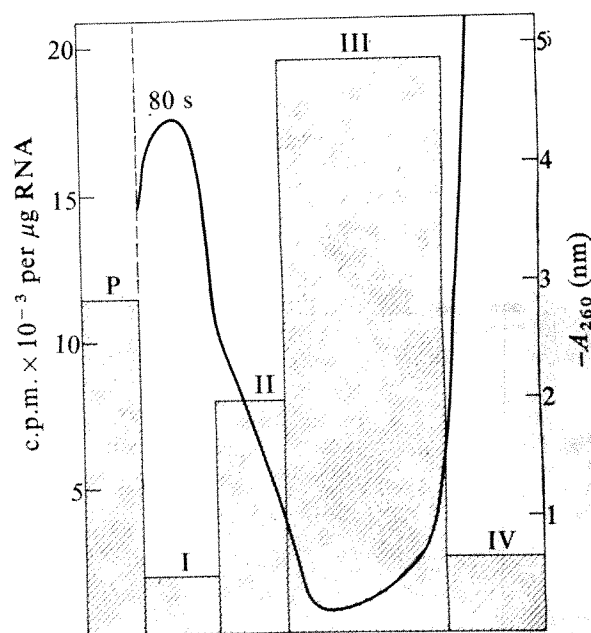


Fig. 1 Fractionation of *S. solidissima* eight-cell embryos homogenate by centrifugation on sucrose gradient. Eggs were collected, washed and fertilised⁴ and embryos were collected by centrifugation and washed as described before⁴. One volume of packed embryos was homogenised with 4 volumes of TNM (20 mM Tris-HCl pH 7.6, 0.2 M NaCl and 5 mM magnesium acetate). The homogenate was centrifuged for 10 min at 12,000 r.p.m. and 4.5 ml of the supernatant were fractionated by 19 h centrifugation at 26,000 r.p.m. through a 34-ml 20–40% sucrose gradient in TNM. The gradients were monitored for A_{260} (solid line) and subdivided into four fractions. Polysomes were recovered in the pellet. RNA was obtained from gradient fractions and from the polysome pellet by phenol extraction and ethanol precipitation¹⁷. The RNA was assayed in a wheat germ cell-free system prepared according to Roberts and Patterson⁹. Each fraction was tested at several RNA concentrations and the stimulation of protein synthesis measured by the incorporation of ³H-lysine into protein in 60-min incubations as previously described¹⁷. The activity of each fraction was calculated from a range of concentrations which gave a stimulation of protein synthesis proportional to the input of RNA. The activity of each fraction is expressed as c.p.m. $\times 10^{-3}$ per μg RNA. Twenty mg of RNA were recovered from the polysome pellet and 5.3 were recovered from fraction III per ml of packed embryos fractionated. Taking into account the relative stimulatory activity and the amount of RNA recovered, it was calculated that the total template activity of polysomal RNA was four times that of fraction III RNA.

directly used in subsequent experiments. About 80% of the template activity of embryo RNA was found in the polysome pellet, and the remaining 20% was found in the fraction sedimenting at 20–60S (Fig. 1).

The translation products of polysomal and non-polysomal embryo mRNA were compared with those of mRNA obtained from whole eggs and purified by absorption of oligo (dT)-cellulose¹⁰. The translation products were fractionated by electrophoresis on polyacrylamide gels containing sodium dodecylsulphate (SDS)¹¹ and detected by fluorography¹² (Fig. 2). The translation products of embryo polysomal mRNA correspond predominantly to marker histones. But, the products of embryo non-polysomal mRNA show in addition to histones some bands which are not detected among the translation products of polysomal mRNA (indicated by arrows in Fig. 2). The translation products of egg mRNA show two bands corresponding to histones H1, several poorly resolved bands migrating with or slightly faster than marker histones, and other bands corresponding to translation products of both polysomal and non-polysomal embryo mRNA. Aliquots of each translation assay were extracted with 0.2 M H_2SO_4 (ref. 13) to further purify histones. The proteins extracted with acid were fractionated by SDS gel electrophoresis (Fig. 2). This analysis showed that

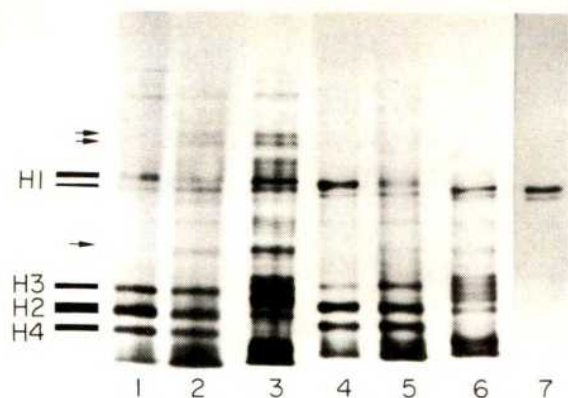


Fig. 2 Analysis of the translation products of polysomal and non-polysomal embryo RNA and of egg RNA. Embryo RNA was prepared as described in Fig. 1. RNA was obtained from eggs homogenised with 4 volumes of TNM, centrifuged 10 min at 12,000 r.p.m., made 1% in SDS and extracted with phenol¹⁷. The RNA was chromatographed on oligo(dT)-cellulose¹⁰ and the poly(A)-containing fraction used for translation assays. The RNA was translated with wheat germ extract containing 0.88 mCi ml⁻¹ of ³H-lysine (40 Ci mol⁻¹). Aliquots of each incubation were analysed by electrophoresis on 12.5% polyacrylamide gels containing SDS¹¹. The radioactive proteins were detected by fluorography of the dried gels¹². The position of marker histones from developing embryos⁴ is shown schematically on the left. Track 1, translation products of polysomal embryo RNA; 2, translation products of fraction III non-polysomal RNA (see Fig. 1); track 3, translation products of egg RNA. An aliquot of each assay was treated with 0.2 M H₂SO₄ to extract the acid-soluble proteins¹³, which were then precipitated with 4 volumes of ethanol and analysed in parallel. Tracks 4, 5 and 6, acid-soluble proteins prepared from the translation assays analysed in tracks 1, 2 and 3, respectively. Track 7, marker H1 histones purified from developing eight-cell embryos incubated with ¹⁴C-lysine⁴; the H1 histones were isolated by precipitating the other histones with 5% perchloric acid²¹. The arrows on the left indicate bands present among the translation products of non-polysomal embryo RNA and of egg RNA.

all the embryo and egg peptides co-migrating with marker histones are acid extractable. Among the egg mRNA translation products, histones H1 are clearly identified, whereas other histones are present in relatively lower amounts. In subsequent experiments egg and embryo RNA were fractionated in parallel into size classes by centrifugation on sucrose gradients (Fig. 3). The RNA sedimenting between 4 and 18S, which is enriched in histone mRNA¹, was isolated and translated in the wheat germ cell-free system. The translation products of 4–18S RNA were analysed by electrophoresis in a gel system devised for the fractionation of histones, which separates proteins primarily on the basis of their charge¹⁴. The two H1 histones, which separate in SDS gel electrophoresis, migrate together in this gel system (Fig. 3). The translation product of 4–18S egg RNA contain H1 histone and two additional peptides, which correspond to translation products of embryo mRNA. One peptide migrates like H4 histone and the other between marker H2A and H1 histones. This peptide also appears among the translation products of embryo non-polysomal mRNA, but not among the products of embryo 4–18S polysomal mRNA (Fig. 3). This peptide represents one further example of a maternal template which is found among non-polysomal mRNA species in the embryo.

The translation products of egg mRNA isolated by oligo(dT)-cellulose chromatography were also analysed in the gel system described above (Fig. 3). By overloading the gel it was possible to show that egg RNA codes for the other histones in addition to H1 histones. This suggests that mRNAs for all histones are present in *S. solidissima* eggs, though in quite different relative amounts, and that these mRNAs may contain poly(A) since they bind to oligo(dT)-cellulose. Histone mRNA obtained from *Xenopus*¹³ and *Triturus*¹⁵ eggs contain poly(A), whereas histone mRNA

obtained from mammalian cells does not contain poly(A)¹⁵ and does not bind to oligo(dT)-cellulose¹⁰.

The analysis of mRNA distribution in eight-cell embryos of *S. solidissima* shows that part of the mRNA is not associated with polysomes and therefore is not actively translated at this stage of development. We have only examined in detail the RNA distribution in eight-cell embryos. In older embryos there is less non-polysomal mRNA (our unpublished observations), whereas in eggs most of the mRNA is non-polysomal¹. A simple interpretation of these observations is that during embryonic development maternal mRNA is translated in an orderly fashion. In the eight-cell embryo some mRNA species are preferentially translated, whereas others are not translated and are therefore found as non-polysomal mRNA species. We have no evidence that these non-polysomal mRNA species are translated later in embryogenesis, though this seems in view of the decrease in non-polysomal mRNA. We suggest, however, that the presence of non-polysomal mRNA is due to translational regulation in eight-cell embryos. Translation of maternal and newly synthesised mRNAs may be regulated in a specific way. This regulation does not involve the activation of maternal templates by addition of the 5'-terminus m⁷Gppp (ref. 17), since *S. solidissima* egg mRNAs already contain this structure (unpublished).

Various mechanisms have been proposed to explain translational regulation (see ref. 18 for review). These mechanisms can be subdivided into two types: (1) regulation involves a change in the components of the translational machinery; (2) regulation results from changes in the species of mRNA present and/or in the activity of the cell to initiate synthesis of new polypeptides. Our experiments have not been directed at studying the mechanisms of translational regulation operating in embryos. An analysis of the events which take place on fertilisation makes it possible, however, to propose an interpretation of the regulatory pattern observed.

The predominant templates translated by embryos are histone mRNAs (Fig. 1). These templates are present in relatively small amounts among maternal mRNA species, with the exception of H1 histone mRNA⁴. Active synthesis of histone mRNA on fertilisation is necessary to account

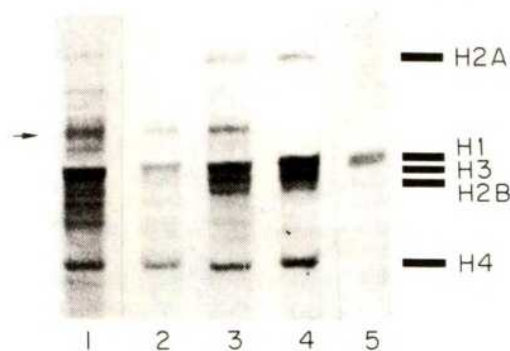


Fig. 3 Analysis of the translation products of embryo and egg RNA. The translation assays are described in Figs 1 and 2. Proteins were fractionated on 12% polyacrylamide gels containing 7.5 M urea, 6 mM Triton X-100, and 0.9 M acetic acid¹⁴. The proteins were detected by fluorography¹². Tracks 1 and 3, translation products of egg RNA containing poly(A) and of fraction III non-polysomal embryo RNA (see Fig. 1). The egg RNA and polysomal embryo RNA were fractionated by centrifugation on sucrose gradients^{1,17} and the fractions containing RNA sedimenting between 4S and 18S pooled. Track 2, translation products of 4–18S egg RNA; track 4, translation products of 4–18S polysomal embryo RNA. Track 5, marker H1 histones prepared as described in Fig. 2. The arrow at the right indicates a prominent band which is found among the translation products of egg 4–18S and of embryo non-polysomal RNA. The position of marker histones prepared from developing embryos⁴ is indicated on the right.

for the synthesis of histones in embryos. Synthesis of mRNA with the sedimentation characteristics of histone mRNA has indeed been reported in *S. solidissima* embryos¹⁹. The presence of histone mRNA among the non-polysomal mRNA species (Fig. 2) suggests that the supply of mRNA exceeds the capacity of the eight-cell embryos to translate this mRNA. This situation leads to competition among different mRNA species for translation. Histone mRNA is one of the 'strongest' mRNA molecules. Treatments of HeLa cells which result in competition among mRNAs for translation suppress the synthesis of most cellular proteins except histone²⁰. The translational regulation observed in *S. solidissima* embryos may be based on a similar mechanism. Initiation may be the limiting step in protein synthesis in embryos and this may lead to competition among different mRNAs for translation. Histone mRNA may therefore be preferentially translated over other 'weaker' mRNAs, which are presumably translated later in development, when initiation is no longer limiting. This regulatory model does not involve any qualitative change in the components of the translational machinery, but only an increase in the activity of embryos to initiate protein synthesis.

This work was supported by grants from the USNIH and the Italian National Research Council. We thank J. V. Ruderman for helpful discussions, and C. Blum, C. Magarian, A. Ruppel, L. Weber and C. Wholers for technical assistance. F.G. was supported by an EMBO short-term fellowship and by a NATO senior short-term fellowship.

FRANCO GABRIELLI
CORRADO BAGLIONI

Institute of Biological Chemistry,
University of Pisa, Italy
and
Department of Biological Sciences,
State University of New York at Albany,
Albany, New York 12222

Received 28 April; accepted 13 August 1977.

- Gross, K. W., Jacobs-Lorena, M., Baglioni, C. & Gross, P. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2614-2618 (1973).
- Skoultschi, A. & Gross, P. R. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2840-2844 (1973).
- Farquhar, M. N. & McCarthy, B. J. *Biochem. biophys. Res. Commun.* **53**, 515-522 (1973).
- Gabrielli, F. & Baglioni, C. *Dev. Biol.* **43**, 254-263 (1975).
- Gross, P. R. & Cousineau, G. H. *Expl. Cell Res.* **33**, 368-395 (1964).
- Gross, P. R. in *Current Topics in Developmental Biology* 2 (eds Moscona, A. A. & Monroy, A.), 1-46 (Academic, New York, 1967).
- Humphreys, T. *Dev. Biol.* **26**, 201-208 (1971).
- Ruderman, J. V. & Gross, P. R. *Dev. Biol.* **36**, 286-298 (1975).
- Roberts, B. E. & Paterson, B. M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2330-2334 (1973).
- Pemberton, R. E., Liberti, P. & Baglioni, C. *Analyt. Biochem.* **66**, 18-28 (1975).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).
- Levenson, R. G. & Marcu, K. B. *Cell* **9**, 311-322 (1976).
- Alfagame, C. R., Zweidler, A., Mahowald, A. & Cohen, C. H. *J. biol. Chem.* **249**, 3729-3733 (1974).
- Ruderman, J. V. & Pardue, M. L. *J. cell. Biol.* **70**, 8a (1976).
- Adesnik, M. & Darnell, J. E. *J. molec. Biol.* **67**, 397-406 (1972).
- Hickey, E. D., Weber, L. A. & Baglioni, C. *Nature* **261**, 71-73 (1976).
- Lodish, H. F. *A. Rev. Biochem.* **45**, 39-72 (1976).
- Firtel, R. A. & Monroy, A. *Dev. Biol.* **21**, 87-104 (1970).
- Stahl, H. & Gallwitz, D. *Eur. J. Biochem.* **72**, 385-392 (1977).
- Johns, E. W. *Biochem. J.* **92**, 55-59 (1964).

Variability of DNA content of murine fibrosarcoma cells

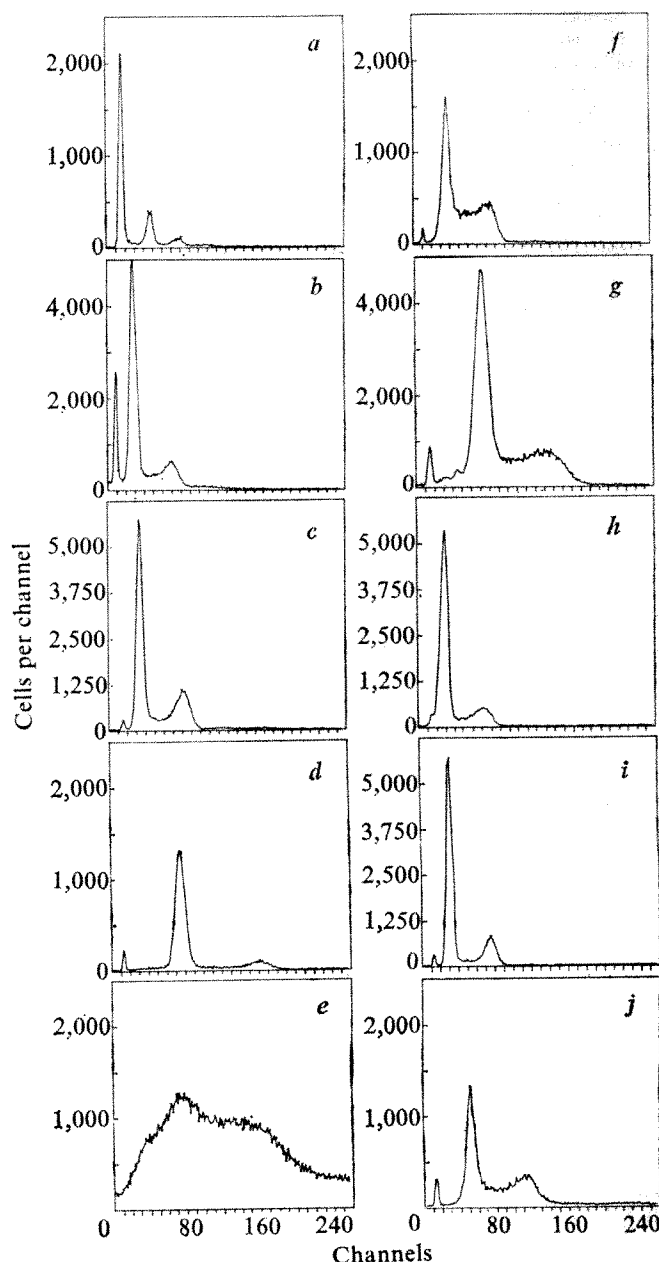
ANEUPLOIDY or heteroploidy and hyperploidy have been observed in tumours of experimental animals and of humans and seem to be a feature of most malignant cells¹⁻⁹. DNA contents seem to correlate with karyotype in these changes or deviations from euploidy. Most tumours are hyperploid and higher in DNA content^{2,8,9}. A rapid and reliable method of analysis of DNA content distribution of a cell population has recently become available with the development of flow microfluorometry (FMF)¹⁰. We report here the use of FMF analysis to measure

DNA content of clones isolated from a fibrosarcoma. All the clones examined had a higher DNA content than did either the normal cells tested or the original fibrosarcoma. There is thus a variability of DNA content in fibrosarcoma cells. This suggests that variability, as well as increase in DNA content in malignant cells may relate to their neoplastic development.

The clones FSA 1231, 1233, 1235, 12310, 12313, 12314, 12316, 12317, and 12318 were isolated from a methycholanthrene-induced fibrosarcoma by repeated cloning in soft agar medium¹¹ under selective pressure for enhanced clonogenicity (N.S. and H.R.W. *J. natn. Cancer Inst.* in the press). The surviving fraction of the original tumour cells was 10^{-7} to 10^{-6} , compared with 0.1 to 0.5 for the resulting highly clonogenic cells. The cells were cultured in a humidified CO₂ incubator with McCoy's 5A medium as modified by Hsu and containing 20% foetal calf serum.

The method of FMF analysis has been described elsewhere¹²⁻¹⁴. Briefly, 2 to 5×10^6 exponentially growing cells or

Fig. 1 Histogram of DNA content distribution obtained using flow microfluorometry. FSA 1231 (c), 1233 (d), 12310 (e), 12313 (f), 12314 (g), 12316 (h), 12317 (i), 12318 (j) were added with normal spleen cells. The spleen peak of FSA 12316 is seen only as a notch, since it includes only a small amount of spleen cells. This was done to avoid an effect on the G₁ peak of the sample. a. Spleen cells; b. FSA original cells.



tumour cells were fixed with 70% ethanol and stained with mithramycin (Pfizer) for DNA. The staining solution contained 50 $\mu\text{g ml}^{-1}$ mithramycin and 7.5 mM MgCl_2 in 12.5% aqueous ethanol. The flow microfluorometer was of the type described by Steinkamp *et al.*¹⁰

Quantitation and comparison of DNA content were carried out by adding spleen cell suspensions to each sample. The spleen cell suspensions were made from normal untreated $\text{C}_3\text{Hf/Bu}$ mice from the specific pathogen-free breeding colony maintained at the M. D. Anderson Hospital, Section of Experimental Radiotherapy. The minced spleen tissues were filtered through a stainless steel mesh (200 wires per inch) screen and washed by centrifugation. The suspension included singlet, doublet and triplet aggregates of spleen cells (Fig. 1). When the relative DNA contents for singlets, doublets and triplets were assigned values of 1, 2 and 3, and were plotted on a linear scale against channel number, a straight line relationship was observed. The line extrapolated across the abscissa at a certain point. This intercept value (channel number) was used to correct zero offset.

Figure 1 represents typical histograms of DNA content in various clones. Spleen cells were added as a standard to each sample except that from the original fibrosarcoma (tumour cell suspension) and FSA 12310. The position of the peak for normal cells in the original fibrosarcoma was not affected by addition of spleen cells although its height was increased, that is, DNA content of normal cells (G_1 peak) in a tumour was the same as that of spleen cells. Intrinsic normal cells were therefore used as a standard. G_1 peak positions of these clones were different from one another. Furthermore, FSA 12310 was completely heterogeneous with respect to DNA content.

Table 1 shows the relative DNA content (G_1 peak) of the various clones relative to spleen cells. These ratios were calculated from the modal G_1 peak positions after correction for zero offset. Modal peak positions were determined from digital records of cell number for each channel. Relative DNA content ranged between 1.49 and 3.07, while the relative DNA content of the original fibrosarcoma was 1.45. All clones so far examined have shown a relative DNA content greater than 1.0, that is, they contained more DNA than did normal cells. DNA content in all except FSA 12310 has remained stable through cell divisions after *in vitro* and *in vivo* growth suggesting a hereditary nature of the DNA content.

The coefficients of variation (c.v., 100 times the standard deviation divided by the mean) of G_1 peaks shown in Fig. 1, were 7.7 (FSA original), 6.8 (FSA 1231), 6.1 (FSA 1233), 8.8 (FSA 12313), 8.5 (FSA 12314), 9.7 (FSA 12316), 6.0 (FSA 12317), 7.3 (FSA 12318), and 5.8 ± 0.3 (spleen cells, \pm s.e. of the mean of eight measurements). The c.v. values of G_1 peaks of the original fibrosarcoma and of these clones were relatively large compared with that of normal spleen cells. The difference between some clones (FSA 1233 and FSA 12317) and spleen cells is not obvious, however.

Kramer *et al.* have investigated DNA content distribution of various established cell lines *in vitro* using FMF^{15,16}. They used c.v. of the G_1 peak to evaluate the heterogeneity of DNA content distribution in cell populations. They reported that DNA content by modal peak position of malignant cells was higher than that of normal cells. Surprisingly, however, the c.v. of the G_1 peak was not greater for malignant than for normal cells, even if a heterogeneity in the karyotype was observed. Thus, they concluded that DNA content distributions of malignant cells were not heterogeneous even if the karyotypes were, and that a change in karyotype does not always reflect an alteration in the DNA content of the cell. This conclusion challenges the classical view of the genetic entity of the chromosome.

We examined, using freshly isolated clones from a fibrosarcoma, whether DNA content of malignant cells varies and/or is greater than that in normal cells. In summary, our results lead us to conclude that these clones from a mouse fibrosarcoma were heterogeneous in DNA content implying that the cells had the ability to vary DNA content. All the clones derived by selective cloning and examined thus far have had a higher DNA content than the original fibrosarcoma cells. The results concerning DNA

Table 1 Relative DNA content of G_1 peaks of various clones

Cells	Relative DNA content	Mean \pm s.e.
FSA original	1.45	—
FSA 1231	1.61, 1.50	1.56 \pm 0.05
FSA 1233	3.03, 3.10	3.07 \pm 0.03
FSA 1235	1.80	—
FSA 12312	1.60	—
FSA 12313	1.93, 2.00, 1.90	1.94 \pm 0.03
FSA 12314	2.84, 2.87, 2.80	2.84 \pm 0.02
FSA 12316	1.50, 1.44, 1.52	1.49 \pm 0.02
FSA 12317	1.62	—
FSA 12318	2.39	—

Relative DNA content is given relative to the spleen singlet peak (G_1) which was assigned a value of 1.

content from this system are consistent with the accumulated findings of aneuploidy and hyperploidy in karyotypes of malignant cells¹⁻⁷. Since higher cellular DNA content was found in clones selected for higher plating efficiency in adverse growth conditions *in vitro*, changes in ploidy during tumour progression¹⁻⁷ may reflect a similar selection process in the host.

We thank Dr B. Barlogie for supplying mithramycin, Ms N. Hunter for technical assistance, Ms R. Goddard for secretarial work, Drs T. C. Hsu and M. L. Meistrich for discussions and Dr D. J. Grdina for critical reading of the manuscript. This study was supported by US NIH-NCI grants CA-06294 and CA-11138.

N. SUZUKI
H. R. WITHERS

Section of Experimental Radiotherapy,
The University of Texas System Cancer Center,
M. D. Anderson Hospital and Tumor Institute,
6723 Bertner Avenue,
Houston, Texas 77030

L. Y. LEE

Department of Physics,
University of Houston,
Houston, Texas 77004

Received 9 May; accepted 22 July 1977.

- Foulds, L. *Neoplastic Developments* 1, 439 (Academic, London & New York, 1969).
- German, J. (ed.) *Chromosomes and Cancer* 756 (Wiley, New York, 1974).
- Hauschka, T. S. *Cancer Res.* **21**, 957-974 (1961).
- Hsu, T. C. *Int. Rev. Cyt.* **12**, 69-161 (1961).
- Makino, S. *Int. Rev. Cyt.* **6**, 26-84 (1957).
- Mitelman, F. *Heredity* **70**, 1-14 (1972).
- Nowell, P. C. *Science* **194**, 23-28 (1976).
- Atkin, N. B., Richards, B. M. & Ross, A. J. *Br. J. Cancer* **13**, 773-787 (1959).
- Stich, H. F. *J. natn. Cancer Inst.* **24**, 1283-1297 (1960).
- Steinkamp, J. A. *et al. Rev. Scient. Instrum.* **44**, 1031-1310 (1973).
- Suzuki, N. & Okada, S. *Mutat. Res.* **34**, 489-506 (1976).
- Barlogie, B. *et al. Cancer Res.* **36**, 1176-1181 (1976).
- Crissman, H. A. & Tobey, R. A. *Science* **184**, 1297-1298 (1974).
- Grdina, D. J., Linde, S. & Mason, K. *Br. J. Radiol.* (in the press).
- Kraemer, P. M., Petersen, D. F. & Van Dilla, M. A. *Science* **174**, 714-717 (1971).
- Kraemer, P. M., Deaven, L. L., Crissman, H. A. & Van Dilla, M. A. *Adv. cell. molec. Biol.* **2**, 47-108 (1972).

Use of coupled transcription and translation to study mRNA production by vaccinia cores

VACCINIA is a double-stranded DNA virus which replicates in the cytoplasm of animal cells. Soon after infection, the outer layers of the virion are removed to yield core particles¹ which make mRNA that is transcribed, capped² and polyadenylated³ by enzymes present in the cores. Later, the cores break down, DNA synthesis begins, and 'late' genes are expressed⁴. Cores can be made *in vitro* by treating virions with non-ionic detergent and mercapto-ethanol. When incubated with nucleoside triphosphates, these particles produce mRNA⁵ which can be translated in cell-free systems to yield authentic vaccinia early proteins^{3,6,7}. It has recently been suggested that this mRNA may be derived from large primary transcripts⁸ which are processed in a manner similar to that proposed for cellular mRNA^{9,10}. I have obtained evidence that early vaccinia

mRNA is made by monocistronic transcription *in vitro*, by comparing the relative sensitivity of the synthesis of individual messages to ultraviolet irradiation. Production of mRNA was assayed indirectly using a coupled cell-free transcription-translation system in which protein synthesis is dependent on added vaccinia cores.

The use of partial ultraviolet inactivation to study transcription depends on the observation that the thymine dimers produced by irradiation cause premature termination of transcription¹¹. Assuming that all DNA is equally susceptible to ultraviolet damage, the sensitivity of synthesis of a given mRNA is proportional to the distance between its promoter and the 3' end of the cistron. In the case of messages which are primary transcripts, the ultraviolet sensitivity should be directly proportional to the length of the mRNA. This would clearly not be the case if several mRNAs were transcribed in tandem from a single promoter. Polycistronic transcription of the latter kind has been detected by ultraviolet irradiation experiments in DNA bacteriophages^{12,13}, adenovirus¹⁴, and the negative strand RNA viruses, vesicular stomatitis virus (VSV)^{15,16} and Sendai¹⁷.

The coupled transcription-translation system used in these experiments is based on the nuclease-treated reticulocyte lysate described previously¹⁸. Fig. 1 shows that when vaccinia cores are added to this system at concentrations similar to those found in infected cells, rapid synthesis of RNA begins after a brief lag. Protein synthesis starts after a further 5–10 min and continues linearly for

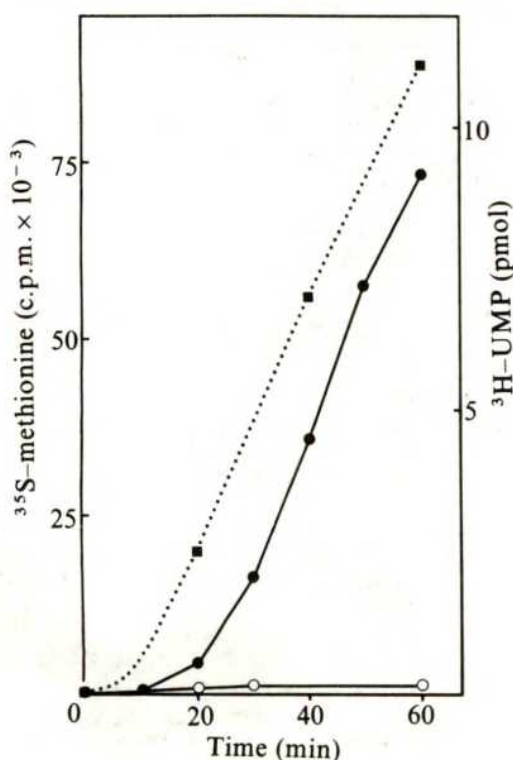


Fig. 1 RNA and protein synthesis directed by vaccinia virus cores in the nuclease-treated reticulocyte lysate. mRNA-dependent reticulocyte lysate prepared as described previously¹⁸ was supplemented by addition of 1 mM each of ATP, GTP, and CTP, 3.5 mM MgCl₂, 60 µg ml⁻¹ mouse liver tRNA, 2 mM glucose and either 20 µM methionine and 50 µM ³H-UTP (10.2 Ci mmol⁻¹) or 1 mM UTP and ³⁵S-methionine (4 × 10⁶ c.p.m. ml⁻¹; about 30 Ci mmol⁻¹ taking into account the unlabelled methionine present in the lysate). Cores, prepared as in ref. 5, were kindly provided by Jane Sykes, and were added at approximately 1.5 × 10¹⁰ ml⁻¹. Samples (5 µl) were removed at intervals for the assay of RNA synthesis by precipitation with cetyltrimethylammonium bromide (CTAB)²¹, or for protein synthesis as described previously¹⁸. Incubation temperature was 30 °C. ■, ³H-UTP incorporation; ●, ³⁵S-methionine incorporation; ○, ³⁵S-methionine incorporation in the presence of 7 µg ml⁻¹ actinomycin D.

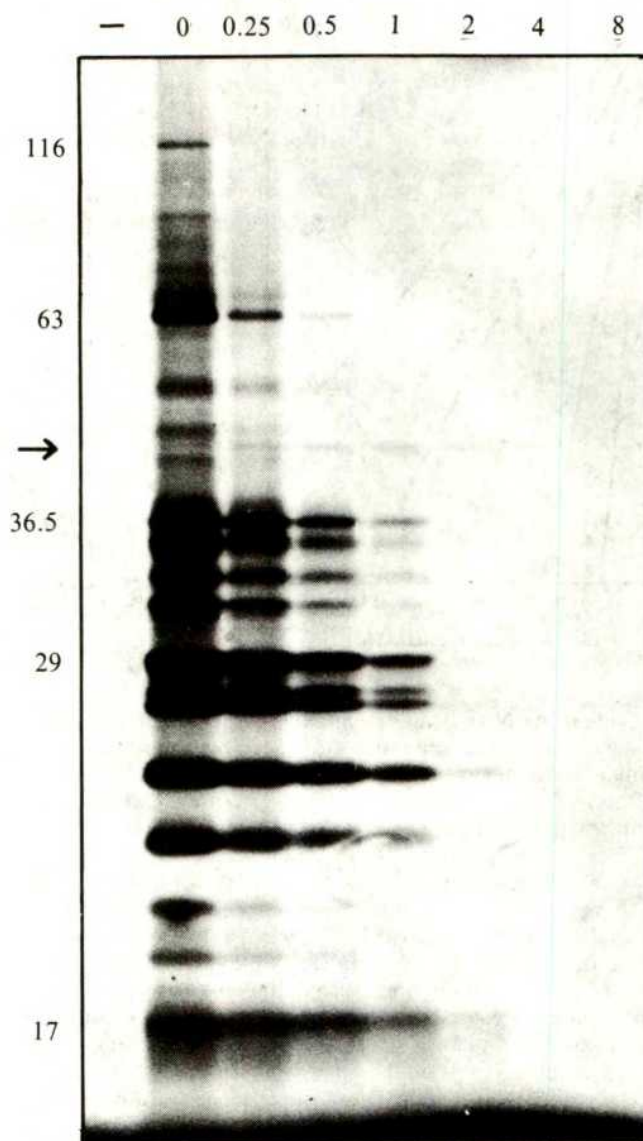


Fig. 2 Protein synthesis directed by ultraviolet-irradiated vaccinia cores. Drops (1 µl) containing (2–3) × 10⁸ vaccinia cores were placed on a sheet of parafilm and held on ice 30 cm from a 15-W germicidal ultraviolet lamp for various times. They were then added to 15 µl of the mixture described in Fig. 1, and incubated at 30 °C. Double-stranded RNA was present at a concentration of 20 µg ml⁻¹ to avoid inhibition of protein synthesis by low levels of endogenous double-stranded RNA present in the core preparations (see ref. 22). After 1 h of incubation, a further 10 µl of translation mixture was added, and the incubation continued for another 1 h. Samples (5 µl) were analysed on a 15% SDS-polyacrylamide gel. A fluorogram of this gel is shown. The molecular weights (× 10⁻³) indicated in the margin were derived by comparison with a set of ¹⁴C-iodoacetate-labelled marker proteins run in parallel with the experimental samples. Arrow indicates a background band which is labelled even in the absence of cores. The sample in the left-hand track contained no cores; others contained cores irradiated for the number of minutes indicated.

about 1 h. About 30–40 discrete polypeptides are made, many of which comigrate with authentic early vaccinia proteins on two-dimensional polyacrylamide gels of the O'Farrell type¹⁹ (data not shown). This protein synthesis is completely prevented by 7 µg ml⁻¹ actinomycin D, whereas the translation of globin or preformed vaccinia mRNA is unaffected by the drug. Protein synthesis in the coupled system is therefore dependent on the transcription of vaccinia DNA. Coupling RNA and protein synthesis in this way avoids the need for an intermediate purification of the RNA, thus eliminating errors due to unequal recovery of different messages. The direct study of

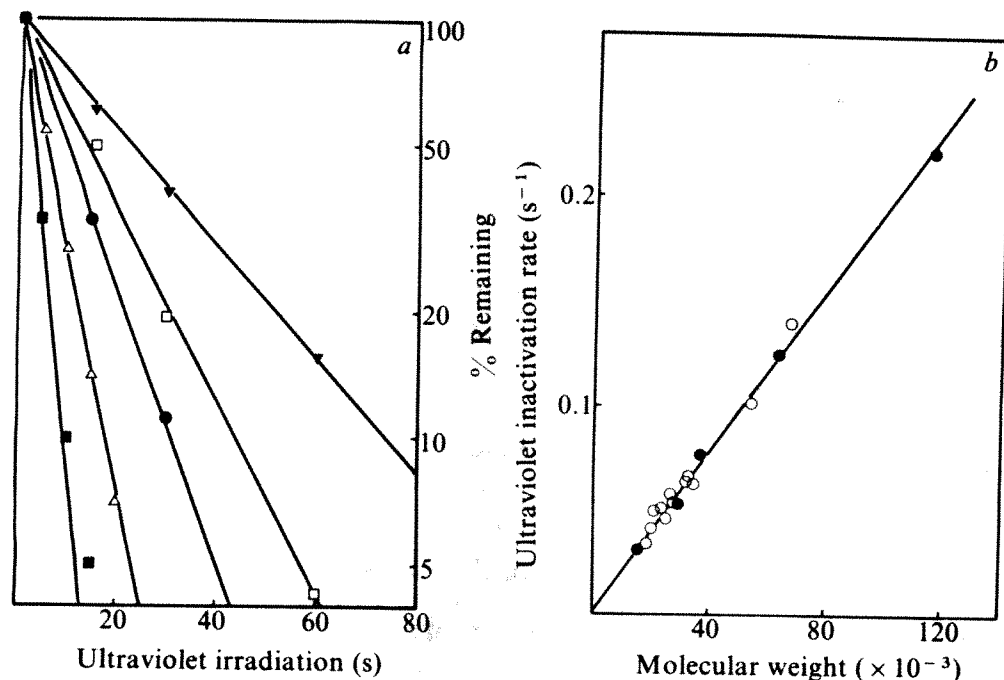


Fig. 3 Rate of ultraviolet light inactivation of synthesis of vaccinia proteins. *a*, Relative amount of radioactivity in five representative polypeptides plotted against the time of exposure of the cores to ultraviolet irradiation. This was determined by densitometry of appropriately exposed fluorograms such as that shown in Fig. 2, and is expressed as a percentage of the radioactivity obtained with non-irradiated cores. Molecular weights of these proteins: \square , 116,000; \triangle , 63,000; \bullet , 36,500; \square , 29,000; ∇ , 17,000. *b*, Plot of 17 first-order rate constants, determined from plots such as those in *a*, against the molecular weights of the corresponding proteins. Solid symbols refer to the five proteins shown in *a*. These figures were compiled from data obtained in two independent experiments.

individual mRNAs is made difficult by their poor resolution on gradients and gels, due to a combination of their similar sizes and their heterogeneous poly(A) tails.

Vaccinia cores were exposed to a germicidal ultraviolet lamp for periods ranging from 5 s to 8 min, and then added to the cell-free system. After incubation for 1 h, fresh lysate was added, and incubation continued for a further 1 h. In these conditions the mRNA produced does not saturate the system, so that competition between mRNAs is avoided. The protein products were analysed on SDS-polyacrylamide gels, and their synthesis measured either by densitometry of appropriately exposed fluorograms²⁰, or by direct counting of excised protein bands. The results are shown in Figs 2 and 3. The decay of the synthesis of each protein obeyed single-hit kinetics, but the rate constant varied according to the protein (Fig. 3a). Fig. 3b plots the rate of inactivation of the synthesis of the 17 most prominent proteins against their molecular weights, which were estimated relative to a set of marker proteins run on the same gel. Thirteen of these bands, including the largest at 116,000, comigrate on SDS gels with proteins labelled in infected cells; at least four correspond to major polypeptides which comigrate on two-dimensional gels with authentic vaccinia-induced proteins (H. R. B. Pelham, unpublished). The other bands are probably not the result of premature termination of translation; analysis of partial digests of the products after labelling with formyl-³⁵S-methionine indicates that they do not share common N termini (H. R. B. Pelham, unpublished).

There is a linear relationship between the molecular weight of the proteins made *in vitro* and the rate of ultraviolet inactivation of their synthesis (Fig. 3b), which argues strongly against tandem polycistronic transcription of their mRNAs. If such transcription were happening, there would be proteins whose synthesis was much more sensitive to ultraviolet light than would be expected from their size. This experiment does not exclude the possibility that the mRNAs are derived from longer monocistronic precursors whose size is directly proportional to the size of the coding regions. This seems intrinsically unlikely, and we have failed to detect any large core-associated precursors to mRNA by pulse-labelling experiments. Such experiments would not, however, detect precursors which were cleaved while still nascent. An argument in favour of the idea that the mRNAs synthesised by vaccinia cores represent primary transcripts has been made by Ball and White¹⁵. Unlike VSV,

vaccinia cores seem to be unable to add caps to the ends of RNA molecules generated by cleavage. Since, as I shall show elsewhere, vaccinia mRNA has a strong requirement for capped 5' ends for its translation, it is difficult to accept that messages produced by a cleavage pathway could be active.

These arguments and the data presented above strongly suggest that the major mRNA species produced by vaccinia cores are uncleaved transcripts of single cistrons, modified by the addition of poly(A) at the 3' end and a cap at the 5' end.

The coupled system used in this work is the first example of a eukaryotic cell-free system in which efficient RNA and protein synthesis are directed by a DNA template. It thus offers an *in vitro* analogue of infected cells, and should be useful for studying further aspects of gene expression in vaccinia virus.

I thank Jane Sykes for supplying the vaccinia cores, and Tim Hunt for help in preparing the manuscript. I am the holder of an MRC studentship, and the work was supported by a grant from the MRC to Dr R. J. Jackson.

HUGH R. B. PELHAM

Department of Biochemistry,
University of Cambridge,
Tennis Court Road,
Cambridge, UK

Received 16 June; accepted 4 August 1977.

- Dales, S. J. *Cell Biol.* **18**, 51-72 (1963).
- Moss, B., Gershowitz, A., Wei, C.-M. & Boone, R. *Virology* **72**, 341-351 (1976).
- Nevins, J. R. & Joklik, W. K. *Virology* **63**, 1-14 (1975).
- Joklik, W. K. *A. Rev. Microbiol.* **22**, 359-390 (1968).
- Kates, J. & Beeson, J. *J. molec. Biol.* **50**, 1-18 (1970).
- Fournier, F. *et al.* *FEBS Lett.* **30**, 268-272 (1973).
- Jaureguierry, G., Ben-Hamida, F., Chapeville, F. & Beaud, G. *J. Virol.* **15**, 1467-1474 (1975).
- Paoletti, E. *J. biol. Chem.* **252**, 866-871 & 872-877 (1977).
- Lewin, B. *Cell* **4**, 11-20 & 77-93 (1975).
- Molloy, G. R., Jelinek, W., Salditt, M. & Darnell, J. E. *Cell* **1**, 43-53 (1974).
- Michalke, H. & Bremer, H. *J. molec. Biol.* **41**, 1-23 (1969).
- Hercules, K. & Sauerbier, W. *J. Virol.* **12**, 872-881 (1973).
- Bräutigam, A. R. & Sauerbier, W. *J. Virol.* **13**, 1110-1117 (1974).
- Goldberg, S., Weber, J. & Darnell, J. E. *Cell* **10**, 617-621 (1977).
- Ball, L. A. & White, C. N. *Proc. natn. Acad. Sci. U.S.A.* **73**, 442-446 (1976).
- Abraham, G. & Banerjee, A. K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1504-1508 (1976).
- Glazier, K., Raghow, R. & Kingsbury, D. W. *J. Virol.* **21**, 863-871 (1977).
- Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247-256 (1976).
- O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).
- Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335-341 (1975).
- Darnbrough, C. H., Legon, S., Hunt, T. & Jackson, R. J. *J. molec. Biol.* **76**, 379-403 (1973).
- Hunter, A. R., Hunt, T., Jackson, R. J. & Robertson, H. D. *J. biol. Chem.* **250**, 409-417 (1975).

Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumours

Agrobacterium tumefaciens incites cancerous growths in dicotyledonous plants called crown gall tumours. Large plasmids in oncogenic *Agrobacterium* strains¹ carry genetic information which is essential for tumour induction^{2,3}. We recently reported that axenic crown gall tumour tissue contains multiple copies of a small part of the oncogenic (Ti) plasmid of the inciting bacterial strain⁴. The mechanism of induction of crown gall tumours thus seems to resemble that of some virally induced animal tumours: the eukaryotic cell is transformed by addition of new genetic information which is stably maintained through subsequent cell divisions⁵⁻⁷. The means by which foreign DNA brings about the tumorous growth pattern of crown gall cells is unknown. The first step in elucidating its mode of action is reported here: we have detected RNA tran-

were resolved into 21 bands by Agarose gel electrophoresis⁴. Plasmid DNA fragments were alkali-denatured in the gel, neutralised and transferred directly to a sheet of cellulose nitrate^{8,9}. The pattern of bands obtained by electrophoresis was thereby reproduced on the sheet, which was then cut into longitudinal strips for separate hybridisation experiments.

Strips were incubated with labelled RNA isolated from clone E9 of a tobacco tumour incited by strain B6-806, and from normal tobacco callus tissue. In addition, a strip was incubated with ³²P-cRNA synthesised⁹ from the whole Ti plasmid genome by *Escherichia coli* RNA polymerase. Autoradiograms of the hybridised filter strips are shown in Fig. 1 together with a photograph of the bands of DNA in the gel used to prepare the cellulose nitrate transfers. Labelled tumour RNA hybridised to a single band of the *Sma* I digest, identified as band 3 by comparison with the photograph of the gel and the autoradiogram of the cRNA hybrid strip. We have shown that this tumour clone contains about 18 copies, per diploid tobacco cell DNA equiv-



Fig. 1 Hybridisation of labelled E9 tumour and control RNAs to strips bearing *Sma* I digest bands. Tumour and normal callus tissues, tested for sterility at the time of labelling, were incubated in Petri dishes with approximately 1 mCi ³²P (neutralised) per g of tissue for 4 h. RNA was isolated by hot phenol extraction, DEAE-cellulose chromatography, DNase digestion and gel filtration on Sephadex G-75 (ref. 12). Specific activities of RNAs were 127,000 c.p.m. per μ g (tumour) and 34,000 c.p.m. per μ g (normal callus). For cRNA synthesis ³²P-UTP (ICN, 75 Ci mMol⁻¹) was used as labelled precursor, and plasmid from strain A277 served as template. Pure virulence plasmid of *A. tumefaciens* B6-806 was isolated from exconjugant strain A277 and digested with *Sma* I as described previously⁴. After electrophoretic separation in 0.5% Agarose, the bands were stained and photographed⁴ before denaturation and transfer of bands to cellulose nitrate sheets (Schleicher and Schuell, B6 membrane). Strips were soaked in Denhardt's¹³ pre-incubation mixture and baked at 55 °C overnight before use. Hybridisation was carried out with 5×10^6 c.p.m. ml⁻¹ labelled RNA and 200 μ g of unlabelled carrier RNA in 0.3 M NaCl, 0.03 M trisodium citrate ($2 \times$ SSC) at 67 °C for 20 h. Filter strips were wetted with $2 \times$ SSC before addition of labelled RNA. Unlabelled carrier RNA was isolated from strain A114, an avirulent plasmidless derivative of strain C58 (ref. 3). After incubation, strips were washed ($2 \times$ SSC 67 °C), treated with RNase (20 μ g ml⁻¹, 1 h, 22 °C), rinsed, dried and autoradiographed for 36 h to 2 weeks. All radioactive label was entirely acid precipitable at the end of the incubation. Autoradiograms of filter strips hybridised with E9 tumour RNA, normal callus RNA and cRNA are presented beside a photograph of the original gel, adjusted to the same scale photographically.

scripts of the foreign genetic information in the tumour cell.

³²P-pulse-labelled RNA was isolated from tumour and normal tobacco callus tissues as described in Fig. 1. To test these RNA preparations for the presence of RNA transcripts of Ti plasmid DNA, we used a filter hybridisation technique developed by Southern⁸. Ti plasmid DNA from the inciting bacterial strain was first cleaved into 25 specific fragments with the restriction endonuclease *Sma* I. These

alent, of part of *Sma* I digest band 3, by direct DNA-DNA hybridisation technique⁴. Our findings show that at least a part of this genetic information is transcribed in the tumour cell. Recent studies have shown that this tumour clone also contains a part of *Sma* I digest band 10 (ref. 10). In three separate experiments using different lots of pulse-labelled tumour RNA from this clone, no hybridisation was noted to band 10 or any other *Sma* I digest bands; only band 3 transcripts were detected. Normal



Fig. 2 Hybridisation of labelled E9 tumour RNA to strips bearing plasmid *Eco* RI digest bands. The labelled E9 tumour RNA (5×10^6 c.p.m. ml⁻¹) was incubated for 16 h (67 °C, $2 \times$ SSC) with cellulose nitrate strips bearing *A. tumefaciens* strain A277 plasmid *Eco* RI/(Miles) digest bands (prepared as for Fig. 1). A line drawing of the bands seen in a photograph of the gel is shown below the autoradiogram of the hybridised strip. Hybridisation is assigned tentatively to bands 12, and 21, 22. (Close neighbouring bands make this assignment equivocal.) The 6,7 doublet has been proposed to bear virulence functions¹¹.



Fig. 3 Hybridisation of labelled E1 tumour RNA to strips bearing plasmid *Hind*III digest bands. Labelled E1 tumour RNA (3 h pulse, 46,000 c.p.m. per μ g) was isolated as described in Fig. 1. Cellulose nitrate strips bearing *Hind*III (New England Biolabs) digest bands of strain A277 plasmid were prepared, and labelled RNA (4×10^6 c.p.m. ml^{-1}) was hybridised with the strips for 16 h ($2 \times \text{SSC}$, 67°C), washed and RNase treated as for Fig. 1. A line drawing of the bands, prepared from a photograph of the gel, is shown below the autoradiogram of the strip. Labelled E1 tumour RNA hybridisation is assigned to *Hind*III bands 1 and 5.

callus tissue RNA was never observed to bind to any fragment of the plasmid, even after fivefold longer exposure of the autoradiograms.

Further experiments were carried out using ^{32}P -RNA form clone E9 and filter strips carrying bands of *Eco* RI digest of the Ti plasmid. The numerous fragments in this digest (Fig. 2) are not well separated by electrophoresis; thus the identification of bands is imprecise. Tumour RNA hybridised well to a band in the region of band 12, and weakly to a band in the region of the band 21,22 doublet. The latter band, while clearly visible in the autoradiogram, is too faint for photographic reproduction in Fig. 2. Doublet *Eco* RI digest band 6,7 has been proposed to be important in tumour initiation because it seems to be common to distantly related virulence plasmids¹¹. We detected no transcript of this band in this tumour clone. Labelled RNA from normal callus tissue did not bind to any bands on filter strips bearing *Eco* RI digest bands (data not shown).

Labelled RNA from tumour clone E1, a single-cell isolate from the same tumour as E9, hybridised to *Sma* I digest band 3 (data not shown). When this RNA was hybridised to cellulose nitrate strips bearing *Hind*III digest fragments of the Ti plasmid, bands 1 and 5 gave positive results (Fig. 3). Thus these singlet bands appear to contain the same genetic information as the relevant member of the *Sma* I band 3 doublet, which we have identified as band 3b as described elsewhere⁴.

Results presented here show that pulse-labelled crown gall tumour RNA can be used to identify DNA fragments, within any restriction endonuclease digest, which contain Ti plasmid DNA base sequences transferred to the tumour cell. Although not all the foreign DNA is transcribed at detectable levels in the tumour, at least part of the transferred DNA can be detected in this manner. DNA fragments shown to be contiguous by conventional mapping procedures may then be tested for homology with tumour DNA.

Our data confirm, by an independent experimental approach, the presence of a specific part of the Ti plasmid in crown gall tumour cells. Also, these data constitute the first convincing evidence that DNA of bacterial origin is transcribed in demonstrably axenic plant tissue. The possibility must be considered, however, that the transcribed genes in question may ultimately be of plant origin, having been picked up by the *A. tumefaciens* Ti plasmid in the course of its evolution. Should this be the case, one might expect these genes to remain untranscribed in the bacterium. Experiments are in progress to determine whether this is in fact the case. If the same RNA transcripts prove to be present in both the bacterial and the crown gall tumour cell, an unsuspected degree of compatibility between transcriptional processes in eukaryotic and prokaryotic systems will be revealed.

This research was supported by grant CA13015 from the National Cancer Institute and grant number NP194 from the American Cancer Society. We thank Mr Chips Armstrong for prints of autoradiograms. We thank Dr

Daniela Sciaky for useful discussions and Dr Benjamin D. Hall for a gift of *E. coli* RNA polymerase.

MARTIN H. DRUMMOND
MILTON P. GORDON
EUGENE W. NESTER
MARY-DELL CHILTON

Department of Microbiology and Immunology,
University of Washington,
Seattle, Washington 98195

Received 14 June; accepted 15 August 1977.

1. Zaenen, I. Van Larebeke, N., Teuchy, H., van Montagu M. & Schell, J. J. *molec. Biol.* **86**, 109-127 (1974).
2. Van Larebeke, N. et al. *Nature* **252**, 169-170 (1974).
3. Watson, B., Currier, T. C., Gordon, M. P., Chilton, M. D. & Nester, E. W. *J. Bact.* **123**, 255-264 (1975).
4. Chilton, M. D. et al. *Cell* **11**, 263-271 (1977).
5. Baltimore, D. *Science* **192**, 632-636 (1976).
6. Dulbecco, R. *Science* **192**, 437-440 (1976).
7. Temin, H. M. *Science* **192**, 1075-1080 (1976).
8. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
9. Keiner, G. & Kelley, J. T. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1102-1106 (1976).
10. Chilton, M. D. et al. *Microbiology* (in the press).
11. Schell, J. et al. *Molecular Biology of Plants* (ed. Rubinstein, I.) (Academic, New York, in the press).
12. Eden, F. C. thesis, Univ. Washington (1974).
13. Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23**, 641-646 (1966).

Decreased initiation factor activity in mouse L cells treated with interferon

INHIBITION of virus-directed protein synthesising events in extracts of cells treated with interferon has been reported¹⁻³. Inhibition of translation of viral mRNAs has been associated with a protein bound to ribosomes of cells treated with interferon^{4,5}. This inhibition may involve the initiation or elongation steps in protein synthesis^{6,7}. Recently, initiation factors for protein synthesis have been isolated from a variety of eukaryotic cells, and it has been established that the initiation factors interact with non-formylated methionyl initiator RNA (Met-tRNA_i) and GTP to form a ternary complex^{8,9}. The experiments presented here show that the activity of initiation factor(s) to form a ternary complex may be impaired or altered in mouse L cells treated with homologous interferon. In preliminary experiments with the initiation factor preparation it was confirmed that the formation of a ternary complex (Met-tRNA_i-eIF-GTP) required GTP for maximal formation (within 2 min at 37°C) and the amount of complex formed was directly proportional to the concentration of initiation factor (Fig. 1). ATP did not substitute for GTP and Mg^{2+} inhibited its formation (data not shown).

When mouse L cells were exposed to (homologous) mouse interferon (100 U ml^{-1}) for 24 h, the initiation factor activity was inhibited about 60%, as compared with untreated controls (Fig. 2). A similar decrease in the activity followed a 6-h treatment of the cells with the same unitage of partially purified mouse interferon (specific activity: 10^7 U mg^{-1} protein). As little as 20 U ml^{-1} mouse interferon resulted in significant inhibition (about 18%). It should be noted that the initiation factor activity of interferon-treated cells reached a plateau at a concentration of $90 \mu\text{g}$ protein and the activity gradually decreased at concentrations higher than

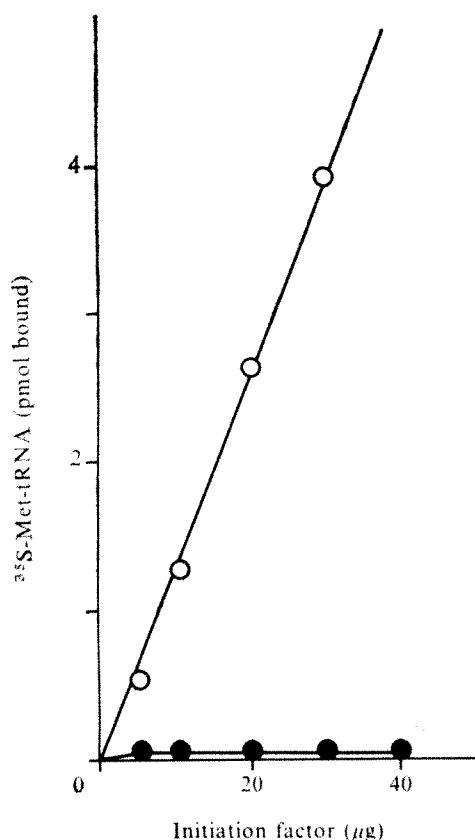


Fig. 1 GTP requirement for formation of ternary complex by initiation factor from mouse L cells. Initiation factor preparation was extracted from exponentially growing mouse L cells treated or untreated with interferon (about 2×10^9 cells). The cells were homogenised in 25 ml 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol, and centrifuged for 20 min at 12,000 r.p.m. Ribosomes from this supernatant were prepared according to the method of Levin *et al.*⁸. The ribosomes were gently stirred in 8 ml 10 mM Tris-HCl (pH 7.5) containing 0.25 M sucrose, 1 mM EDTA, 2 mM dithiothreitol and 0.5 M KCl for 60 min at 4 °C. After centrifugation at 105,000g for 3 h, the supernatant was precipitated with solid ammonium sulphate (0.361 g ml⁻¹), then re-dissolved in 1.0 ml 10 mM Tris-HCl (pH 7.5), containing 0.1 M KCl, 2 mM dithiothreitol and 5% glycerol, and dialysed against 300 ml of the same buffer for 4 h. All procedures were carried out at 0–4 °C. Rat liver Met-tRNA_f was purified from rat liver using DEAE-cellulose¹⁰ and BD-cellulose¹¹ chromatography successively and then charged with ³⁵S-methionine (22.3 Ci mmol⁻¹) using Met-tRNA synthetase purified from *Escherichia coli*⁸. Initiation factor activity was assayed as follows: each 0.1 ml of the reaction mixture contained 40 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 0.1 M KCl, 50 pmol ³⁵S-Met-tRNA_f (3,500 c.p.m. pmol⁻¹) and the amount of initiation factor indicated in the text. The solution was mixed on a vortex mixer before and after the addition of initiation factor, then incubated at 37 °C for 5 min in the presence or absence of 5 mM GTP. The complex formation (³⁵S-Met-tRNA_f.eIF.GTP) was determined by dilution with 1.5 ml 20 mM Tris-HCl (pH 7.5), containing 50 mM KCl, 10 mM $MgCl_2$ and 1 mM DL-methionine and passage through a nitrocellulose membrane filter (Millipore, type HA). The filter was washed with four additional 2.0-ml aliquots of buffer. After drying, the radioactivity associated with the filter was measured. Protein was determined by the methods of Bücher¹² and Groves *et al.*¹³. The initiation factor from the ribosomes of mouse L cells was prepared and assayed in the presence (○) or absence (●) of GTP. The initiation factor preparation contains initiation factor(s) and ribosomal components which elute from ribosomes and purify in the present conditions.

130 mg (Fig. 2). No effect was detected when mouse L cells were treated with 100 U ml⁻¹ heterologous human leukocyte interferon for 24 h. The reduction in initiation factor activity induced by interferon was prevented by inhibition of cellular RNA synthesis with actinomycin D (98% inhibition of cellular RNA synthesis) or by inhibition of cellular protein synthesis with cycloheximide (93% inhibition of cellular protein synthesis) (Fig. 3a and b). Furthermore, the activity of initiation factor was not inhibited by mouse

interferon added directly to the assay reaction mixture. This finding is not consistent with the proposal that interferon itself is a modified initiation factor¹⁴.

Evidence indicating that the interferon in the preparation was responsible for the altered initiation factor activity comes from the above findings that: (a) partially purified interferon (which originated from a different mouse cell source) had the same effect as crude interferon; (b) heterologous human interferon at levels which did not induce antiviral activity in mouse cells had no effect on the activity of initiation factor; (c) inhibition of cellular RNA synthesis by actinomycin D prevents both the antiviral activity and the alteration of initiation factor activity by interferon; (d) inhibition of protein synthesis by cycloheximide blocks both the antiviral activity and the change of initiation factor activity; and (e) interferon which does not directly inhibit virus, when added directly to the initiation factor assay system did not inhibit the activity (data not shown). Taken together, these findings show that the initiation factor activity from interferon treated mouse L cells was consistently and significantly reduced when compared with the initiation factor activity obtained from control cells. These characteristics coincide with those which are considered to be specific for interferon¹⁵.

A preliminary attempt to compare the physical properties of the initiation factor extracted from control and from interferon-treated cells was carried out by studying their heat stabilities. Initiation factor from cells treated and untreated with interferon were incubated at 50 °C for the times indicated in Fig. 4, and then

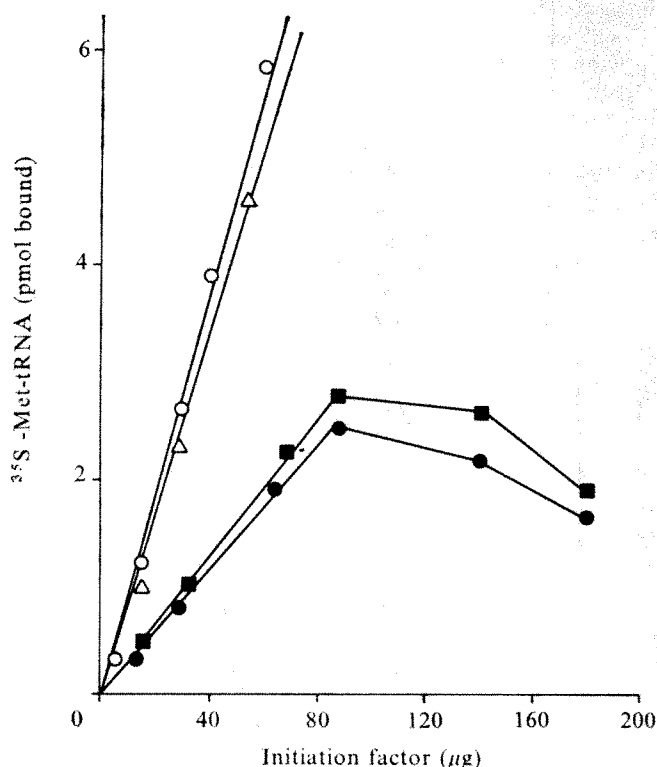


Fig. 2 Comparison of initiation factor activity from mouse L cells treated and untreated with interferon. Exponentially growing monolayers of mouse L cells were treated with 100 U ml⁻¹ of either mouse interferon (Bionetics Corporation) or human leukocyte interferon for the indicated periods in the presence of 2% foetal calf thymus. After treatment of the cells with interferon, they were collected and washed with 10 mM Tris-HCl (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$ and 2 mM dithiothreitol. The initiation factors from the cells were prepared and assayed as described in Fig. 1. Initiation factor activity of the cells exposed to mouse interferon (●), human interferon (Δ), or partially purified mouse interferon 100 U ml⁻¹ for 6 h (■), ○, untreated cells. The difference between the average values of initiation factor activity from interferon-treated and control cells observed in eight different experiments are statistically significant ($P < 0.001$ using Student's *t* test).

assayed for residual activity. With a little as 15 min of heating, the initiation factor from interferon treated cells showed greater stability than initiation factor from untreated cells or from cells treated with both actinomycin D and interferon. This finding suggests that interferon either induces an alteration of the normal initiation factor or it induces an inhibitory material which combines with and alters the normal factor. The latter possibility is consistent with the finding that increasing concentration of initiation factor from interferon treated cells results in increasing inhibition of activity (Fig. 2).

Several previous reports have indicated that the translation of added viral mRNAs is impaired in a mixture of extracts from interferon-treated cells more than that of cellular mRNAs³⁻⁵. This inhibitory effect is thought to be mediated by the induction with interferon of a new, cellular antiviral protein which may act by one or more mechanisms: impairment of the binding of viral mRNAs to ribosomes¹⁶; alteration of the methylation of mRNAs¹⁷; restriction of the availability of a minor species of tRNA required for translation^{18,19}; and induction of a nuclease which impairs translation²⁰. The present finding that the initiation factor from interferon-treated cells has diminished activity to form a ternary complex narrows the possible mechanisms in favour of an effect on initiation and will be used in our further studies to help define the basis of selective inhibition of viral translation in interferon-treated cells. Note that the effect of interferon on activity of initiation factor is the earliest event in the sequence of protein synthesis which has been shown to be inhibited by interferon. Other important questions which merit further study include: (a) Can the impairment of initiation factor activity be observed in intact cells treated with interferon? (b) Is the degree of inhibition of initiation factor activity in cells treated with interferon sufficient to account for the reported impairment of the functions of resting, rapidly metabolising and tumour cells? (c) Can the decreased initiation factor activity be overcome by formylated initiator tRNA as has been described for encephalomyocarditis virus polypeptide chains⁹? The latter mechanism may provide a

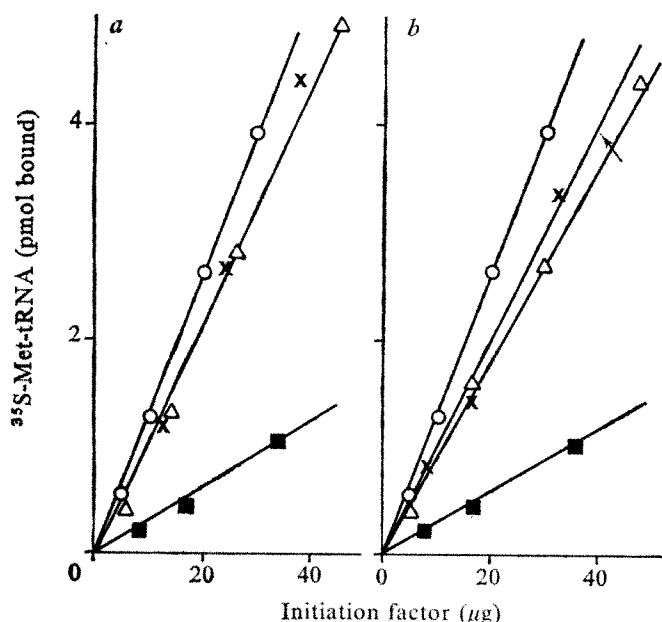


Fig. 3 Effect of actinomycin D and cycloheximide on the impairment of initiation factor activity by interferon. *a*, Activity of initiation factor preparation from L cells exposed to partially purified mouse interferon 100 U ml⁻¹ for 6 h (●), pretreated with actinomycin D 2 μg ml⁻¹ for 60 min, or incubated for 6 h in the absence (×) or presence (Δ) of interferon. Untreated cells (○). *b*, Activity of initiation factor from L cells exposed to partially purified mouse interferon 100 U ml⁻¹ for 6 h (●), pretreated with cycloheximide 100 μg ml⁻¹ for 30 min, or incubated for 6 h in the presence of interferon and cycloheximide (Δ) or in the presence of cycloheximide (×). Untreated cells (○).

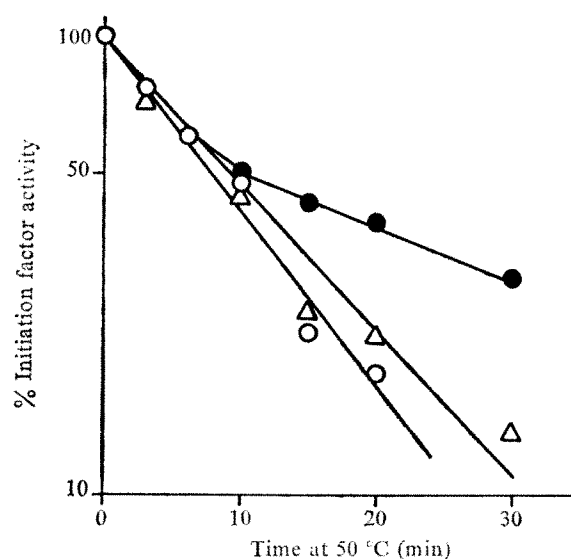


Fig. 4 Temperature sensitivity of initiation factor activity from cells treated and untreated with interferon. Each initiation factor preparation (0.5 mg protein) was incubated at 50 °C for the indicated periods. After heating the initiation factor preparation was tested for ability to form a ternary complex. Initiation factor activities from cells treated for 6 h with partially purified mouse interferon 100 U ml⁻¹ (●), and from interferon-treated cells after treatment with actinomycin D 2 μg ml⁻¹ for 60 min (Δ), ○. Untreated cells.

discrimination between viral mRNAs and host mRNAs at initiation level, because the ternary complex formation in eukaryotic system requires Met-tRNA_i as described previously^{8,9}.

This work was supported in part by the McLaughlin Foundation and Grant No. NIH RR 05427 and DHEW S 00170. We thank Drs E. Stringer and V. Holoubek for discussions.

KENZO OHTSUKI
FERDINANDO DIANZANI
SAMUEL BARON

Department of Microbiology,
University of Texas Medical Branch
Galveston, Texas 77550

Received 27 May; accepted 22 July 1977.

- ¹ Carter, W. A. & Levy, H. B. *Science* **155**, 1254-1257 (1967).
- ² Friedman, R. M. *et al.* *J. Virol.* **10**, 1184-1198 (1972).
- ³ Falcoff, F., Falcoff, R., Leblou, B. & Revel, M. *Nature new Biol.* **240**, 145-147 (1972a).
- ⁴ Gupta, S. L., Sopori, M. L. & Lengyel, P. *Biochem. biophys. Res. Commun.* **54**, 777-783 (1973).
- ⁵ Samuel, C. E. & Joklik, W. K. *Virology* **58**, 476-491 (1974).
- ⁶ Kerr, J. M., Friedman, R. M., Brown, R. F., Ball, L. A. & Brown, J. C. *J. Virol.* **13**, 9-21 (1974).
- ⁷ Content, J. *et al.* *Proc. Natl. Acad. Sci. USA* **74**, 1041 (1977).
- ⁸ Levin, D. H., Kyner, D. & Acs, G. *J. Biol. Chem.* **248**, 6416-6425 (1973).
- ⁹ Suter, B., Adams, S. L., Anderson, W. F. & Merrick, W. C. *J. Biol. Chem.* **250**, 9076-9082 (1975).
- ¹⁰ Smith, A. E. & Marcker, K. A. *Nature* **226**, 607-612 (1970).
- ¹¹ Petermann, G., Boissard, M. & Puissant, C. *Biochimie* **53**, 1105-1109 (1971).
- ¹² Bucher, X. X. *Biochim. biophys. Acta* **1**, 292-314 (1974).
- ¹³ Groves, W. L., Davis, F. C. & Sells, B. H. *Analyt. Biochem.* **22**, 195-210 (1968).
- ¹⁴ Kosower, N. S. & Kosower, E. M. *J. molec. Med.* **1**, 11-17 (1975).
- ¹⁵ Lockart, R. Z. in *Interferon Induced by Interferon* (Ed. Finter, N. B.) 11-27 (American Elsevier, New York, 1973).
- ¹⁶ Marcus, P. I. & Salh, J. M. *Virology* **30**, 502-516 (1966).
- ¹⁷ Sen, C. G. *et al.* *J. Virol.* **21**, 69-83 (1977).
- ¹⁸ Gupta, S. L., Sopori, M. L. & Lengyel, P. *Biochem. biophys. Res. Commun.* **57**, 763-770 (1974).
- ¹⁹ Zilberstein, A., Dudock, B., Berissi, H. & Revel, M. *J. molec. Biol.* **108**, 43-45 (1976).
- ²⁰ Marcus, P. I., Terry, T. M. & Levine, S. *Proc. natl. Acad. Sci. U.S.A.* **72**, 182-186 (1975).

Nature Index and Binders

The complete Index for 1976 is available, price £2.50 (UK), US\$5.00 (Rest of World). Copies of the 1975 index are still on sale, price £2.25 (UK), US\$5.00 (Rest of World).

Binders for the journal are also available at £8.00 (UK), US\$16.00 (Rest of World) for three: a year of *Nature* fits into three binders.

All prices include postage. Payment may be made in any currency at the prevailing exchange rate. Orders should be sent, accompanied by remittance, to Macmillan Journals Ltd, Brunel Road, Basingstoke, Hampshire, RG21 2XS, England.

reviews

Nuclear theory

D. F. Jackson

A Unified Theory of the Nucleus. By K. Wildermuth and Y. C. Tang. Pp. x+389. (Academic London and New York, 1977) \$38.50; £27.35.

In nuclear theory the essential problem is to reduce the description of a many-particle system to manageable proportions without the introduction of too many arbitrary assumptions and with the possibility of estimating the effects of approximations made. In the unified theory of nuclear reactions, particularly associated with the name of Feshbach, projection operators are used to reduce the true many-particle Hamiltonian to effective Hamiltonians, which belong to subspaces of the total Hilbert space but contain the correct asymptotic boundary conditions for the final states. In contrast, Wildermuth and Tang propose a theory in which the total wavefunction for the many-particle system is represented as a sum of non-orthogonal terms each corresponding to a final system with the correct boundary conditions. Thus, the subspaces of the total Hilbert space are defined through the wavefunction and, it is argued, this provides the correct asymptotic behaviour for the initial state and all final states.

The authors apply their approach to a variety of bound-state and reaction problems. The terms of the wavefunction each correspond to a possible cluster structure of the many-particle system, although, as the authors repeatedly stress, it is inappropriate to take these cluster structures too literally because of the effect of the anti-symmetrisation operator which exchanges particles between the clusters. Among the problems discussed are the construction of bound-state wavefunctions and the evaluation of charge density distributions, ground-state energies and transition probabilities, the scattering of light systems at low and medium energies, resonance reactions, the microscopic formulation of optical potentials, and fission.

This book covers a very wide range of nuclear physics and contains some penetrating observations about aspects of the theory. It is, however, a rather frustrating book to read. So many topics are started and reach a premature conclusion with a rather trivial

example based on a very light system such as ^4Be or ^4Li . There is a useful discussion of the generator coordinate technique but recent developments in the resonating group method and the orthogonality condition model are ignored. The discussion of optical potentials for composite particles completely ignores extensive work done with folding models. The most serious weakness of the approach is that, although great emphasis is laid on the

need for correct asymptotic behaviour of wavefunctions, all the examples fall back on oscillator functions which are notoriously incorrect at large separation distances. Until the practical application of the theory can be extended to yield the correct asymptotic behaviour the formal theory is unlikely to lead to major advance. □

D. F. Jackson is Professor of Physics at the University of Surrey, UK

Cancer chemotherapy

Biosynthetic Products for Cancer Chemotherapy Vol. 1. By G. R. Pettit. Pp. xii+215. (Plenum: New York and London, 1977.) \$23.40

THE author states that his main aim is "to provide a current overall view of the cancer problem and the development of cancer chemotherapeutic drugs of biosynthetic origin". If you want a reasonably detailed summary of the chemical and biological properties of the kinds of cytotoxic products which have been isolated from plants, micro-organisms, marine organisms, reptiles and other sources, then this book, from p47 on, may supply your needs.

It is less easy to know for whom the whole book was written. The first chapter (Introduction and Perspective) is the longest (46 pages); in it, the author discusses the nature, causation, and treatment of cancer with special emphasis on the role of the National Cancer Institute. Such a survey seems out of place and belies the title of the book. Dr Pettit could undoubtedly write a useful review or two on different aspects of cancer chemotherapy, but not, as here, by trying to cram a quart into an inappropriate pint pot.

In his introductory chapter, the author refers to the ten or so malignant conditions particularly amenable to drug treatment, without making it clear that these are relatively rare cancers. Their control is a commendable achievement, but is only a scratch on the surface of the cancer problem.

On the credit side, the author and publishers are to be complimented on the clear and numerous structural formulae. They occupy, however, all

of 80 pages, leaving only about 75 pages of text for the natural products section of the book. Dr Pettit has succeeded in compressing a large amount of information into these pages, and the comprehensive tabular survey which volume 2 will provide, promises to be a useful supplement.

The author's enthusiasm often comes through, insufficiently tempered, perhaps, by an acknowledgement of the limitations of cancer chemotherapy in man at the present time. He clearly feels that much more money could usefully be spent on the exploration of natural products as potential anti-tumour agents. He may be right, but some of his statements would not find universal acceptance. Take, for example, the dogmatic comment on p175: "Given sufficient financial support and time, the lower animals will prove to be a particularly good source of unusual and valuable drugs for cancer chemotherapy". Again, the author seems to imply (p58) that, with sufficient knowledge, one could design a single substance effective against human cancer in its various forms, a view now held by few.

Such criticism does not invalidate the work as a handy reference book on a range of naturally occurring cytotoxic compounds, although—if the reviewer may now be dogmatic—the very great majority of these products will never become established as clinically useful drugs. One must therefore not take the title of the book too literally.

J. A. Stock

J. A. Stock is Senior Lecturer in Chemistry at the Chester Beatty Institute of Cancer Research, London, UK.

Inorganic azides

Energetic Materials Vol. 1: Physics and Chemistry of the Inorganic Azides Pp. 503. \$59.40. Vol. 2. Technology of the Inorganic Azides. Pp. 296. \$90. Edited by H. D. Fair and R. F. Walker (Plenum: New York and London, 1977).

THESE volumes review recent research and development work in the physics, chemistry, and technology of the inorganic azides. Summarising fundamental advances over the past two decades, emphasis is placed on investigations supported by the United States Army through its Office of the Chief of Research and Development and its Materiel Command. The contributing authors are, for the most part, members of the Energetic Materials Division (formerly Picatinny Arsenal), Armament Research and Development Command, New Jersey.

The wide variation in crystal bonding characteristics and stability of the inorganic azides present problems of unique interest in solid state physics and chemistry. In the reactive state, the inorganic azides exhibit a remarkable range of reactivities from low order decomposition, deflagration, to high order detonation. These properties have led to the commercial development of azides in a wide field, including particularly gas-generating devices and explosives, as well as photographic emulsions.

One of the central and fundamental problems in the study of azides lies in the development of valid equations of state applicable to the explosive decomposition of these materials. This requires the elucidation of interatomic forces from data on vibrational levels obtained from crystal spectra. Considerable success along these lines obtained with the solid alkali metal halides, using dynamic models, has very recently been applied to the inorganic azides, using laser Raman and thermal neutron spectroscopy. The results of these studies have contributed greatly to our understanding of lattice and phase transition dynamics in the crystalline metal azides. Another important area concerns the calculation of activation energies of decomposition, and the correlation of these with ground and excited energy levels in the azide crystal.

These major aspects are reviewed comprehensively in Volume 1, which deals with azides across the whole range of the Periodic Table, with particular reference to azide crystals and crystal structure; molecular vibrations in lattices; electronic structure of the

solid state, and all aspects of decomposition, initiation and propagation in solid metal azides.

Volume 2 is devoted to the technology of those metal azides used in detonators, and as such is largely concerned with lead and silver azides. The aspects covered comprise manufacturing techniques and process control; analysis of azides, handling and safety; sensitiveness and the roles of azides in detonating trains. The section on electrostatic sensitivity and dielectric properties of lead azide and other primary explosives is of great current interest. The final section on the initiation of detonating trains by azides, also merits comment. In these situations lead azide functions commonly both as

acceptor and as donor. These two processes together determine the pulse time and shape of the impulse integral on which the detonation threshold of the secondary charge depends. Recent high precision data are presented on these phenomena.

In conclusion, these volumes offer an extremely comprehensive, well-documented review of the most significant aspects of the science and technology of the inorganic azides. This work will be of inestimable value, particularly to those concerned with the development of military and commercial explosives. **J. H. Turnbull**

J. H. Turnbull is Professor and Head of the Chemistry Branch, Royal Military College of Science, Shrivenham, UK.

Aerosol research and technology

Smoke, Dust and Haze: Fundamentals of Aerosol Behaviour By S. K. Friedlander. Pp. xvii+317. (Wiley: New York and London, 1977) \$19.50; £11.35.

AEROSOL research and technology is, like most modern fields of interest, complex and interdisciplinary. Whereas transport, deposition, collision and coagulation are merely governed by physical laws, the formation of particles is largely a chemical problem. Meteorological processes such as rain-out, wash-out, and boundary layer physics are involved as well as engineering aspects for the design of sampling and measuring devices.

The available literature is specialised on certain aspects of the complex field, and there is a strong need for a comprehensive presentation especially for those who want to get into this field. Friedlander's book is likely to close a great deal of this gap. Written as a classroom text for graduate students in environmental engineering it mainly covers the physical and engineering aspects of aerosol behaviour.

The first third of the book is devoted to particle transport and deposition. Excellent fundamentals of problems, such as diffusion and deposition for different flow parameters, filter efficiencies, electrical precipitation, inertial and turbulent deposition, are given.

The second third deals with fundamentals of particle generation and growth. Collision and coagulation processes, thermodynamic processes, such as condensation, formation and stability of droplets and clusters, and a brief outline on gas-to-particle conversions, are presented.

In the last part, a general dynamic equation for the continuous distribution function is set up taking into account all processes of particle formation, growth, diffusion, coagulation, convection, and sedimentation discussed before. Special cases, in particular the dynamics of turbulent stack plumes, are discussed and compared with observational data. The techniques for setting up predictive models for air quality-emission source relationship are briefly described.

A short chapter on optical properties is confined to light scattering and visibility. For the complete problem of radiative transfer the reader is referred to the specialist literature. In another short chapter on experimental methods, descriptions of sampling, filtration, particle counters, the cascade impactor, devices for chemical analysis, and aerosol generators, are given.

The book is clearly written and beautifully made. At the end of every chapter carefully selected examples and problems provide excellent applications of the fundamentals treated. Friedlander concentrates mainly on the physical aspects of aerosol behaviour. Important chemical processes such as gas-to-particle conversion through heterogeneous reactions and meteorological aspects of particle transport and precipitation are only briefly outlined for completeness. It would be highly desirable to see equally well produced books covering the chemical and meteorological aspects being published.

This book is more than a textbook for graduate students in environmental engineering. It should be on the bookshelf of every scientist and engineer directly or indirectly involved in aerosol research and technology. **P. Fabian**

P. Fabian is Head of the Department of Atmospheric Minor Constituents, Max-Planck-Institut für Aeronomie, Katlenburg-Lindau, West Germany.

Molecular weight determination

Molar Mass Measurements in Polymer Science. By N. C. Billingham. Pp.254. (Kogan Page: London, 1977.) £13.50.

DISCUSSION of the theories underlying molecular weight determinations is almost an incidental part of most textbooks of polymer science. In contrast, practical aspects of the various techniques are most commonly discussed in the instruction manuals issued by appropriate manufacturers. The author of this book has made a highly successful attempt to combine the two sources of information into a most useful and reasonably concise reference text.

As the title indicates, the book is written with a fairly rigid adherence to the SI system of units; molar mass, a fundamental unit with dimensions, being preferred to the more generally used, dimensionless quantity, molecular weight.

In the early part of the book the various molar mass averages, and their relationships, are discussed as a preliminary to a most enlightening survey of the nature and behaviour of polymer molecules in solution. Membrane

osmometry, vapour pressure, light scattering, ultracentrifuge, viscosity, and gel permeation chromatography techniques are fully described in later chapters and, for each, there is a discussion of both underlying theory and practical laboratory procedures. Different types of instruments supplied by various manufacturers are individually described and compared, and full details of equipment suppliers and a list of suitable solvents for most common polymers are included as appendices. There is also a very short discussion of chemical methods for assay of endgroups in polymers.

In total, the contents of this book are rather similar to those of two edited volumes (*Polymer Molecular Weights*, ed. P. E. Slade, Jr, recently published) and indicate the requirement for a convenient reference text of this kind. The present single volume, however, has the advantages of both lower price and a more coordinated treatment of the subject. It will be valuable to anyone interested in polymer characterisation and especially to research workers who may be abruptly faced with the problems of molecular weight determination.

A. Ledwith

A. Ledwith is Professor of Chemistry at the University of Liverpool, UK.

Liquid-state chemistry

Introduction to Liquid State Chemistry. By Y. Marcus. Pp. viii+357. (Wiley-Interscience: London and New York, 1977.) £12.50; \$24.00.

LIQUID-STATE PHYSICS is a flourishing branch of science which has been reviewed recently in several good books. The liquids discussed were those whose structure is the most easily determined, and whose properties provide the most direct test of physical theories; argon is the archetype. Professor Marcus has a different aim—to give an account of the equilibrium properties and structure of those liquids that are of more concern to the chemist. "Crude petroleum, sea water, aqueous acetone and molten cryolite" are his examples.

Before tackling his main subject, he rightly supposes that his reader will need first to know something of the physics of simple liquids and their mixtures, and so the first half of his book is essentially liquid-state physics. Unfortunately, 160 pages is too small a space for this to be anything but a string of theories, models, approximations, and references. Those familiar with the field might wish that he had been more critical in his choice of material; those who are not, will probably gain little but a list of references and useful tables, since the text is not only too condensed but abounds with errors and examples of misplaced emphasis.

The second half of the book covers his main interest; there are chapters on mixtures of molecular liquids, solutions of electrolytes, molten salt mixtures, and mixtures of liquid metals. Here, the level of rigour of the physics is necessarily lower than in the earlier chapters; often a well chosen correlation of physical properties is more valuable than a neat trick with the grand partition function. Chemists who work with liquids will welcome the last four chapters, with their extensive tables and full references.

The units of the book are said to SI, but this system implies not only the use of metres, kilogrammes, seconds, and so on, but also the use of the rationalised electromagnetic system. Unfortunately, the author has married the sizes of the units of the SI with equations of the unrationalised electrostatic system, so that we have, for example, a polarisability in $\text{m}^3 \text{mol}^{-1}$. This combination will probably confuse students reared on the *Système International*, and not only on its units.

J. S. Rowlinson

J. S. Rowlinson is Professor of Chemistry at the University of Oxford, UK.

Advances in Invertebrate Reproduction

Volume I

Edited by K. G. Adiyodi & Rita G. Adiyodi
1977; xii+514pp.; art paper; hard bound; £23/\$40
(inclusive of postage)

Proceedings of the First Symposium of the International Society for Invertebrate Reproduction, Calicut, September 10-12, 1975. Updated and revised. The 39 original contributions and overviews by leading authorities in the field cover several fascinating aspects of sexuality, reproduction and behaviour of a wide range of invertebrates. Some of the comparative data given on vertebrates add to the usefulness of this volume. Profusely illustrated and complete with author, subject and species indexes.

Enclose payment with your order

Publishers:

Peralam-Kenoth
Karivellur, Kerala 670521, India

Precambrian formations

Precambrian of the Northern Hemisphere. By L. J. Salop. Pp.ix+378. (Elsevier Scientific: Amsterdam, New York and Oxford, 1977.) Dfl.145; \$59.25.

THIS book provides an "inter-regional correlation of Precambrian formations", the subdivisions of which are based on the "natural stages in the earth's evolution". Essentially the book is a correlated list of the Precambrian formations of the northern hemisphere.

Three introductory chapters are largely concerned with methods and principles of correlation and subdivision, and the two final chapters review briefly the major features of geological evolution during the Precambrian (tectonic models and plate tectonics are avoided). The six main chapters list the principal rock groups in Salop's six subdivisions, as follows: (1) The Archaean era includes all rocks formed more than 3500 Myr ago (largely gneisses and granulites). (2) The Paleoprotozoic ("Protozoic" is Salop's new term) (3500–2600 Myr) which concerns the well-known greenstone belts. (3) The Mesoprotozoic (2600–1900 Myr) including geosynclines like Krivoi Rog, the Huronian and the Labrador Trough. (4) The Neoprotozoic (1900–1000 Myr) which deals with clastics and red beds in platforms and aulacogens (for example, Gardar and Keweenaw) and the gabbro-anorthosite-rapakivi granite-alkali syenite complexes. (5) The Epi-protozoic (1000–650 Myr) including platform and miogeosynclinal, mostly terrigenous (especially red bed) facies and the prominent tillites (for example, lower Dalradian, upper Briovarian). (6) The Eocambrian (650–570 Myr), dealing with platform clastics and the Ediacara fauna.

There are about 800 references, at least half of which relate to the USSR and China, but most date from the 1960s. A disappointing aspect of the book is the paucity of figures—only 36 with an additional four tables. Two

very useful charts in a back pocket correlate formations in 78 regions across North America and Greenland, and 78 across north-western Europe and the USSR (Britain is missing); and a large table gives a synopsis of all aspects of geological evolution.

Salop is very much better on the younger than the older Precambrian—in fact, his interpretations of some Archaean formations will raise many eyebrows. All gneissic-granulite areas have to be Archaean and their stratigraphy is sedimentary (V. R. McGregor's tectonic intercalations are not considered). Isochrons are said to indicate "rejuvenated" ages dating the period of late recrystallisation. Thus, the Fiskenaesset complex, the Scourian

and the Moldanubian are considered to have "formed" more than 3500 Myr ago, but the Isua supracrustals are included in the Paleoprotozoic. Salop's classification is so rigid that it does not allow greenstone belts to form in one region while high-grade gneisses are forming in another.

The book provides, however, for the first time in English a very valuable correlation of the main formations and rock groups throughout the USSR and China with those "in the west". This is its main contribution.

B. Windley

B. Windley is Reader in Geology at the University of Leicester, UK.

Modern knowledge of cyclic nucleotides

Cyclic Nucleotides in the Nervous System. By John Daly. Pp.401. (Plenum: New York and London, 1977.) \$39.

ONE of the first papers from Earl Sutherland's laboratory about the new nucleotide, cyclic AMP, included observations on the distribution in mammalian tissues of adenylate cyclase, the enzyme responsible for its synthesis. The highest concentration was found in the brain. Thus, it was clear from the start that Sutherland's epoch-making discovery would figure prominently in neurobiology. Development of the significance of this observation was initially due to Sutherland's co-worker, T. W. Rall, who observed that in the nervous system cyclic AMP functions as second messenger not to hormones circulating in the blood stream, but to certain neurotransmitters. Rall's work may be said to have set the stage for the tremendous expansion in the literature on cyclic nucleotides in the nervous system so extensively documented in John Daly's monograph.

Dr Daly's book is entirely orientated to the nervous system; no concessions are made to the general reader unfamiliar with the background to the subject. After a cursory introduction of no more than two pages, he launches into the first of three long chapters, entitled "Enzymatic Formation, Degradation and Action of Cyclic Nucleotides". This deals mainly with the pro-

perties and distribution of adenylate and guanylate cyclases and phosphodiesterases in brain, ganglia and cell cultures, together with a description of enzyme systems concerned in the cyclic AMP-dependent phosphorylation and dephosphorylation of tissue proteins. Next comes an extensive account of the *in vitro* accumulation of cyclic nucleotides in cell-containing preparations of brain, ganglia and other cells of neural or glial origin. The detail here is exemplary, reflecting the author's personal research interests. The final chapter deals with "Functional Roles for Cyclic Nucleotides in the Nervous System". Understandably at this stage of our knowledge it concentrates on actions and effects rather than the physiological functions, of which Dr Daly would be the first to recognise very little is known at present.

The book should prove an excellent text for researchers about to enter or already working in the field. It contains over 1,300 references and is written in a straightforward style that expounds facts and eschews speculation. Such an approach does not always make for easy reading, especially since very little effort is made to summarise or synthesise the mass of information, or indeed critically assess its relative importance. This leads to some repetition and the occasional uncritical citation of dubious work. Dr Daly's book is therefore about the 'trees' rather than the 'wood' but is nevertheless invaluable as an exhaustive source of up-to-date information on modern knowledge of cyclic nucleotides in the nervous system.

R. Rodnight

R. Rodnight is Professor of Neurochemistry at the Institute of Psychiatry, London, UK.

Erratum

● In the review of *Spectrochemical Analysis of Pure Substances* (Nature, 11 August, 268, 572, 1977) the publisher was incorrectly quoted. This should have read: Adam Hilger: Bristol; Crane, Russak: New York.

obituary

Lord Adrian, 1889–1977

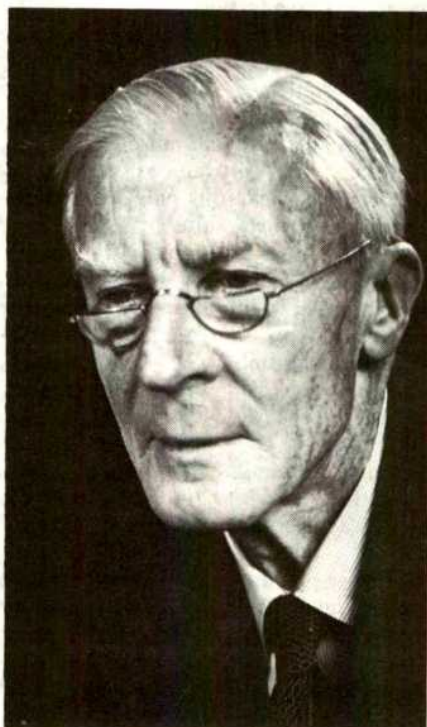
LORD ADRIAN disliked his christian names, Edgar Douglas, and was invariably known to his family and friends as Adrian; he will be called Adrian in this article which is concerned mainly with his scientific work, and particularly with the events which led up to the discoveries described in his first two books *The Basis of Sensation* (1928) and *The Mechanism of Nervous Action* (1932).

In order to understand the full extent of Adrian's contribution to science and medicine we must first consider the changes which have come over physiology since he started work with Keith Lucas in the Physiological Laboratory at Cambridge in 1911. It is easy to appreciate the effect of the development of electronic equipment for, as Adrian himself once said, 'The history of electrophysiology has been decided by the history of electric recording instruments'. But there have also been overwhelming changes in ideas and these are harder to take in, for, as historians of science know only too well, it is extraordinarily difficult to put ourselves in the position of our scientific ancestors who did not accept some scientific law or principle of nature that we now regard as self-evident.

One must, for example, remember that although Bowditch established the all-or-nothing law for heart muscle in 1871, in 1911 it was still uncertain whether the same law applied to nerve. The work which Adrian did before 1914 helped to show that in a single nerve fibre the size of the impulse is independent of the strength of the stimulus, provided the latter is large enough to evoke a response; it also removed some of the difficulties which had persuaded scientists as perceptive as Max Cremer or F. Hofmann of the opposite.

Then there was a complete muddle about the contraction of skeletal muscle and it was widely held that the quick and slow movements of voluntary muscle were brought about by different contractile mechanisms activated by two types of nerve fibre, or even by two forms of nerve impulse. This situation was not cleared up until Adrian and Bronk carried out their famous experiments in the late 1920s.

Adrian, who was not at all fussy about laboratory accommodation, has



Douglas Glass

given an entertaining description about life in the old Physiological Laboratory at the time when he started work there. 'In those days the Cambridge School of Physiology was at the height of its fame. It was housed deplorably, judged by modern standards, and run so economically that there was no mechanic and no common stock of tools or apparatus. The research rooms were barely rooms at all. Keith Lucas was lucky to have a small cellar all to himself. It was approached through a larger cellar which housed A. V. Hill and many cages of rats on which Hopkins was carrying out his classical work on the vitamins. A side door led to a dark chamber in which all the frogs were kept and beyond this was the centrifuge driven by a large gas engine of obsolete design which shook the building and added the smell of warm oil and half-burnt gas to that of frog and rat; upstairs worked Barcroft, Mines, [W. B.] Hardy, Anderson, Fletcher, Langley, to name only those who were in permanent occupation, and their accommodation was little better.'

In 1913 Adrian wrote a thesis on *The conduction of the impulse in the nerve fibre* which won him a Fellowship at Trinity College, of which he was later to become Master (1951–1965).

In July 1914 Adrian went to St Bartholomew's Hospital where he qualified in record time, just over a year; he then worked on nerve injuries and shell shock, first at Queen Square and later at the Connaught Military Hospital at Aldershot where he remained to the end of the war in spite of strenuous efforts to get to France.

There are several interesting papers of a clinical nature from this period, but much the most remarkable is one published in 1917 in the *Lancet* with L. R. Yealland on the treatment of some common war neuroses. The paper describes a highly successful method of dealing with hysterical paralysis, which the authors applied with at least 95% success to more than 250 cases, including all the common hysterical disorders such as mutism, deafness, paralysis of limbs and so on. No great originality was claimed for the method which depended on persuasion and suggestion, usually employing electrical stimulation to evoke sensation or movement, but it is evident that the authors applied the method with much ingenuity and understanding of human nature. The article is also remarkable for sympathetic references to psychoanalysis which bear witness to the width of Adrian's interests, as does the manuscript of an unpublished lecture 'Freud without tears' given in 1919.

After the war, Adrian continued for several years with analytical electrophysiology of the type which he had started with Keith Lucas (who was killed in an aeroplane accident in 1916). But about 1925 he began to use valve amplifiers and made what in the jargon of today (which Adrian detested) would be called a break-through.

The transformation is best described in his own words, 'Alexander Forbes had been working with me in Cambridge and I had learned a great deal from him about string galvanometers and about mammalian preparations, but the experiments I had started became more and more unprofitable. You know the sort of thing that happens—they became more and more complicated and the evidence more indirect, and after a time it was quite clear that I was getting nowhere at all.'

Adrian then describes how he had built a 3-stage valve amplifier to the design given him by Gasser and had

connected it up to a capillary-electrometer recording from the sciatic nerve of a nerve-muscle preparation. He was distressed by a rapid electrical oscillation which appeared whenever the muscle was hanging down freely and disappeared when it was supported. Then "The explanation suddenly dawned on me, and that was a time when I was very pleased indeed. A stretched muscle, a muscle hanging under its own weight, ought, if you come to think of it, to be sending sensory impulses up the nerves coming from the muscle spindles, signalling the stretch on the muscle. When you relax the stretched muscle, when you support it, those impulses ought to cease.

"I don't think it took more than an hour or so to show that that was what the little oscillations were. I was able to make photographic records of them, and within about a week I was nearly certain that many of these oscillations were action potentials coming up sensory fibres in the nerve, and what was more, that many of them came from single nerve fibres and that by some extension of the technique it ought to be possible to find out exactly what was happening in single nerve fibres when the sense organs attached to them were stimulated.

"That particular day's work, I think, had all the elements that one could wish for. The new apparatus seemed to be misbehaving very badly indeed, and I suddenly found that it was behaving so well that it was opening up an entire new range of data. I'd been bogged down in a series of very unprofitable experiments and here suddenly was the prospect of getting direct evidence instead of indirect, and direct evidence about all sorts of problems which I had set aside as outside the range of the techniques that one could use. The other point about it was that, as I said, it didn't involve any particular hard work, or any particular intelligence on my part. It was one of those things which sometimes just happens in a laboratory if you stick apparatus together and see what results you get."

The comment that one wants to make about the last sentence is that when most people stick apparatus together and look around they do not make discoveries of the same importance as those of Adrian.

The initial advance made by Adrian was rapidly followed by three important papers with Zotterman which showed beyond doubt that the nerve impulse was invariant, that the intensity of sensation was conveyed by the frequency of nerve impulses and the quality by the type of nerve fibre in action. Another very important point is that adaptation to a steady stimulus takes place peripherally and that some

sense organs, like those concerned with touch, are rapidly adapting, whereas others like muscle spindles are slowly adapting.

These early experiments on sense organs are beautifully described in *The Basis of Sensation* which is written in such simple and elegant language that it is difficult for a student of today to appreciate the novelty and importance of what is being said.

At about this time Adrian's small group was joined by B. H. C. (now Sir Bryan) Matthews who followed up Adrian and Zotterman's work in two classical papers on muscle spindles. Matthews also introduced the oscilloscope which bears his name and which proved extremely suitable for the type of work done by Adrian and his school. Later Adrian and B. H. C. Matthews collaborated in important studies of the Berger rhythm and on the interpretation of potential waves in the cortex; these papers laid the foundation of modern work on electroencephalography in man.

Other famous collaborative papers from the period 1926–1944 were those with Rachel Matthews on the action of light on the eye, with Bronk on the discharge of impulses in motor nerve fibres and with Moruzzi on the motor cortex and pyramidal tract. The paper with Bronk cleared up the long-standing puzzle of the origin of 'tonus' in mammalian skeletal muscle by showing that during weak discharges motoneurons fire at slow rates, ten per second or so, giving an incomplete tetanus in individual motor units but a smooth movement of the whole muscle because the discharges are not synchronised.

In 1937 Adrian who had previously held a Foulerton Research Professorship of the Royal Society, succeeded Sir Joseph Barcroft as Head of the Department of Physiology, a post which required much teaching and administration and which he held until 1951 when he became Master of Trinity.

In addition, Adrian was President of the Royal Society from 1950–1955, of the British Association in 1954 and of the Royal Society of Medicine from 1960–1961, Chancellor of Leicester University from 1957–1971, Vice-Chancellor of Cambridge from 1957–1959 and Chancellor of Cambridge from 1968–1976.

Adrian shared the Nobel Prize with C. S. Sherrington in 1932, was awarded the Order of Merit in 1942 and received a host of other honours which testify to the esteem in which he was held throughout the world; he was made a peer in 1955. He was a superb lecturer and after-dinner speaker and was much in demand in both capacities;

in addition he served on several time-consuming and important committees such as the Medical Research Council, the University Grants Committee and the Chemical Board.

After contemplating the list of positions held by Adrian from 1937 onwards one might be forgiven for thinking that he had little time for research in the Laboratory, or at least that he would be forced to rely mainly on help from colleagues. This was very far from the truth. From 1937 till 1959 Adrian was constantly in the Laboratory and published a number of excellent papers dealing with subjects as diverse as hearing, the sense of smell, vision, the cortex and the thalamus, and using animals as large as a pig or a Shetland pony or as unusual in a physiological laboratory as an alligator or a hedgehog. Much of this work was done alone, Adrian's custom being to dispense with the help of his faithful assistant Hatton as soon as the animal was anaesthetised and a single pair of hands could cope.

Edgar Douglas Adrian was born on 30 November 1889. He read classics at Westminster School and became interested in philosophy, partly at least through his friendship with C. D. Broad. This interest can be seen in all three of his books but most strikingly in *The physical basis of perception* which skates round the problems of mind, brain and consciousness in a most engaging manner. The book also contains a fascinating summary of the experiments which he carried out during the latter part of his scientific life.

In 1923 Adrian married Hester Pinsent who later became famous for her work on mental health; she died in 1967 to the sorrow of a wide circle of friends. Adrian relied greatly on her enterprise and ability and there is no doubt that she made an important if indirect contribution to his scientific achievements. They had three children; Mrs Anne Keynes, Mrs Jennet Campbell and Richard Hume Adrian, FRS who succeeds to his father's title.

After Hester's death, Adrian returned to Trinity, the College which he loved so well, to live in a beautiful set of rooms in the corner of Neville's Court. Until failing health intervened, it was his custom to entertain scholars and many other undergraduates to lunch or dinner about once a week. He remained in Trinity until a few weeks before his death in the Evelyn Nursing Home at the age of 87 on 4 August 1977.

A. L. Hodgkin

¹ *The Mechanism of Nervous Action*, 2 (Oxford University Press, 1932).

² From Adrian's chapter in the account of Keith Lucas published in 1934 (W. Heffer Cambridge), 90–91.

³ *Memorable experiences in research* by E. D. Adrian and others. (*Diabetes*, 3, 17–27, 1954).

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Card faces the inside front cover.

Peristaltic pumps. ChemLab. Models CPP15SB and CPP30SB for continuous flow analysis incorporate a 'standby' facility which enables the pump to be set to operate for 1 s every 15 s. This gives a minimal flow of liquid to maintain a continuous flow system in readiness for operation but avoids wasting reagents with the pump running at full speed while analyses are not actually being carried out. As soon as samples are ready for processing, the 'normal' button on the pump can be pressed and the peristaltic pump then operates continuously at its standard speed.

Circle No. 42 on Reader Enquiry Card.

Digital thermometer. Markson. A new digital thermometer which combines the convenience of battery operation, portability and laboratory accuracy has a selection of thermistor-type probes which permit a variety of laboratory application such as oesophageal, rectal, skin, and oral temperatures as well as water bath, incubator, cuvette, and air surface determinations. The 3-digit instrument offers a measurement range from -30°C to $+100^{\circ}\text{C}$ in two stages.

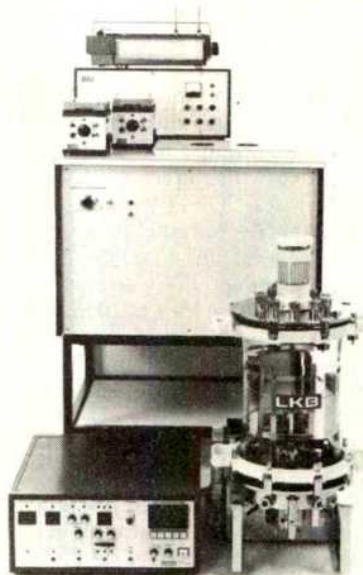
Circle No. 43 on Reader Enquiry Card.

Miniature welding torch. British Roto-therm. A precision engineered miniature welding torch. The Little Torch is not much larger than a fountain pen yet has the capabilities of more conventional welding equipment while having a considerably higher degree of accuracy. With tip orifice sizes as small as 0.003 inch, which are jewelled for precise flame control, and the ability to achieve flame temperatures up to $6,300^{\circ}\text{F}$, it is capable of welding the majority of 'weldable' metals and alloys ranging from 0.001 inch wire to 16 gauge sheet. Also, it can be successfully used for welding many other materials including glass and ceramics.

Circle No. 44 on Reader Enquiry Card.

Microbiological Flow Calorimeter. LKB. This instrument provides non-destructive, continuous, direct monitoring of processes in micro-organisms in the conditions found in a culture vessel. Microorganisms are pumped continuously from the culture vessel through the microcalorimeter's flow-through cell. Because of the high sensitivity of the detection method (requires only 10^5 – 10^6 cells per ml), reactions can be assayed directly: only the microorganism and its substrate(s) are required. Microorganisms are unchanged by the assay, and can thus be returned to the culture vessel or used for further assays.

Circle No. 45 on Reader Enquiry Card.



LKB Microbiological Flow Calorimeter

Portable flocculator. Voss. This portable flocculator measures $52\text{ cm} \times 33\text{ cm} \times 18\text{ cm}$, is lightweight and can be carried easily. When the case is opened it acts as a base for the flocculator. It consists of a 4-bank stirring unit with flat propeller blades $60\text{ mm} \times 25\text{ mm}$. The diameter of the stirrer shafts is 6.3 mm and the centre between paddles is 10 cm. When paddles are withdrawn there is a clear height from base to underside of paddle of 15 cm. The stirrers are driven by a geared motor at speeds between 25 and 250 r.p.m. A thyristor control enables fine speed control.

Circle No. 46 on Reader Enquiry Card.

Replica plating set. Embio Equipment.

A labour-saving equipment for the replica plating technique has now been developed which uses glass beads, a picking device, a bead sieve and a new type of replicator. A sterile glass bead is sucked by vacuum on to the tip of the picking device, by covering a hole in the device with a finger. The bead, so held, is dipped into a bacterial colony and immediately transferred to a hole in the sieve, which is in position on a nutrient agar plate. The glass bead is left in the sieve and the procedure is repeated with another glass bead. The bead sieve can accommodate up to 100 beads on a single 9-cm plate. The bacteria-carrying beads are thus inoculating the plate. The beads and the sieve are then easily removed from this plate. Additional replicates of the colonies that appear on this master plate after incubation can be made by using the replicator. Twenty successive replicas are easily obtained.

Circle No. 47 on Reader Enquiry Card.

Digital film thickness monitor. Nanotech. Many vacuum deposition processes require the control and indication of increasing film thickness during the deposition cycle. The model NQC1 is an economical instrument for this purpose. The deposited film thickness is read directly on a 4 digit LED display, a thumbwheel switch allows the density of the material being deposited to be entered to ensure the correct thickness reading using any deposition material. The thickness of the deposited material is measured by the alteration in the resonant frequency of a quartz crystal located close to the material being coated. The maximum thickness that can be measured before replacing the crystal is equivalent to $12\text{ }\mu\text{m}$ of aluminium or proportionately less for more dense materials. Alternative configurations of crystal holder are available to suit different applications. These include water cooled sensors for high temperature ambients, also special filter arrangements to extend the quartz crystal life in high rate deposition processes such as Ion Plating.

Circle No. 48 on Reader Enquiry Card.

Plasma etching unit for electron microscopy. Nanotech. The Plasmaprep 100 includes a Pyrex chamber 150 mm × 100 mm diameter which is externally excited by a variable supply of up to 100 W RF power. Access to the chamber is gained by a vacuum sealed hinged door, the pressure inside the process chamber is controlled by a gas inlet needle valve with associated flow meter. The chamber is evacuated by a rotary vacuum pump to a normal working pressure in the 0.5 to 1.0 torr region. Control of the etching process is simplified as the equipment is semi-automated; when the chamber has been evacuated the RF power can be varied and tuned to match the chamber pressure and loading. The choice of reagent gas dictates the reaction with the specimen; typical gases used are oxygen, hydrogen and fluorinated and chlorinated hydrocarbons. Reaction products are removed in the gas stream.

Circle No. 49 on Reader Enquiry Card.



Nanotech plasma etching unit

Rotating anode X-ray generator. Marconi-Elliott. The new rotating anode X-ray generator operates at currents up to 300 mA at 50 kV on a focus of 0.5 mm. This new high power (15 mA kW) generator greatly extends the field of application and brings the advantage of short counting and exposure times as well as focal spot sizes down to 0.1 mm to all crystallographers in both research and industrial laboratories. It is the first X-ray diffraction equipment to comply in every way with the forthcoming statutory safety regulations under the Health and Safety Act. The GX21 generator has been specially designed to prevent the occurrence of any accidental exposure of personnel to a significant level of dangerous radiation. A transparent radiation shield can be provided to protect the operator from hazards from all diffraction instruments which are not themselves shielded.

Circle No. 50 on Reader Enquiry Card.

Disposable filter units. Variable Volumetrics. Completely disposable miniature liquid and gas filters, Balston Microfibre Disposable Filter Units are

specially packaged for laboratory use in boxes of five filters each and are available in a range of filtration grades which make them suitable for such laboratory functions as filtration of reagents and samples to liquid or gas analysers, filtration of liquids fed to liquid chromatographic columns, filtration of precious metal solutions without danger of leakage, sterilisation of compressed air and other gases, and complete removal of oil, dirt and water from compressed air and other gases. The units are also ideal for use as vent filters on microbiological reactors to prevent loss or escape of microorganisms.

Circle No. 51 on Reader Enquiry Card.

Industrial conductivity transmitter. Philips. The PW 9521, can be used with the Philips PW 9570 flow cell to provide accurate conductivity measurement even when the monitoring electrodes become contaminated. Designed for flow line applications, the PW 9521 automatically compensates for contamination caused by impurities such as oils, fats and emulsions and eliminates polarisation effects, making possible reliable long term measurements with greatly reduced maintenance. When used with a platinum or nickel resistance thermometer, the PW 9521 can be adjusted to provide automatic temperature compensation for a wide variety of solutions with differing temperature characteristics. The PW 9521 can accommodate measurements from 1 ms cm⁻¹ full scale to 1,000 ms cm⁻¹ full scale; the conductivity range being converted to a 0–20 mA or 4–20 mA signal, providing standard inputs to associated monitoring or control systems.

Circle No. 52 on Reader Enquiry Card.

Refrigerated bench top centrifuge. MSE. The Chilspin centrifuge has a refrigeration unit built in and although designed as a bench top model it is easily converted to a floor-standing unit. Chilspin has a maximum speed of 6,100 r.p.m. (6,030g), and four rotors with trunnion carriers and adaptors provide the versatility needed beyond the standard swing-out capacity of 4 × 100 ml, 8 × 50 ml or 16 × 15 ml. Controls include a temperature regulator from +5 to 15 °C, speed indicator, automatic timer and electric brake. Chilspin complies with latest international safety requirements including a positive lid lock, high grade steel guard ring and a hydraulic lid stay.

Circle No. 53 on Reader Enquiry Card.

Column packer. Micromeritics. One of the major recurring costs to the user of liquid chromatographs is column replacement and packing. The Model 705 stirred-slurry column with micro-

particle materials. The pumping unit of any HPLC system can be readily adapted to pressurise the Column Packer which is best used at 6,000 p.s.i. In the 705 the packing material is dispersed in a suitable liquid and then stirred vigorously while being fed into the column. The continuous stirring ensures that a continual homogenous suspension is fed into the column. The technique is simple and fast and it takes about 30 min to pack a 4 mm i.d. × 25 cm column with 10 μm silica gel.

Circle No. 54 on Reader Enquiry Card.



Beckman analyser

Glucose and B.U.N. analysers. Beckman. The Glucose Analyser 2 and BUN Analyser 2 incorporate clinically proven methodologies and advanced circuitry for easier, faster, clinical determination of glucose or blood urea nitrogen. Both instruments are simplified to one-step operation. After calibration the operator injects a 10 μl sample, which automatically initiates analysis. In 15 s the reading in mg dl⁻¹ or mmol l⁻¹ is indicated on the digital display. Three pumps assure fresh reagent in exact measure. A drain pump empties the sample cup, a fill pump refills it with fresh reagent, and a sip pump removes excess fluid until the precise amount remains. Lights on the control panel indicate when to sample and when to read. Instrument chemistries are clinically proven and accepted. The Glucose 2 is based on the oxygen rate method; the BUN 2 uses the conductivity rate method. Both instruments provide answers in 15 s, an advantage in STAT and routine laboratories. Routine output is up to 67 determinations per hour. The 10 μl sample requirement is a further advantage.

Circle No. 55 on Reader Enquiry Card.

Automatic wow and flutter meter. B & K Laboratories Ltd. The 6203 is a small, easy to use but accurate instrument using analogue and digital techniques to measure peak flutter and drift in sound recording and reproduction equipment. It will measure flutter and drift down to 0.001% and 0.01% peak respectively.

Circle No. 56 on Reader Enquiry Card.

nature

13 October 1977

Scientists in public

It is traditional for scientists to return to Europe from visits to the United States with their batteries recharged and a whole host of new ideas picked up in haste to be tried out at leisure. And it is equally traditional for those first few days after re-entry to be days of frustration, with limited funds, petty bureaucracy and inferior telephones. Gradually, however, there occurs a readjustment to the Old World and its range of values, almost like a shift of cultural Circadian rhythms. And the British can console themselves with the often-heard assertion that we may not have the greatest industrial vigour in the world, or a very significant growth in Gross National Product, but when it comes to the arts and sciences there are immense riches, and these are continually being fed by bright youngsters who, by some pleasing quirk of genetics or environment, seem to keep on coming in their droves.

Now much of this may well be true, although certainly as far as the sciences are concerned lack of industrial vigour is leading to a lack of new opportunities for young people to use their talents, which in a very short time could lead to young British scientific talent being famed mostly through its widespread presence in the laboratories of other countries. But one area in which the trans-Atlantic traveller certainly ought to notice that the United Kingdom is already painfully lacking in comparison with the United States is in the matter of the scientist and public life. It is not just that science has a more visible rôle to play in the United States, although this is undoubtedly true of a country with a vigorous space programme, a highly technologised military, serious energy problems, a commitment to the latest in telecommunications, micro-processors and so on. This inevitably means a wide, mature exposure for science in the media, with all the concomitant benefits. But it is also that the individual scientist is welcomed much more openly and equally into the discussion of public affairs and is expected not just to make specialist inputs appropriate to his or her discipline, but also to be the broad and balanced person that in Britain the arts graduate is expected to be.

This phenomenon not only occurs through the

existence of the in-and-outer in the higher ranks of public service in Washington—a category, incidentally, which will probably diminish as President Carter's campaign for purity on conflict-of-interest bites more deeply. It is to be seen also in the proliferation of quasi-governmental and private institutions around the nation devoted to public policy, which think it entirely natural to hire—and fire—scientists in numbers. It is certain that amongst them are going to be many who will just conform to the seedy wheeler-dealer image. But the system has thrown up and will continue to throw up many scientists who acquire a grasp of generalities and an overall profundity rarely to be seen in British circles.

Now many scientists on both sides of the Atlantic undeniably want, and should be granted the right to stay perfectly happily out of the public eye, getting on with their research. But as science and society cross each others paths more and more, society ought to be asking a growing number of scientists to abandon their specialisations, even temporarily, and to worry about broader issues.

We have, of course, in Britain an excellent and highly professional civil service, and it performs many functions admirably in the matters of science and public policy. But we pay a price for its very professionalism and that is a resistance to outside pressures. And with negligible movement into or out of the service either by administrators or 'professionals' above the age of about 30 there must be the most serious questions about whether the service doesn't waste genuine human resources. Here is a question which the Commons Select Committee on Science and Technology, if it were to be looking for some new and difficult ground to break, could well investigate. The role of the scientist in British society and the problem of too much compartmentalisation, which discriminates against the free flow of ideas, is a subject of broad interest. The results of an investigation would hardly be spectacular, nor given to immediate and drastic action. But long-term benefits could be substantial if the scientist who so wishes could be encouraged to lead a fuller life and to provide more in the way of public service. □

GDR's state of science

Scientific exchanges with the German Democratic Republic (GDR) are rare, and our knowledge of the state of its science is consequently sparse. A correspondent with some experience of East European countries, who recently completed a trip to several biochemistry and microbiology laboratories in the GDR, returned with these impressions.

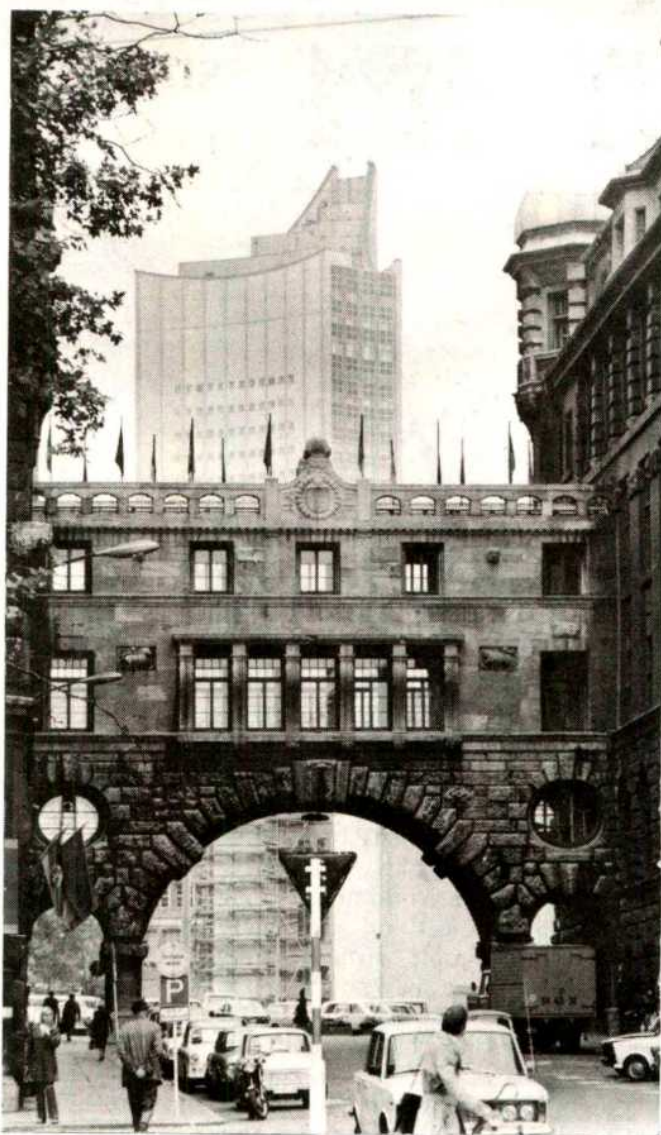
THE mood among the scientists with whom I spoke is one of frustration and pessimism. The scientific community is thwarted and repressed by the government, and the situation is, if anything, worsening. I would put it as more critical than, say, in Poland or the Soviet Union. The very possibility of doing research in the GDR was a subject of discussion. The problems are of two sorts, financial and political.

One of my stops was a large city with a prestigious university. The contrast between the newly constructed university skyscraper, to be used primarily for the study of Marxism-Leninism, and the shabby buildings of the science complex made a striking impression. The biochemistry laboratory which I visited was not adequately equipped. The laboratory, with a staff of some 20 researchers, had, for example, no scintillation counter. In addition, some of the equipment which they did possess was of inferior quality. In particular, the ultracentrifuges, of East German manufacture, can be run for only a few hours at a time, or the drive will fail. Aggravating these financial problems is the peculiar bureaucratic regulation that purchases be made only once a year. This both wastes the meagre resources available to the laboratory—chemicals are ordered which are never used—but also leads to spot shortages which may abort an ongoing project.

Hard currency restrictions make subscriptions to Western scientific journals difficult to obtain. The biochemistry group had subscriptions neither to the *Journal of Biological Chemistry* nor to the *Journal of Molecular Biology*. The restrictions also greatly reduce the number of GDR scientists who can attend conferences in the West. At the time of my visit to another laboratory, for example, only one scientist out of some 800 total staff members was in the West. While science was a low priority area, the support of athletics was lavish. The new municipal stadium was monumental. Furthermore, much hard currency goes to support the hordes of javelin throwers, swimmers and runners who are treated to expensive trips to Western Europe and North America.

The political situation complements the economic one. The government plays a very direct role in laboratory affairs. Needless to say, permission is needed to attend a scientific conference outside the GDR, but is also needed, for example, to publish an article in a Western scientific journal. The government can and does direct a laboratory programme; a microbiology laboratory on my itinerary had been ordered to abandon its programme of basic research and to commence work on a project which the administrators felt had industrial applications. Even in basic research, 'plans' are delivered to the researchers detailing experimental 'goals'. With the exception of the Communist Party members of the staff, these 'plans' are largely ignored. Recently, however, it was decided that the laboratories must hire more Party members. For one group, this meant that all future appointments at the level of Laboratory Chief or above would have to be filled by Party men.

The authorities have also found that science administration is a good place to put retired military personnel. In



Camera press

Leipzig, with Karl Marx university in background

some cases these ex-colonels have chosen to classify a laboratory as 'secret', shutting it off from communication with the rest of the scientific community.

Pressure is applied on the scientists to join the Communist Party, but it is my impression that this is far from successful. A valid reason for not joining is the profession of a religious belief, and this excuse is used frequently. Although promotions, trips abroad and other rewards are given preferentially to Party men, these inducements are not sufficient to overcome the moral repugnance which most of my informants felt towards Party membership. Other scientists, less estranged from Communist ideology, spoke of "compromises" that have to be made in order to work effectively.

In spite of these difficulties, science has not been abandoned; research of good quality is still going on. Efforts to keep up with scientific progress are evident. Especially apparent is the enthusiasm with which Western visitors are greeted; the GDR scientists clearly want to establish contacts and exchange ideas with their Western counterparts. If we wish to aid them, we must visit their laboratories and attend meetings in the GDR, since our Eastern colleagues cannot easily leave their country, or initiate contacts with Western scientists. The exchange of scientific information, as well as gifts of subscriptions to journals or of chemicals and laboratory supplies could help science survive. □

International science (1)

IMS at the crossroads

The success of next week's ISEE satellite mission is crucial to the IMS. Judy Redfearn outlines why

PART of the International Sun-Earth Explorer (ISEE), the second major satellite mission in the International Magnetospheric Study (IMS), was due for launch this week aboard one of NASA's Delta launch vehicles. But because of delays to NASA's launch programme caused by the failure last month of the Delta rocket carrying the European Space Agency's (ESA) Orbital Test Satellite (OTS), the ISEE launch has been put back, probably until 19 October. If the launch fails to go ahead then, or at least by the end of the month, it will face a lengthy hold-up because the current period of short daily launch windows will then have passed by; the ISEE mission would have to remain earthbound until next spring.

Magnetospheric scientists will be watching the launch with a degree of apprehension for another reason. Earlier this year, a Delta launcher failed to send Europe's Geos, the first IMS satellite mission, into geostationary orbit. Although the consequences are not as severe as was first thought, the IMS programme has suffered a setback. So the success of ISEE is now more important than ever to the success of the IMS. If all goes well next week, a unique programme will begin to find out the precise mechanisms governing the magnetosphere, the area of the near-earth environment where the solar wind interacts with the earth's magnetic field.

For the first time two satellites, NASA's ISEE A and ESA's ISEE B, are to be launched in tandem aboard a Delta 2914 rocket into the same highly elliptical orbit. ISEE B, the 'daughter', will follow ISEE A, the 'mother', at a distance varying between 100 km and 5,000 km. The satellites will carry carefully matched experiments allowing them both to make comparable measurements of the same phenomena. Because they will be moving at a known speed and distance apart, they will be able to distinguish between transient phenomena and those which are permanent but vary in space. A single satellite suffers from not knowing whether a phenomenon has moved past it or has died out.

Crossing boundaries

Although many phenomena are known to exist in the magnetosphere, they are not well understood because of the problem of distinguishing spatial from

temporal variations. In particular little is known about the processes which occur at the boundary where the solar wind meets the magnetosphere. The highly elliptical orbit of ISEE A and B was designed to maximise the number of crossings of this boundary, or bow-shock, during the satellites' three-year lifetime. The orbit chosen has a 59-hour period with a closest distance from earth of 280 km and a furthest distance of about 140,000 km. It will also carry the satellites through other boundaries, such as the magnetopause, plasmopause and neutral current sheet.

In June next year, ISEE A and B will be joined by the third and final part of the ISEE mission, ISEE C. Unlike A and B, C will be launched into an orbit around the sun at a distance of about 1.6 million km from the earth. For fuel economy, the orbit was chosen to lie on a libration point where the gravitational forces between the sun, earth and moon almost cancel out.

While A and B are measuring processes in the magnetosphere, from its position in deep space C will be monitoring variations in the solar wind and sending information on solar particles back to earth an hour in advance of the particles reaching earth themselves. The data will give a picture of the solar wind which will help in understanding observations made by the A and B satellites.

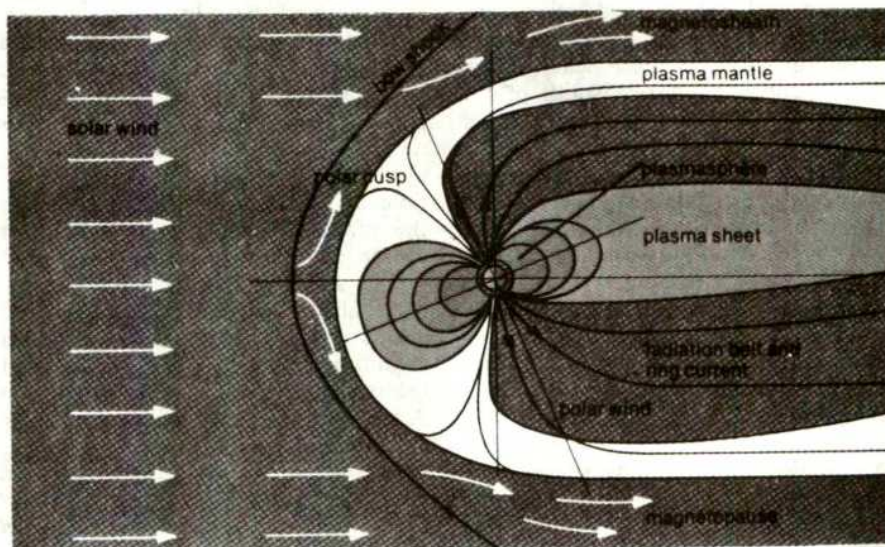
At the end of the mission, scientists should know a lot more about the solar wind and the processes governing the magnetosphere, which may in turn lead to a better understanding of radio

transmission on earth and some of the mechanisms effecting climate. The magnetosphere, a natural plasma laboratory surrounding the earth, is like the earth's own small universe, the solar wind taking the part of the hot plasma which makes up most of the matter in space and the earth's magnetic field taking the part of the magnetic fields found in space. The study of how ionised particles interact with the earth's magnetic field should yield much about the fundamental processes which govern the behaviour of cosmological objects such as radiogalaxies, pulsars and radio and X-ray stars.

Setting up the IMS

By the late 1960s scientists had a good picture of the magnetosphere, but they knew little of the dynamic processes controlling it. The International Magnetospheric Study was set up as a co-ordinated attack on the remaining problems. To organise the study, the International Council of Scientific Unions (ICSU) Special Committee on Solar-Terrestrial Physics set up an IMS steering committee with representatives from various scientific unions, COSPAR and satellite launching agencies. Thirty-nine countries are now taking part in the IMS, which began in 1976 and is due to run until December 1979.

It is the first international programme of its kind to be based on spacecraft flown by several different agencies. Fourteen spacecraft launched during the IMS period are planned to carry experiments directly relevant to the study and 25 launched before 1976 are expected to add valuable data. But at the centre of the IMS are two spacecraft missions devoted entirely to magnetospheric science—Geos and the ISEE. In addition, Japan is contributing with its magnetospheric satellite, Exos, and Russia with a near-earth satellite.



Cross-section of magnetospheric plasma distribution

The spacecraft programme, however, would be of little value without the numerous ground-based rocket and balloon projects which will continue throughout the IMS period. Earth-based measurements of variations in the magnetic field can be used to monitor magnetic storms and plasma waves and even to predict changes in the magnetosphere. Correlating these measurements with satellite observations should indicate how changes in field strength affect the shape and dynamics of the magnetosphere. Many of the 39 countries which could not afford to be involved in the space programme are contributing to the IMS through a sophisticated world-wide network of ground observatories. Even at the beginning of 1976, more than 1,000 individual research projects were registered on the IMS computerised file.

Many different experimental programmes working towards the same end thus need coordinating. Occasionally, for example, two or more satellites may be in such a configuration that

simultaneous observations would be very valuable. Similarly, satellite observations should sometimes be timed in with ground-based or rocket experiments. To coordinate observations, NASA helped set up an IMS Satellite Situation Centre at the beginning of the study at the World Data Center A for Rockets and Satellites at the Goddard Space Flight Center, Boulder, Colorado. Its function is to report on satellite positions, recommend periods of special interest, compile and up-date information on satellite experiments and answer inquiries on special projects, experiments and the whereabouts of satellites. Every month, the IMS Central Information Exchange Office, also at Boulder, produces an IMS news letter.

Regaining ground

Hopes for regaining the ground lost by the partial failure of Geos hang on the possibility of launching the second Geos satellite into geostationary orbit early next year. The intention had been to place the first Geos in a geo-

stationary orbit 36,000 km above the equator along a field line connecting the north and south auroral zones so that particles on their way to the aurora would pass through it. Ground-based experiments and rocket-borne observations of the aurora could then have been coordinated with experiments on board Geos.

But Geos is not in a geostationary orbit and coordinating observations is very difficult. It would have been impossible had the Geos ground control at Darmstadt not managed to manoeuvre the satellite into a doubly geosynchronous orbit shortly after the unsuccessful launch. Geos now passes through the neighbourhood of the planned geostationary orbit twice a day, meaning that coordinated observations are possible, but only for a very short time. If Geos 2 were launched next year, it could take the place of Geos 1. It would have to be launched on another Delta rocket, however, and ESA might decide to wait until the end of 1979, when it could be launched on Europe's launcher, Ariane. □

International science (2)

WHO's show on the road

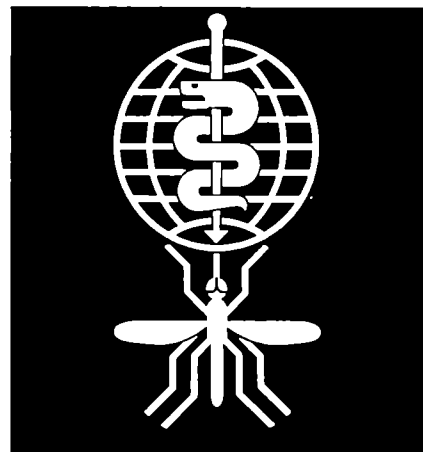
Peter Collins describes the gestation of the World Health Organisation's tropical diseases programme

THE temptation to launch global campaigns against specific diseases or conditions of mankind is one the World Health Organisation (WHO) seems unable to resist. The most recent and from all appearances most successful is the smallpox eradication campaign. Pockets of the disease remain, principally in the horn of Africa, but the campaign's objectives are in sight in under ten years. By contrast, in spite of the malaria eradication campaign, which started in 1955, malaria has never been anywhere near eradication in much of Africa south of the Sahara, and is now increasing in other regions and countries where eradication was thought to have been achieved. Now WHO's thinking has turned towards a more broadly-based approach. It is well exemplified by the Special Programme on Research and Training in Tropical Diseases which is tackling six diseases (malaria, schistosomiasis, the filariases, trypanosomiasis, Leishmaniasis and leprosy) that affect some 700 million men, women and children.

Perhaps the greatest single constraint on the development of the tropics lies in the burden of disease borne by their human inhabitants. Probably one in every four people alive

today is at risk from one or more of the six diseases with which the programme is concerned. The position worsens every year as population increases and the world's pharmaceutical industry stands back. No new drug against trypanosomiasis has appeared in the past 20 years; the only drugs available against the filariases have side effects so serious that their use for mass treatment is out of the question; and the drugs used for treating malaria are meeting with more and more resistance on the part of the parasites. Added to this is the increasing resistance of many of the vectors of these diseases to the chemicals used against them, and the many reservations about the use of DDT, still often the most effective weapon for insect vector control.

The sheer lack of scientific knowledge about many of these diseases is also a problem. Not enough is known about the biology of the parasite and the mechanisms by which it eludes the defences of the human host to allow intelligent intervention to be planned. But great advances in such fields as immunology have led to the evolution of a whole armoury of new weapons for the prevention, diagnosis and treatment of disease afflicting richer



WHO anti-malaria emblem

countries, and the question is how these can be made available or adapted to help control the great endemic diseases of the tropical countries

WHO's answer

The present programme is WHO's answer to this question, but it originated within WHO from the conjunction of two quite separate ideas. In 1964 an expert group of immunologists and parasitologists was assembled for the first time in Geneva. Little common ground seemed to exist between these two disciplines thereto: the parasitologists considered that there was little if any immune response to the diseases with which they were concerned, and the immunologists had found ample scope, backed by adequate funds, in fields like cancer research. After this meeting, however, things began to change, and while the immunology of

parasitic diseases was being examined, another initiative was being taken within WHO—the establishment in certain African countries of a network of biomedical research centres, eventually to be staffed and run by local personnel. The feeling was that only research by nationals of the countries concerned could achieve real control of tropical diseases.

It was from a 'meeting of minds' among senior staff involved in these two developments that the present programme evolved. By 1974 their ideas were ready for presentation to the World Health Assembly and subsequent examination by WHO's Advisory Committee on Medical Research (ACMR). The basic plan, further discussed with prospective participants, was developed in time for a major 'housekeeping' meeting in December last year. The pattern is that of a co-operative effort of donor and recipient governments, international agencies and specialised institutions, collaborating with and coordinated by WHO. A major part as co-sponsors will be played by the United Nations Development Programme (UNDP) and (though its precise rôle has yet to be determined) the World Bank. Because the programme involves a number of different WHO departments, a small Tropical Diseases Research Group (TDR) has been set up for overall scientific coordination and management. Progress will be reviewed annually by a Scientific and Technical Advisory Committee composed of scientists, selected on an individual basis, to make the best existing knowledge and experience available to those directing the programme. General oversight will be ensured by a Joint Consultative Board, expected to be convened later this year and designed to represent all the various groups of participants in the programme.

The programme is expected to cost between \$10 and \$20 million a year as presently foreseen. This money will come from governments through their development aid or technical assistance allocations, and from other sources of international funding. It is here that WHO might have anticipated some difficulty, for those concerned with development financing are naturally somewhat cautious when asked to support what is, admittedly, a vast experiment, at a time when funds for development projects are increasingly tight. Several different approaches to this type of financing are already apparent. The Scandinavians prefer to hand over funds directly, often seeming to take little interest in their management, although they may now be looking harder at the results than hitherto. The USA, on the other hand,

appears to favour complete control of the programme funds, preferably by the World Bank. Britain's fairly neutral stand on that point is tempered by an insistence on continuing and critical evaluation of both the selection and progress of individual projects. Even at this early stage, several governments, Britain's included, have been highly critical of the potential of certain projects to which programme funds have been allocated by WHO. Intriguingly, it does seem that one problem is to find sufficient projects to absorb the funds already available, a situation that should reassure those who fear that the programme will prove to be yet another drain down which development funds are being uselessly poured.

Two lines

The two main lines of action that make up the agreed programme reflect its dual origin within WHO. The first is a vastly increased research effort directed at some of the main problems of the six selected diseases, and involving many of the ideas and techniques developed by biomedical research into the diseases of the industrialised, affluent world. Where possible, this research should be done where the diseases occur, and eventually (though this is not yet feasible) by nationals of the countries concerned. The second recognises that research, however successful, is of little use unless it can be properly exploited. This means training nationals of the tropical countries in the design, administration and application down to village level of whatever control system is finally evolved.

The underlying approach is not without precedent as far as WHO is concerned. Its programme for research into human reproduction was set up on much the same lines: a group of scientists was assembled, presented with certain ideas, asked if they thought a programme based on these ideas could be made to work, and urged to indicate the steps to be taken. This in itself was something rather new; biologists are not usually asked to do research with a definite end in view, and the human reproduction programme is one of WHO's current successes. That the system can work is also shown by the Scientific Working Group on the immunology of leprosy, which was set up in the same way and is already yielding results.

The parasitic diseases programme is of a different order of magnitude, though. Each of the scientific working groups is in itself an experiment directed to separate objectives. Apart from those dealing with leprosy, groups on filariasis, schistosomiasis and the immunology of malaria have been set up; others will be concerned with

such broad fields as vector biology and the tissue culture of parasites. Each group examines its particular disease under headings like immunology, chemotherapy, epidemiology and training, and identifies desirable research projects and the institutes and individuals to tackle them. WHO envisages a network with a coordinating group at headquarters. On the one side are the 'collaborating institutes', the established centres of biomedical research, mostly located in the industrialised countries. Their collaboration will be sought for the more sophisticated research especially where new and special equipment and facilities are needed, and for training at the higher levels. On the other side are 'collaborating centres', at various levels from large university departments to small laboratories employing only a handful of qualified staff, and located mostly where the diseases are prevalent. It is in this group that the 'institution strengthening and training activities' of the programme will have first priority.

No cutting

As WHO sees it, there is no need for the programme to cut across the activities of the established institutions in the industrialised countries. But with too many people doing research into tropical diseases who have had little or no contact with those suffering from them, a properly coordinated operation of such a network should help progress. WHO would make a further point, that the programme offers opportunities that are, scientifically speaking, exciting, with the use of the new tools and high technology of modern biological science. The different approach to publicising the programme reflects this. In the past, publicity has been given to global programmes of public concern, with the aim of telling people about something being done for their welfare—the conquest or alleviation of rheumatism, for example (1977 is World Rheumatism Year). For the tropical diseases programme, WHO's aim is rather to interest the scientific community.

With the gradual run-down of the great colonial medical services, tropical disease research has been increasingly less popular. But now, the more people who know of this vast new programme and of its funding, the greater likelihood there is of interesting young research workers in the opportunities it has to offer. And at some \$20 million a year for, say, 20 years, it is not that expensive. A major pharmaceutical company can spend five times that annual sum in a single year, and some countries spend as much on cancer research as the total budget for this research on tropical diseases. □

BRITAIN

Blunt then blunted

The UK Medical Research Council (MRC) published its latest annual report earlier this week. Chris Sherwell reports

APART from being mere snapshots of the period they describe, annual reports suffer by emerging halfway through the following year. Introducing the first report since he became MRC Secretary, James Gowans quickly addressed one problem by communicating a vivid impression of the dynamic quality that informs current medical research. But the need to bring everyone up to date blunted the impact of the report's potent, if measured, criticisms of the way some of that research is now run.

The report, which is for the twelve months to March, describes the impact of a £1 million (10%) cut in commissioned research from the Department of Health and Social Security (DHSS). It says the Council was "deeply concerned" at the size and suddenness of the reduction, which "disrupts financial planning and frustrates initiatives" and affects both commissioned and other research. But the outlook was now less bleak, Gowans told his audience. The DHSS would be giving back £700,000 next year, and the MRC "firmly expected" a 1.6% real increase in its share of the Science Budget in the coming year.

Gowans was apparently concerned also to moderate the tone of the report's criticisms of the operation of the customer-contractor principle, under which the DHSS acquired control of one-fifth of the MRC's funds. A series of rhetorical questions in the report suggests that responsibility for any lack of orientation of research work to social objectives cannot be laid at the MRC's door. The report also describes at length how administrative work and accountability requirements have imposed heavy burdens and taken experts away from research—"a high price to pay" for arrangements which could be achieved "with less

formality and administrative expense"

Gowans amplified the first of these points and dwelt not at all on the second. He described specific cases—the latest being the Blood Group Reference Lab—of the MRC deciding to start something new and then handing it on to an enthusiastic government; the MRC, he said, was anticipating national needs long before the customer-contractor principle came along. The ease with which the MRC programme merged with DHSS requirements, he added, also vindicated the MRC's judgment; the DHSS wanted the MRC to do what it was doing anyway. But the conclusion he drew was that DHSS-MRC relations were very close. The "one proviso" was a complaint about bureaucracy.

If these pulled punches reflect the happy amelioration of financial problems, other thrusts in the report indicate that additional anxieties remain. The organisation of clinical research is "becoming increasingly difficult" as the incentives for young doctors to do research have diminished, and there is an "apparent tendency to lose sight of research as a proper component of clinical training". An appropriate pattern of support for university research when universities are not expanding has also yet to emerge, and is "of crucial importance" if the flow of able young scientists into research is to continue; holding research teams of high quality together will be "increasingly difficult" unless the problem of low numbers being absorbed into permanent university appointments is solved.

In the stringent economic circumstances the Council describes its first priority as reducing long-term commitments in order to free money for new work. Competition for long-term support will thus be fierce, it says, but short-term awards will now increase, and it urges the scientific community to continue to apply for these.

On the subject of recombinant DNA experiments, the MRC says it is looking forward to receiving proposals (four

large project grants have already been made to universities); "high priority" should be given to the provision of centralised facilities for category III and IV experiments. The MRC in fact has no plans to build a category IV facility. The future of the only one in the country, the Microbiological Research Establishment at Porton, is now under consideration by the government. An MRC committee set up to examine the possibilities it offers is due to report soon □

Research report

THE massive UK Department of the Environment (DOE) last week released a slim fourth annual report on research and development—covering a period that ended a full 18 months ago. A press release accompanying the report, which deals with the last full year before the department was divided into the Departments of Environment and Transport and before Martin Holdgate succeeded Dennis Lyons as Director General of Research, does not explain the delay. But it does advise, without amplification, that recent organisational changes under Holdgate will be described in the 1976-77 report. Holdgate is also expected to outline them to the press next week.

In the meantime, some of the details are available in the September issue of *Environment and Transport World*, the newspaper of the two departments. They are not exactly minor but, unsurprisingly perhaps, a couple are apparently mere name changes; thus, the Directorate of Research Requirements has become the Directorate of Research Policy, and the Research Secretariat has become the Research Administration Division. The Systems Analysis Research Unit (SARU), on the other hand, which has been looking at global problems (see page 356), has not changed its name but is, according to the paper, "increasingly turning its attention to problems on the domestic front".

Perhaps the most important change is the reduction from fifteen to six in the number of internal Research Requirements Committees. These will consist of what Holdgate calls "triads", three-man teams with a representative from the policy side who want particular work done ('customers'), from department research establishments or other research centres ('contractors'), and from the central research policy division. The six committees embrace areas controlled by senior civil servants like planning, countryside and resources, transport, construction and environmental protection.

As for last week's report, it says expenditure on research (at a peak of some £45 million for 1976-77, of which about a quarter went on environmental pollution and resources) is expected to decline sharply to a level in 1978-79 close to that five years ago. This, it says, is a result of constraints on public expenditure and reductions in civil service manpower.

Chris Sherwell

Five-year pattern of MRC expenditure

	Total Expenditure (£million) ¹	Change % ²	Real change % ³
1972-73	28.510 (1.737)	+14.3	+4.1
1973-74	30.330 (5.765)	+ 6.4	+2.5
1974-75	36.293 (8.745)	+19.7	-1.3
1975-76	47.140 (13.148)	+29.9	-0.9
1976-77	52.166 (13.315)	+10.7	+0.9

¹ Figures in brackets are income within the total from sources other than the MRC's allocation from the Science Budget (eg. government departments, health authorities)

² Change in total expenditure over previous year (undeflated)

³ Change, in real terms, of MRC allocation from the Science Budget, over previous year

USSR

Exchange agreement

In spite of new agreements, there is scope for more scientific visits between the UK and USSR. Vera Rich reports

A NEW agreement on the exchange of scientific visits was signed in Moscow last month by Lord Todd on behalf of the Royal Society and Academician Anatolii A. Aleksandrov for the Soviet Academy of Sciences. Although the negotiations took place in what Aleksandrov described as a "friendly and constructive" atmosphere, and although Lord Todd told *Nature* that he felt that "good progress had been made", the details of the latest agreements reveal just how much progress in scientific exchange still remains to be made.

The new agreement provides for a two-way traffic of seven senior and four junior visits per year. Senior scientists will visit the host country for 3–6 weeks and junior scientists for 8–10 months at a time, the total yearly

quota being 40 man-months. This seems negligible compared with the 50–100 short-term visits per year between the UK and the major EEC countries or with UK–Poland exchanges, where there is an annual flow of some 80 visits to Britain and 40 visits to Poland. Leaving aside the special case of the English-speaking countries, one would expect the flow of exchange visits to be roughly proportional to the size and scientific importance of the country concerned. The anomalous position of Soviet–UK exchanges is apparently recognised by both parties; according to Lord Todd, both sides agreed that in so far as finances permit, they would like to see the number of exchanges increased, especially among junior scientists.

There is no great enthusiasm in the UK, however, to take up even the number of exchange places available. One reason is obviously language; most Soviet scientists have at least a reading knowledge of English, but the need for a reciprocal knowledge of Russian has

largely been obviated in recent years by the expansion of cover-to-cover journal translation programmes. It is also extremely difficult for young British scientists to discover how they would benefit from an exchange visit.

The Royal Society delegation pressed for more explicit information on the work being done at the various Soviet Institutes. They were assured that intending visitors could find all they needed to know from journals such as *Priroda* (Nature) and *Nauka i Zhizn* (Science and Life). These semi-popular journals are, in fact, designed to give a general idea of current progress rather than to specify the work of individual scientists or institutes; moreover, they are in Russian (the translation programmes deal with specialist journals only), as is the more informative *Vestnik* (Bulletin) of the Academy of Sciences.

The new agreement does, however, include one rather promising provision—the extension of joint research projects. Working visits between the teams involved would not necessarily come out of the 40 man-months of the basic programme. □

USA

Rock energy

David Davies reports from Washington on the US programme to exploit geothermal energy

WITH the United States importing nearly half of its petroleum now, it is no wonder that even modest contributions to the nation's energy budget assume a growing importance. The geothermal energy programme in the US Department of Energy (DOE) could only displace a few per cent. of the nation's fossil fuel requirements by the year 2000 but is authorised this financial year to spend \$116 million, double last year's authorisation and nearly five times the figure of four years ago.

Resources are divided into three categories of increasing magnitude but also of increasing difficulty of exploitation. Convective hydrothermal resources comprise both dry-steam systems, of which the Geysers field in California (extracting 500 MWe at present but eventually 2,000 MWe) is probably the only US example, and liquid-dominated reservoirs, of which there are many at temperatures upward of 90° C. Geopressured resources consist of methane dissolved in water at

moderate temperatures. The water is in isolated reservoirs at the pressure of the overlying rock, much greater than the hydrothermal pressure, and heat, methane and hydraulic power can all be extracted. The resource is concentrated around the Gulf states. Finally there are 'hot dry rocks', rocks at relatively high temperatures near the surface into which water must be introduced to extract the heat.

Dry steam already provides 5% of California's electrical needs at a very attractive price and has needed little government support. Liquid-dominated reservoirs, on the other hand, have needed a stimulus; DOE has just issued a request for bids to build a 50 MWe demonstration plant in the western United States that will be economic and socially and environmentally acceptable. The department hopes to sign a contract for this in mid-1978. But the real and growing optimism, according to Dr James Bresee, director of the programme, is in the potential of the hydrothermal resource for direct-heat applications.

Geopressured resources have a much larger question mark against them. There are major unanswered issues of reservoir producibility and longevity, and so far there has been little com-

mercial interest. Nevertheless, DOE plans to go ahead this year with well-tests in Texas and Louisiana, and has proposed pilot plants for energy recovery.

The hot-dry-rock experiment at Los Alamos, although it has attracted the widest attention, must at present be regarded as the most speculative of all. A fractured zone has been created at a depth of 3 km in granite, and water is run down one pipe, through the granite, and up through a second. The rising hot water flashes to steam. Less than 10% of the water is lost underground. So far the heat extracted (about 3 MWt) is less than had been expected, so the next few months will be a crucial time for this programme.

Not all the obstacles to geothermal energy are technical. DOE employs about six professionals simply to look at the so-called institutional barriers—tax laws, environmental regulations and so on. At last count at least six governmental departments and over ten major agencies are involved in some way or another. Further, although the federal government has established loan guaranties for up to 75% of any loans to the geothermal industry, the widely-expected flood of applicants has not materialised; so far only two guaranties have been made, apparently because large companies would rather act as their own guarantor. □

CANADA

Add minister

The Canadian cabinet has a new science minister. David Spurgeon reports from Ottawa

CANADIAN scientists are once again feeling hard done by as a result of a federal government cabinet shuffle. Hugh Faulkner, Minister of State for Science and Technology since 1976, has been shifted to the portfolio for Indian and Northern Affairs, and a new science minister, J. Judd Buchanan, appointed in his place. He is the fifth minister to have been appointed since the ministry was established about six years ago.

Grumbling was also heard when Faulkner was appointed just two years ago. But in his short time at the post he earned the respect of many in the scientific community by his obviously sincere concern, his interest in the field and his desire to learn. He did not come to the ministry with what many scientists felt was the ideal background. He nevertheless took time to listen, and many scientists feel they have now lost an ally if not a friend. All Faulkner's homework will now go for naught, and Buchanan will have to start over again.

The shift is still more galling for the scientists inasmuch as it indicates once again that the federal government does not take science seriously. For apart from Buchanan's own lack of familiarity with the field, the task becomes once more a part-time concern of its minister. When C. M. Drury held the portfolio, he also held that of Public Works.

Buchanan will now revert to that arrangement, but he'll have even less time than Drury to think about science because his job has been as a sort of 'lieutenant' for the Liberal party in Ontario. Buchanan went from the ministry of Indian and Northern Affairs to the less onerous Public Works portfolio before coming to Science and Technology in order to have time for these political duties. Now some are wondering how he is going to deal effectively with all three areas.

Some very senior scientists, indeed, are beginning to question the usefulness of a system that depends on firm and effective communications lines being established between a minister and the scientific community, and then breaks these lines with impunity just when they have become established. Perhaps the new minister will be able to prove they are wrong, and that such a system can work well after all. It is a heavy responsibility. □

WEST GERMANY

Solar sell

Werner Gries reports from Bonn on state support for energy research

WEST GERMANY'S support for energy research developed a stage further last month with the announcement from the federal Minister of Research and Technology, Hans Matthöffer, of a large programme of support in the field of solar energy. The federal government has supported research into direct and indirect utilisation of solar energy since 1974, and the new programme, *Technologies for the Utilisation of Solar Energy*, makes available a total of DM166 million over the four years 1977-80 to supplement expenditure by private industry.

Some 76% of the country's total energy requirement goes on heating, and 24% for lighting and power. Space heating alone absorbs 40%, mainly through burning mineral oil. Solar energy is therefore important because it can be used for such low-temperature purposes as hot water supply and heating in residential buildings; but its low intensity and its variability mean an efficient technology is needed. Solar radiation per square metre in Germany is only half that in the USA.

The government's measures to promote solar energy hitherto have extended to 50 projects, requiring a total contribution of DM50 million of government money. Chief of these have been the development and long-term testing of novel types of solar collectors. At the same time mass production processes for solar collectors are being developed and a few demonstration plants both in public installations as well as in the private sector have been built with government funds.

Results so far show that it is technically possible even in West Germany to satisfy fully the heating requirements of a well insulated single-family house with solar energy. The necessary systems and appliances are already being offered on the market but the financial outlay is so high that it does not pay to put them in at present. Therefore, in addition to their R&D measures, the government introduced at the beginning of September a programme to encourage energy-saving investments. This grants allowances of 20% of installation costs for insulation, heat pumps and solar appliances.

The conversion of solar energy for purposes other than low-temperature heating has lower priority, but the federal government is promoting R&D in electricity generation to open up new export prospects for German in-

dustry in countries which have plenty of sun. Solar collectors for hot water supply will receive support in the future. Private investment will be rewarded by allowances, model public building measures will explain the new techniques, and standardisation of the most favourable appliances will have an important place in government promotion measures. But the extension of solar installations for space heating depends on the development of energy storage systems, and R&D in this area is being intensified. Solar engineering systems for heat production up to 200 °C are another important focus of research, and the government is making funds available for demonstration plants to show their practical application.

In another trench on the energy front, meanwhile, discussion of the advantages and disadvantages of breeder reactors and the risks attached to them is becoming more heated, notwithstanding the recent unexpectedly quiescent demonstration at Kalkar, the site for a 300 MW prototype breeder. A recent court judgment called into question the construction of the reactor when the judges requested the Federal Constitutional Court to inquire into whether the building of breeder reactors is consistent with the law as it stands at present.

Arguments have persisted in parliament. The Minister of Research and Technology is responsible for financing the development of breeder reactors, and he has given the Bundestag a detailed account in support of breeder development. A total of DM3,000 million has been distributed by the state for research work in this field, annual expenditure is running at DM470 million, and 8,000 people are employed in research, development and construction. Development is marked out up to 1982 with the prototype at Kalkar and the Franco-German *Superphénix* in France.

Though the idea is being aired of severely reducing breeder reactor development to no more than a minimum, the minister has come to the conclusion that cutting the building work on the prototype would cost just as much as terminating it. Many contracts have still to be fulfilled and high social costs would accrue in paying off workers and paying the stipulated penalties for the co-partners in Belgium and the Netherlands. The government maintains that plutonium presents no unjustified risks, contending that the technology can be mastered and that the potential danger is no greater than with a lot of other materials. □

IN BRIEF

USSR space set-back

The cancellation this week of the *Soyuz 25/Salyut 6* link-up is a serious set-back to the Soviet space-programme and a blow to its prestige, especially as some major exploit to mark the 60th anniversary of the October Revolution was widely expected. It also stresses, once again, the great reliance placed by Soviet planners on automatic control even of manned spacecraft. According to the Tass announcement, the two craft were automatically brought close together, and then a docking operation was carried out from 20 metres. "Deviations from the planned docking regime" led to the decision to abort the mission.

Antarctic meeting

The main achievement of the Antarctic Treaty meeting, which ended in London last weekend, was to agree on interim arrangements for conserving marine living resources. Details were not immediately available but the drift of the discussions has been clear.

First the agreement is voluntary. Second it looks at this stage as if it will be confined to restraints on catch quotas of krill and the exchange of statistics. The agreement can only be temporary because West Germany, which has been active in the field, is not a signatory of the Treaty.

The Treaty powers have also agreed to extend their voluntary moratorium on minerals exploration and exploita-

tion. Experts attending the meeting think that it will be at least five years before enough scientific data has been accumulated to assess the likely environmental effects of any exploration and exploitation.

Telescope agreements

ESA is to contribute a faint-object camera and a solar array to NASA's Space Telescope programme under an agreement signed last weekend. The telescope is due for launch in 1983.

Progress has also been made in the Dutch-American Infra-red Astronomical Satellite (IRAS) project with the award last week of a contract for construction of the satellite by the NIVR in Holland.

EVEN the sophisticated generally believe what they hear on the radio, or see on television. A few days ago these media prominently featured a report by the UK Department of the Environment which apparently dealt with the world's future. Listeners or viewers (I was both) received the impression that a group of economists and scientists had engaged in a complicated and detailed exercise, using sophisticated mathematical models and advanced computers, and after years of hard grind had come up with the conclusion that if the world's population continued to grow exponentially at something approaching 3%, in fifty or more years the forebodings of Malthus, first put forward nearly two hundred years ago, would be realised, and mass starvation would overtake mankind. Other shortages, including that of energy, would be less serious.

My reaction was that here was yet another expensive and pointless exercise, over-supported by government funds, discovering something which was obvious to anyone with a little knowledge of these problems. We have seen many such useless exercises in the past, the most notorious being a Cabinet Office report on Energy; this was obviously just another example of governmental irresponsibility wasting the taxpayers' money.

Before expressing this view, however, I waited until the next day, expecting to find a fuller summary in the serious daily press. I found no mention in the *London Times*, the *Financial Times*, the *Guardian* or the *Daily Telegraph*. I did not consult the tabloids. Now I've received the volume which stimulated the radio and television but did not interest the newspapers. It is Research Report 19 from the Systems Analysis Research Unit

(SARU) of the UK Departments of the Environment and Transport, and is called "Sarum 76: Global Modelling Project". It extends to some 230

Model report



KENNETH MELLANBY

pages, and as it costs no less than £15, is not expected to have a mass circulation.

My reactions to the document are quite different to those stimulated by the radio. It is no facile statement of the obvious. I assume that it did not interest the newspapers because it draws no conclusions, contains no pithy summary forecasting doom, but is a serious and careful study, adding another useful volume to the series of reports from these two government departments.

The writers of Sarum 76 (anonymously, only the Director's name appears) have got together an immense amount of information about

world incomes, populations and their changes, energy, renewable and non-renewable resources and agricultural productivity. The figures and tables alone make the volume worth its inflated price. The team has produced a sophisticated mathematical model which, if you think modelling is worthwhile, is itself a model (in another meaning of this over-used word) for this type of study, and it will clearly be useful for others with similar interests.

The report shows that Sarum 76 can be used to test many reasonable assumptions for the effects of any number of permutations and combinations of the changes in population, productivity, climatic conditions and all the other known factors which may affect our future. It makes no claims to foretell the future, and admits that this is not the ultimate, perfect model for this purpose. There is indeed a figure, reminiscent of some in *Limits to Growth*, which shows that if the world population grows at 2.27% a year, food supplies per head could increase for fifty years and then fall rapidly, but this should be seen in the context of many other possible forecasts.

We are now wedded to acronyms, but where those have existing meanings they should be used with care. ASH is undoubtedly suitable for Action on Smoking and Health, but in England Sarum is the old name for the city of Salisbury, and our Established Church adopted the Sarum Liturgy in 1542. Its use brought back to my mind a limerick—in England, incidentally, Hampshire is commonly abbreviated to Hants:

There was a young curate of Salisbury
Who used to be most harum-scalisbury
He went through Hampshire
Without any pampshire
'till his bishop said he must walisbury

correspondence

Research 'ridiculous'

SIR,—Regarding your editorial of 8 September, it seems to me that the Psychological Association and Dr Shockley and Dr Huxley have all missed the point. The proposed research may be desirable or deplorable, but above all it is ridiculous.

Dr Shockley proposes the existence of close linkage between the (unknown) polygenes for skin colour and the (unknown) genes that determine (unmeasurable) absolute intelligence. Even the existence of "absolute intelligence" is unproven and debatable.

For present day genetics and psychology to tackle this problem is like Antonie von Leeuwenhoek studying the structure of the ribosome. Maybe it will be possible some day, in the far future, by which time, hopefully, we will be getting excited about something other than race.

S. W. BOWNE

Edinboro State College,
Edinboro, Pennsylvania 16444

Using hyphens

SIR,—I heartily endorse J. Faber's plea (19 May, page 202) that semantically essential hyphens be reinstated in compound adjectival phrases. The current practice of abandoning this punctuation nearly always generates serious ambiguity, or even utter gibberish. Thus, a printed label on a recent pharmaceutical product advises me: "Do not take with milk or calcium containing antacids." (In this instance one must presume the reference is to *low* calcium, since a recent report in a reputable journal refers to "high calcium containing phospholipids".)

Faber rather charitably attributes this widespread offence to "sheer sloppiness", but my experience has been that the problem stems largely from a deliberate editorial conspiracy. Critical hyphenation is commonly deleted between manuscript and galley, and frequently the deletion is enforced over the authors' protests concerning the resultant semantic atrocities. If one encounters the phrase "men chasing women" in print, the prevailing editorial policy makes it impossible to conclude which group of participants is pretending to be in flight.

PAUL G. LEFEVRE

Health Sciences Center,
State University of New York,
New York 11794

Solar nuclear waste disposal

SIR,—It has been recognised for some years that nuclear (fission) reactors are power sources for which there is an increasing need in view of growing world demands for power and depleted fossil fuel supplies. Such power reactors provide a reliable bridge to new power technologies, such as fusion and solar energy collection, which are under long term development.

However, serious questions are rightly asked about the real and imagined safety aspects of the increased proliferation and use of fission reactors of various types. One principal area of concern is the need to find acceptable and safe long term methods for disposal of spent nuclear fuel. Many have yet to be convinced of the conventional wisdom of burial of such wastes in deep ocean trenches or in deep stable rock formations.

In the light of our undoubted need for increasing access to nuclear power, coupled with parallel needs for safe disposal of nuclear wastes, I suggest that serious consideration be given to the application of existing space technology to transport such wastes to the sun.

Reliable space transport systems have been developed in a number of countries during the past two decades. They culminate in the Space Shuttle System. Within the next few years the Shuttle will be making regular, and almost routine, manned journeys between the earth and earth orbits to perform a wide variety of tasks. These include transport and operation of Spacelab, launch of satellites, recovery and manipulation of payloads in space using the Remote Manipulator Facility, deployment of Space Telescope and so on.

It is thus suggested that serious thought should be given, at an international level, to the possibility of using Shuttle or Shuttle technology to carry, on a regular basis, safely encapsulated containers of nuclear waste into temporary parking orbits above the earth from which they can be assembled into payloads for vehicles (also carried into orbit by Shuttle) to carry them on to the sun. The development of such a 'sun-bus' system including its propulsion and guidance components is well within the capability of contemporary space technology.

The writer does not overlook the

many economic and safety aspects of this proposal which will have to be considered in the development of this application of space technology to the service of all mankind. It would appear to be as attractive, if not more attractive, from many points of view, as the current terrestrial nuclear waste disposal methods which are used or which have been proposed. It has the strong advantage of not imposing a social burden lasting for many centuries for the monitoring and safeguarding of our legacy of nuclear waste.

R. W. NICHOLLS

Centre for Research in Experimental
Space Science, Ontario

Farm energy

SIR,—In his recent contribution Kenneth Mellanby seems determined to show us that everything is for the best in the best (ours) of all possible worlds. When it comes to energy use in farming he should be more careful: one man's objective view is another's blind prejudice.

Although our use of energy in farming looks modest by comparison with our profligacy in other directions, it is monstrous by world standards. If all those bushmen, whose energy economy he derides, were to adopt our farming methods they would use all their countries' usual energy supply in the process. If they were foolish enough to adopt our food processing habits as well 40% of the global energy supply would be required.

Although it is possible for several people to be fed from 1 ha, farmed by modern methods, that is not what happens; something like 0.7 ha and half a tonne of oil a year are needed to feed each and every Briton. Of course we have got used to all this, and it would be politically awkward to change, but that is not to say we have a God-given right to carry on. As Gerald Leach says in his book *Energy and Food Production*, "this is quite clearly not a viable system for all time".

Finally, I cannot share Mellanby's optimism that we are responding adequately to the problem he seems so intent to minimise. The main driving force of technical change in food production is towards economy of labour use. I see no sign of significant reductions in energy requirements.

MICHAEL KNEE

Kent, UK

news and views

New look at the aminergic nervous system

from Key Dismukes

THE aminergic neurotransmitters—noradrenaline, dopamine, and 5-hydroxytryptamine—have been studied with an intensity far disproportionate to the tiny fraction of brain cells which contain them. This attention has been drawn by the realisation that many clinically important psychoactive drugs apparently operate by altering availability of one or more of these transmitters. New histochemical techniques for mapping aminergic projections are revealing striking anatomical features and hint at mechanisms by which these neuronal systems may shape emotion, vigilance, and learning processes. For instance, almost all of the brain's noradrenaline is contained in a few thousand neurones originating in tiny nuclei in the brain stem. These few cells, however, innervate the entire cerebral cortex and much of the underlying diencephalon, as well as the cerebellum. To accomplish this diffuse innervation, the axons from each noradrenergic neurone must branch perhaps a hundred thousand times.

Several laboratories have reported a startling feature of these ascending aminergic projections. Only about 5% of the axonal varicosities from which neurotransmitter would appear to be released are associated with postsynaptic neurones. The remainder lack the close cellular apposition, subsynaptic web and postsynaptic membrane thickening which characterise classical synapses in electron micrographs. The implication is that the majority of amine-containing varicosities release neurotransmitter which must diffuse to remote receptors, a mode of operation intermediate between the private addressing of classical synaptic messengers and the broadcasting of neuroendocrine secretion.

The technique used in these studies was adopted by the Descarries group at the Université de Montréal from a

procedure developed by Aghajanian at Yale University. Radiolabelled noradrenaline or 5-HT is topically applied (*in vivo*) to a region of the brain for just long enough to be preferentially accumulated by noradrenergic or serotonergic neurones. The tissue is then prepared for autoradiography and examined by electron microscopy. Specificity of labelling was verified by demonstrating that neuronal accumulation of label was blocked by inhibitors of specific uptake or by lesions which selectively destroy one class of neurones.

³H-noradrenaline applied to rat cerebral cortex was found to be taken up and distributed in noradrenergic neurones in a pattern closely similar to that of endogenous noradrenaline (Descarries *et al.* *Brain Res.* **133**, 197; 1977). The radioactive label dispersed along tiny diffusely projecting unmyelinated axons, which have small enlargements spaced at frequent intervals (1–3 μ m). Label was highly concentrated in these axonal varicosities, and was mainly associated with numerous small agranular synaptic vesicles. Although these varicosities have the appearance and inner structure typical of classical synaptic boutons, less than 5% showed signs of being connected to postsynaptic membranes (as opposed to 50% of unlabelled boutons in the surrounding neuropil). Labelled boutons making synaptic contact could not be distinguished morphologically from those without synaptic junctions.

Such non-synaptic boutons may turn out to be a common characteristic of brain aminergic systems. The axonal projections of cerebral serotonergic neurones closely resemble those of noradrenergic systems, with only about 5% of boutons marked by ³H-5-HT evincing synaptic contact (Descarries *et al.* *Brain Res.* **100**, 563; 1975). Chan-Palay has shown that the extensive plexuses of serotonergic axons lying on both sides of the ependymal walls of the ventricles, in the arachnoid sheath around cerebral blood vessels,

and in the pia over the spinal cord have numerous axonal boutons but lack post-synaptic structure (*Brain Res.* **102**, 103; 1976). Earlier approaches to tagging aminergic projections suggested the existence of non-synaptic varicosities in (presumably) dopaminergic endings in the striatum and in catecholamine and indolamine terminals of the median eminence (see references in Chan-Palay *op cit.*).

It is important to note that in none of these systems have the axonal varicosities yet been shown to release their sequestered amines. However, indirect evidence suggests that release does occur. The non-synaptic boutons have all the components normally associated with neurotransmitter secretion. Furthermore, it seems unlikely that the large fraction of aminergic neurotransmitter content which can be released by depolarisation *in vitro* or stimulation *in vivo* could be accounted for by the small fraction of boutons with synaptic contact. Release from non-synaptic boutons is known to occur in sympathetic neuromuscular junctions, such as in the vas deferens.

What is the functional significance of these non-synaptic axonal boutons? The lack of close apposition to postsynaptic membranes implies that receptors for released amines must be distributed farther afield than in classical junctions. 5-Hydroxytryptamine released into the cerebrospinal fluid of the ventricles could be carried to wide areas of the brain. Similarly, the diffuseness of ascending noradrenergic and serotonergic projections would allow distribution of aminergic neurotransmitters throughout the forebrain. Descarries and coworkers estimate that the cortex contains some 330,000 noradrenaline varicosities per mm³, with remarkably uniform distribution. Thus, every cell in the cerebral cortex may lie within less than 30 μ m of a non-synaptic noradrenergic bouton. This arrangement suggests that release of noradrenaline would alter the activity

of large populations of cells, rather than controlling individual targets on a one-to-one basis. Both Chan-Palay (*Cerebellar Dentate Nucleus* Springer-Verlag, 1977) and Descarries *et al.* (*op. cit.*) speculate that these aminergic systems may be used as neuromodulators, biasing the responses of target cells to classical neurotransmitters and shaping the activities of large neuronal regions.

Regardless of whether non-synaptic boutons turn out to release neurotransmitter, a neuromodulatory role for these diffuse aminergic projections is suggested, both by their anatomical arrangement and by neurophysiological data. Freedman and coworkers for example, recently reported that noradrenergic activation, although suppressing spontaneous firing of cerebellar Purkinje cells produced a relative enhancement of the Purkinje response to synaptic inputs from other cells (*Expl Neurol.* 55, 269; 1977). Noradrenergic projections to the forebrain contrast with the fibre pathways of classic neuroanatomy not only in the diffuseness of their distribution, but also in topographic organisation. In classically described long pathways the terminal projections of individual cells

are generally arranged so that a map of the total projection field corresponds topographically with the distribution of cell bodies. R Y Moore and coworkers have found that this sort of organisation does not occur in noradrenergic projections from the locus coeruleus to the neocortex; rather, projections from a single cell are widespread, with vast overlapping (*A. Rev. Neurosci.* in the press). Interestingly, many of the brain regions receiving noradrenergic input make reciprocal connections back to the tiny locus coeruleus nucleus. These observations suggest that the diffuse ascending aminergic systems represent a distinct category of neuronal organisation.

A neuromodulatory role is concordant with a massive literature implicating amines in various aspects of behaviour. A leading biochemical theory of schizophrenia suggests malfunction of dopaminergic systems. Alterations in mood are correlated with neuronal availability of noradrenaline. Many studies have linked catecholamines and indolamines to vigilance, reward, and learning, yet it has not been possible to identify a specific aminergic role in any of these behavioural processes. That elucidation may now be a step closer.

Egg activation

from Duncan O'Dell

WHEN an egg is fertilised, many of its properties change promptly. Most species show an increase in the rate of respiration, protein synthesis is turned on, permeability and transport characteristics of the surface membrane change, a block to the entry of supernumary sperm is established and meiosis, where appropriate, is resumed. It has long been considered that during oogenesis an egg acquires all the machinery necessary to respond rapidly to fertilisation, but that these several responses become 'blocked' until they are 'activated' by the entry of a sperm. For many years, there has been a search for the 'switch' or 'switches' that would serve to activate eggs and explain why so many different processes get turned on more or less together. This search was complicated by the fact that some eggs, such as those of sea urchins, can be parthenogenetically activated by any of a long and confusing list of treatments whereas others, such as those of sea squirts, are refractory to most procedures tried. Matters became much clearer in 1974

when Steinhardt and Epel (*Proc. natn. Acad. Sci. U.S.A.* 71, 1915) showed that the ionophore for divalent cations, A23187, would activate echinoderm eggs; subsequent work by these and other authors has shown both that many other eggs can be similarly activated and that the movement of calcium ions is intimately involved in the process.

In a more recent analysis (Johnson *et al.* *Nature* 262, 661; 1976) the activation of sea urchin eggs was divided into two stages. The first, which occurs in the minute after fertilisation or activation, involves the exocytosis of the granules under the surface of the egg leading to various surface changes and it is this event that can be brought about by the calcium ionophore. The second stage, about three or four minutes later, involves the onset of macromolecular syntheses and the most interesting feature here is that the first stage is not a prerequisite for the second. A number of treatments—ammonia, procaine, nicotine—all lead directly to the metabolic activation of the egg. Johnson *et al.* showed that all these agents caused an efflux of H^+ ions with a subsequent rise in the intracellular pH of the eggs. If this efflux

of acid was blocked in various ways, the second stage of activation did not occur. It was then suggested that low intracellular pH served as the natural 'block' of metabolism before fertilisation, with the caveat that this sort of analysis has not yet been performed on many species of egg. Johnson *et al.* concluded by speculating that such shifts of intracellular pH could be regulating mechanisms elsewhere when quiescent cells resume division.

In this issue of *Nature* (page 590) Lopo and Vacquier take up the challenge presented by this speculation. First, the observation that intracellular pH rises after fertilisation is confirmed. But the changes in pH have also been followed for much longer during development, and it has been found that the higher pH of the newly fertilised egg is not maintained for long. Since the actively dividing cells of cleavage stage embryos seem to have an intracellular pH lower than that of the 'blocked' mature egg cell, it would seem that low pH alone is no absolute barrier to the processes involved in preparing for mitosis. The initial rise in pH, then somehow serves irreversibly to 'unblock' the egg. However there may still be a link between DNA synthesis and gross intracellular pH as Lopo and Vacquier show by use of procaine. This drug promotes DNA synthesis and a high intracellular pH; when procaine is removed, both fall.

It seems established, then, that at least in sea urchin eggs an early event following fertilisation is a rise in intracellular pH and that this rise is associated with the onset of the processes of early development, but the higher pH is not necessary for the completion of these processes. Clearly, there is scope for further analysis here. All these pH measurements were made on egg homogenates and subtle local variations within the cell would have been missed. The mode of action of a drug such as procaine is by no means exactly certain. More work needs to be done before one can interpret the consequences of a rise in intracellular pH with confidence. However, the past three years, starting with Steinhardt and Epel's experiments on A23187, have taught us a great deal about fertilisation and egg activation. Although the definitive analysis is not yet quite in sight important experiments are being done and there is a feeling that an interpretation will be made. At the beginning of this century good scientists worked hard on the problem but mainly produced a baffling list of egg activating treatments, there is now hope that the scientific great-grandchildren of pioneers like J. Loeb will be able to interpret that list in terms of calcium levels, pH shifts, surface membrane changes and so on. □

Ciliar dyneins

from Roy Burns

THE similarities between the molecular basis of muscle contraction and the beating of cilia flagella have been evident for several years. Both mechanisms involve the sliding of parallel filaments, resulting from the hydrolysis of ATP by enzymes which transiently form crossbridges between the adjacent filaments: the myosin heads in the case of muscle and the dynein-containing side arms of cilia and flagella. Furthermore, the demonstration by Gibbons and Gibbons (*J. Cell Biol.* **63**, 970; 1974) that the crossbridges of sea urchin sperm flagella are frozen when the ATP concentration is suddenly lowered, resulting in rigor, strongly suggests that the dynein-ATPase crossbridge cycle resembles the Lymn-Taylor cycle of muscle (see *News and Views* **235**, 362; 1972). It is however dangerous to extend the analogy too far, and Warner, Mitchell and Perkins (*J. molec. Biol.* **114**, 367; 1977) have recently shown that the outer sidearms of cilia are structurally much more complex than the myosin head crossbridge.

Each of the outer doublets of cilia and flagella bear a pair of sidearms which tangentially approach the neighbouring doublet. These sidearms can in general be removed by dialysis against low ionic strength buffer, and the outer sidearm selectively extracted with 0.5 M KCl (Kincaid, Gibbons & Gibbons *J. supramolec. Struct.* **1**, 461; 1973), and the principal component present in such high salt extracts from sea urchin sperm tail axonemes is the high molecular weight ATPase termed dynein-A. Warner *et al.* have examined such a preparation of dynein-A from cilia from both *Tetrahymena* and the gills of the lamellibranch *Unio* by SDS-gel electrophoresis and by negative staining.

SDS-gel electrophoresis of the *Unio* gill axonemes shows two principal high molecular weight bands, present in a 2:1 ratio, and four additional components with molecular weights greater than 300,000 daltons. Extraction with high salt selectively removes approximately half of the major component (Band-3) which has an estimated molecular weight of 360,000 daltons. When the high salt supernatant from either *Unio* or *Tetrahymena* is examined by negative staining, three principal structures are observed: a 9.3 nm spherical particle with or without a centrally located accumulation

of stain, and aggregates of 2-6 of these 9.3-nm subunits. A fourth unrelated particle is occasionally observed in the *Unio* preparations with dimensions of 9.4×12.7 nm together with some solubilised tubulin and some unidentified 200×3.5 nm filaments. The 9.3-nm particle is consistent with a protein of molecular weight 348,000 daltons, and in view of the extraction conditions is considered to be the monomeric form of dynein-A.

This particle is, however, significantly smaller than the attached sidearm, which in *Tetrahymena* is hook-shaped and measures $20-22 \times 8-10$ nm (Allen *J. cell Biol.* **37**, 825; 1968). Warner *et al.* find that the outer sidearm can be resolved by negative staining into at least three morphological subunits, with a centre-to-centre spacing of 7.5 nm. What then is the relationship between these smaller subunits and the 9.3-nm extracted particles? Three possibilities are suggested; first, that the outer sidearm is composed of one or more non-enzymatic molecules with an identical molecular weight to dynein-A. This possibility should not be discounted as the resolution of SDS-gel electrophoresis is low in the high molecular weight range ($\pm 10,000$ daltons), and the high salt extraction removes only half of the Band-3 component, the remainder presumably being a different protein with the same molecular weight. The second possibility is that the sidearm is a polymer of the 9.3-nm particle. In order to account for the lower centre-to-centre spacing of the subunits of the intact arm it is necessary for a conformational change to occur during extraction, or for the subunits to be superimposed *in situ*, and indeed the sidearms are tilted towards the base at an angle of 28° from the perpendicular. The third possibility is that only the distal portion of the sidearm is extracted, the proximal part remaining attached to the outer doublets but in a form undetected by either negative staining or conventional electron microscopy. However, all of these alternatives indicate that the sidearm is much more complex than the myosin head, and support the view that although the two contractile mechanisms share certain properties, they are unrelated.

Finally, Warner *et al.* have numbered the *Unio* and *Tetrahymena* high molecular weight axonemal proteins 1-6, while Gibbons *et al.* (in *Cell Motility, Cold Spring Harbor Conferences on Cell Proliferation* (eds Goldman, Pollard & Rosenbaum) **3**, 915; 1976) had labelled the sea urchin proteins Band-C, A, D, and B, where Band-A corresponds to dynein-A (also termed dynein-1) and to Warner's

Band-3. At first sight this suggests a proliferation of cumbersome nomenclature. Two points should be made: first, Gibbons has defined the dyneins as high molecular weight (above 300,000 daltons) microtubule-associated ATPases so that certain of the sea urchin high molecular weight proteins, for example Band-B, which lack ATPase activity are not by definition dyneins. Second, it is clear that the same experimental procedure does not necessarily extract the same components from all cilia and flagella; for instance the cilia and flagella of *Aquipten* differ in their extraction properties (Linck *J. Cell Sci.* **12**, 951; 1973) and not all species of sea urchin behave identically. It is probably wise therefore to retain separate nomenclatures until the function and location of each of the high molecular weight components is clear. The differences in the extraction of different cilia and flagella suggest also that the elucidation of the structure and mechanism of movement may be hastened by exploiting obscure organisms rather than confining our attention to a single widely available species. □

Coulson rules OK

from John Walker

THE problem of calculating the electronic energy levels of defects in semiconductors has not been solved, and is currently the subject of much controversy; some new results and re-interpretations of data on point defects in diamond by Gordon Davies (*Nature* **269**, 498; 1977) strongly suggest that the method of calculation proposed by Coulson as far back as 1957 (Coulson & Kearsley *Proc. R. Soc. A* **241**, 433; 1957; see also Coulson & Larkins *J. Phys. Chem. Solids* **32**, 2245; 1971) is the most appropriate one.

Since the 1920s and the development of quantum mechanics it has been possible to calculate with reasonable (and in some cases extreme) accuracy the electronic energy levels of atoms and molecules, and hence their characteristic spectra. The calculations are simplified because an atom is an isolated system; the electrons do not interact with other atoms. Molecules are more difficult, but only a few atoms are involved, at least in the case of simple molecules.

In a solid the analogous problem to the hydrogen atom is an electron bound to a lattice defect such as a vacancy. Here the situation is complex; the surrounding lattice atoms must be taken into account, since they are

intimately connected to the defect by chemical bonds. They affect the magnitude and symmetry of the potential seen by the electron, and this influence is complicated by the fact that they can vibrate and become distorted.

In semiconductors there are simplifications in some cases—the so-called 'shallow' centres—whose energy levels lie close to the edges of the forbidden gap. These can be treated in a hydrogenic approximation. For 'deeper' levels the approximate treatment does not work, and diamond is particularly difficult because its forbidden gap is wide (which is why diamond is transparent) and all levels are deep.

On the other hand, a diamond is effectively a giant in theoretical chemistry? So why not apply to it the techniques used in theoretical chemistry? This is what Coulson and Kearsley did. They treated the neutral vacancy (a lattice site from which the carbon atom has been removed) as a 'defect molecule' containing four electrons—the 'dangling bonds' from the four nearest-neighbour carbon atoms which were broken in creating the vacancy. (For a positive or a negative vacancy there are three or five electrons.)

The calculations predicted a 1E groundstate with a 1T_2 state about 2 eV higher in energy. (E and T_2 denote doublet and triplet orbital states. The superscript $=2S+1$, where S is spin, as in atomic spectroscopy. Hence S is zero in this case.) The transition 1E to 1T_2 is allowed. It so happens that an absorption band in irradiated diamond—the GR1 band—occurs at about 2 eV and has a non-paramagnetic ($S=0$) ground state. Since irradiation produces vacancies it was reasonable to assume that this absorption band was due to the neutral vacancy. Experimental work 16 years later (Clark & Walker *Proc. R. Soc. A* **334**, 249; 1973; Davies & Pechina *Proc. R. Soc. A* **338**, 359; 1974) confirmed that the GR1 transition was indeed between an E ground state and a T excited state. So the Coulson calculations seem to work.

In his paper Davies has suggested that the calculations work equally well for the negatively-charged vacancy (V^-). He has pointed out that irradiation of some diamonds (the so-called type Ib) gives a much weaker GR1 band than normal, but produces very strong absorption in the ultraviolet—the ND1 band. Since it is unreasonable to assume that irradiation produces vacancies in some diamonds but not in others, Davies argues that they are being produced in a different charge state. Type Ib diamonds contain nitrogen, which should be a donor, so vacancies are likely to be in the

negative rather than the neutral charge state. The calculations predict that V^- probably has a 1A_2 ground state, with an allowed transition to a 1T_1 excited state several eV higher in energy. Earlier work (Davies & Lightowers *J. Phys. C: Solid State Physics* **3**, 638; 1970) has shown that ND1 is indeed due to an A to T transition and that the defect is tetrahedral, as one would expect for a vacancy. Furthermore, Dyer and Du Preez (*J. chem. Phys.* **42**, 1898; 1965) have demonstrated that ND1 is paramagnetic, which it would be with a 1A_2 ground state. They have also shown that the ND1 absorption can be strengthened, and GR1 reduced, or *vice versa*, by shining light of appropriate wavelengths on the

diamond. This phenomenon is easier to explain if ND1 and GR1 are different charge states of the same defect, rather than being different defects, as was formerly assumed.

Davies's suggestions therefore fit the facts. They indicate that further work is desirable on the ND1 centre in EPR. They imply that the sign of charge carriers involved in photoconductivity at ND1 and GR1 should differ, which can be checked. And most important of all, they imply that the 'defect molecule' approach of Coulson and his coworkers is the best way to calculate the electronic properties of defects in semiconductors. It is to be hoped that Davies's work will stimulate further activity amongst the theoreticians. □

Primaeval heavy leptons

from P. C. W. Davies

DISCOVERIES in subatomic particle physics take place against a continual struggle for more technology and money. The Universe enjoys complete freedom from these worries and produces for free events which may tax human resources to the limit. During the big bang, exceedingly high temperatures and energies stimulated subatomic processes of all varieties, some of which have left relics for us to observe today, so it has become increasingly attractive to confront the theoretical work on these processes with astrophysical and cosmological, as well as technological, data.

A famous example of this approach concerns the unknown rest mass of the neutrino, which most physicists would like to be zero. Laboratory experiments are not particularly sensitive to the neutrino mass, but cosmology is. A study of the fireball resulting from the big bang indicates that the entire Universe should be filled with primaeval neutrinos, about three hundred of them per cm^3 —a billion times more numerous than atoms. The existence of a similar background of photons was confirmed by observation in 1965, but neutrinos interact so weakly with matter (it would take a lump of lead several thousand light years thick to absorb them) that their presence has remained undetected. Nevertheless, their energy will gravitate like any other, and thereby influence the characteristics of the expanding Universe. If they do possess even a tiny rest mass, they are present in such profusion that the cumulative gravity of all the

neutrinos could dominate the cosmological motion. It has been estimated that a neutrino mass in excess of 40 eV (about 10^{-4} of the electron mass) would be sufficient to cause the expansion of the Universe to decelerate more rapidly than observations permit, and eventually to bring about its demise by gravitational collapse.

In two recent papers in *Physical Review Letters*, similar arguments have been used to place mass limits on a hypothetical new particle: a neutral heavy lepton. This entity is predicted by some recent gauge theories of weak interactions and is being actively sought by particle physicists. Its properties would be rather like those of a super-heavy neutrino. If these particles exist, they would have been present along with their antiparticles in the primaeval fireball, but their high mass would have greatly reduced their abundance over that of ordinary neutrinos. In the first paper, the late Benjamin Lee of Fermilab, and Steven Weinberg of Harvard University (currently on leave at Stanford) consider the thermal equilibrium abundance of the heavy leptons when the temperature of the Universe was about 10^{10} K (about one second after the big bang). Before this point the cosmic matter density was so high that even the feebly-interacting neutrinos would have felt its presence and been held in thermal equilibrium with it. This fact enables the abundance of neutrinos and other particles to be calculated from the common temperature of all the primordial material. However, when it comes to calculating the abundance of the heavy leptons, it turns out that their density would have been so low that their natural incli-

P. C. W. Davies is a lecturer in the Department of Applied Mathematics at King's College, London.



A hundred years ago

THE GELADA.—Several living specimens of this extremely rare Abyssinian monkey first described by Dr Ruppell in 1835, have quite recently reached this country for the first time and are being exhibited at the Alexandra Park. The exact affinities of the species have never been fully determined, different biologists placing it, some with the Macaques, others with the Baboons. It is peculiar in that the male is covered with very lengthy hair like that of the Wanderoo, whilst the female is a much more ordinary-looking monkey. In the male, also, there is a bare spot, in shape like an inverted T, upon the breast which is of a light-pink colour, becoming red and expanded into an inverted heart-shaped patch upon excitement. The tail is long and like that of a lion, having a bushy tuft at the extremity. The colour is a sooty dark-grey brown, verging upon black; the hands and face are black; the nails are powerful and long. The size of the male is about that of a Chimpanzee four years old. The eyes are close together, and the snout prolonged. The living animal has a habit of everting the whole upper lip when irritated and thus exposing its formidable array of teeth.

From *Nature* 16, 11 October, 512; 1876.

nation to annihilate with their antiparticles was inhibited, simply due to the rarity of particle-antiparticle encounters. Consequently, relatively more of them would have escaped this fate and survived to populate the Universe. Using a simple model of the primaeval fireball, Lee and Weinberg are able to show that the mass of these heavies must be at least about 2 GeV (four thousand times the mass of the electron) to avoid the gravity of this residue drastically decelerating the Universe. Curiously, the same argument which places an upper bound on the neutrino mass places a lower bound on the heavy lepton mass. The reason for this is that the heavier the particles the more they gravitate individually, but the less abundantly they are produced at a given fireball temperature. Because of these competing effects, the combined gravity of all the particles is greater if either the mass is especially low or high—very many light particles or a few very heavy ones. In the neutrino case, we know from laboratory experiments that the mass is tiny, so the individual-gravity factor dominates,

but the reverse is true of the heavy lepton, and it is the abundance factor which is more important.

A fascinating consequence of this calculation is that the abundance of these particles, if they exist and are not too massive, could still be high enough to contain nearly all the mass of the Universe, which opens up the prospect of dozens of times more matter in the form of weakly interacting, virtually unobservable, heavy neutrinos than in all the galaxies. If this is correct, then it might indeed be sufficient to cause the cosmological expansion eventually to reverse into collapse.

In the second paper, Duane Dicus and Edward Kolb of the University of Texas at Austin, and Vigdor Teplitz of the Virginia Polytechnic Institute consider how the Lee-Weinberg result would be modified if the heavy neutrinos are weakly unstable, and decay after a while into ordinary neutrinos. The essence of their calculation is the observation that cosmic neutrinos resulting from recent heavy lepton decays are more energetic, and hence gravitate more strongly, than the primordial ones, because the latter have been redshifted to low energies by the expansion ever since the big bang. Hence the cosmological measurements provide a limit on the lifetime of the lepton. However, this quantity

can also be inferred from the theory of weak interactions if the mass of the lepton is known. Turning the argument round, the lifetime limit can be used to place an upper bound on the mass, which works out at about 10 MeV, or less than one hundredth of the Lee-Weinberg value for a stable heavy lepton. The particle lifetime corresponding to this is about a year.

The authors have checked that any high energy photons also produced in the decay events during the first few hundred million years would have been degraded (thermalised) by scattering. Those produced after this would be swamped by the cosmic X-ray background, so are not detectable. Nor do the energetic decay neutrinos affect the nucleosynthesis in the primaeval fireball, because at 200 s this process occurs too early for the decay products to have accumulated significantly. Unfortunately, the presence of the decay neutrinos, in spite of their higher energy, is still too ephemeral to register in neutrino detectors or noticeably to affect protons in cosmic rays so that, intriguing though these ideas are, no positive experimental verification of cosmic heavy leptons appears likely. Nevertheless, the imposition of mass bounds at least lets the laboratory physicist know what to expect from terrestrial ones. □

What future for Antarctic geology?

from Peter Barker

The 3rd Symposium on Antarctic Geology and Geophysics held at Madison, Wisconsin, on 22–27 August, was cosponsored by SCAR, IUGS and ICG. The organising committees, chaired by Campbell Craddock (University of Wisconsin-Madison), ensured a friendly and interesting meeting.

ANTARCTIC Earth science can be a slow, difficult business, at sea as well as on land. The weather is often foul and the ratio of outcrop to ice vanishingly small. The great expense of putting a party into the field can generate years of delay, and tends particularly to discourage the return visit which a mature assessment of the first season's work often recommends. Along with delay, these conditions breed unconscious error, over-reached speculation and a parochial viewpoint, and ensure their prolonged survival in the literature. Nevertheless, the past few years have seen a revolution in the earth sciences generally, and considerable

funds allocated for Antarctic research. The importance of the symposium at Madison, therefore, lay not in its being larger, better attended and broader in scope than its predecessors at Oslo (1970) and Cape Town (1963), but in the opportunity it provided to assess the quality and global relevance of contemporary Antarctic earth science, and consider its future.

Continental geology, the traditional core topic, occupied less of the programme and produced few of the kind of surprises which must have animated past meetings: the heroic age of exploration and reconnaissance mapping is past, the emphasis shifted towards re-mapping anomalous areas and questioning the reality of the classically-defined widespread orogenic episodes (in North Victoria Land, for example). Better descriptions of the oldest Antarctic rocks and more confident (although conflicting) comparisons with neighbouring Gondwana continents

Peter Barker works in the Department of Geological Sciences, University of Birmingham.

were evident than at Oslo, but for most of Antarctica the isolated coastal exposures and ice-covered interior seem a crucial impediment to further understanding. Radar ice-thickness measurements are revealing something of sub-ice morphology and, in those rare cases where allied geophysical data are available, seem capable of generating some small, speculative understanding of the interior. There is no doubt, however, that the geology of most of Antarctica, East and West, and its important glacial history, will remain obscure without an expensive and time-consuming programme of survey-controlled, regional sub-ice drilling.

Perhaps the liveliest part of the symposium, in contrast, was a two-day section on the geodynamics of the Scotia Arc. Considerable progress has been made since 1970, particularly in understanding southern South America's evolution and strengthening its connection with South Georgia. A Cretaceous age for almost all the Scotia Sea is now established, but the earlier history of the region remains uncertain. A crucial unknown, the positions of the Antarctic Peninsula and South America at the Gondwanaland margin, should emerge shortly; magnetic anomaly-based Gondwanaland reconstructions at Madison were conflicting, but obviously convergent, and the growth of marine geophysical interest in the Weddell Sea should complete the puzzle.

Under the influence of geodynamics, some Scotia Arc studies are oriented towards the processes involved in formation. The area is particularly well-endowed with back-arc basins, and a geochemical data base for these and other active margin igneous processes as broad as any in the world is now available.

Mineral resources, presently occupying the attention of Antarctic Treaty signatories were considered in only six out of about 150 papers. This dearth does not reflect wilful ignorance (the academic ostrich!), nor in the informal opinion of the meeting does it hide a much greater actual knowledge. Simply, reconnaissance mapping is complete and any resource detectable by such means is known; none is economic to extract at present, and such more detailed, follow-up surveys as have been carried out have proved no more fruitful. Offshore petroleum prospects were not discussed at Madison but this case is essentially similar, although there has been less reconnaissance.

The main goal of Southern Ocean marine geology (a topic hardly considered at Oslo) is an understanding of the interdependence of climate, circulation, sedimentation and Antarctic

Have gunk, will travel

from Peter J. Smith

A *nuée ardente* is a rapidly flowing, turbulent and often incandescent cloud of gas and ash originating in an explosive volcanic eruption. The first one to come to the attention of scientists, but surely not the first ever to be seen, was that from Mt Pelée (Martinique), which in 1902 overwhelmed the town of St Pierre, killing all but two of the inhabitants. This still remains the most famous of all, although many others, including much larger ones, have been observed since, not least from Mt Pelée itself.

But observation is one thing and scientific investigation is another; by their very nature *nuées ardentes* in action (as opposed to the deposits they leave behind) are not particularly amenable to the latter. The pioneer in this field was Frank Perret, who studied hundreds of *nuées ardentes* at close quarters after the eruption of Mt Pelée in 1929, captured their visual beauty in a remarkable series of photographs, and almost ended as a victim of one. Today, however, some aspects of glowing clouds may be studied from the relative safety of an aeroplane, which is how Moore and Melson (*Bull. Volcan.* **33**, 100; 1969) observed the 1968 eruption of Mt Mayon (Philippines) and how Stith *et al.* (*Geophys. Res. Lett.* **4**, 259; 1977) approached the eruption of St Augustine Volcano (Alaska) in 1976.

One of the most conspicuous characteristics of a *nuée ardente* is, of course, its speed, which reference books usually give as "perhaps 100 kilometres an hour" or something equally vague but which Stith and his colleagues have now determined much more accurately using aerial photography. Thus the *nuée ardente* produced by St Augustine Volcano on 8 February 1976 began moving down the initial 1:3 slope with a speed of 180 km per hour, slowed to 75.6 km per hour down the subsequent 1:7 gradient, then to 46.8 km per hour (1:12) and finally to 21.6 km per hour (1:28) before entering the sea 6 km from the volcanic cone. The average speed was 54.0 km per hour, which means that the 6 km was covered in less than 6.7 minutes.

The chief reason for such astonishing speeds is the 'fluidisation' of the volcanic particles brought about by the release of gases, a process which greatly reduces the frictional resistance of the mass as it moves under gravity. The dangers are obvious. A *nuée ardente* travels not only rapidly but with very little noise. A community in its path may be overcome without ever knowing what happened. □

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

glaciation in a ocean basin of changing shape and with the most vigorous oceanic current system in the world. At Madison, papers based mainly on Deep Sea Drilling data and the Eltanin piston core collection attempted partial analyses of some of these relationships over time intervals varying from 1,000 years to 150 Ma. Individual papers were interesting but the pervading impression was of a huge field largely unexplored, with the gaps between contributions at least as important.

The four disparate fields considered above, with very few exceptions, contain all that is of wider interest in Antarctic earth science at present. They are at very different stages of development, from youth to old age, and their futures too are likely to diverge. Marine geology should prosper independently, with the driving force of the Southern Ocean's importance to world climate and primary productivity, and the basic unity of circum-Antarctic circulation as a constraint on the multi-disciplinary approach. One looks, perhaps, for an

injection of numerical modelling, to link through the huge range of periodicities being examined and to direct further sampling.

The future of Antarctic geology is less certain. Some aspects, such as Scotia Arc studies, may continue to develop and, as the emphasis in geology changes, remain as fertile ground for the study of processes. For much of Antarctica, however, a similar transition from unmapped wilderness to geological laboratory may not be possible; without sub-ice drilling too little of the context may ever be learnt. The value of Antarctica to global geology will then decline. Economic geology has reached a similar impasse. Workable deposits within and around Antarctica must exist and, given the increasing world pressure on known resources, continued interest is inevitable. There are no easy, short-term prospects, however, so the opportunity is still there for a balanced, disinterested political decision on the course of further exploration and exploitation. □

articles

Upper limits on the Faraday rotation in variable radio sources

J. F. C. Wardle

Department of Physics, Brandeis University, Waltham, Massachusetts 02154

Data on the linear polarisation of variable radio sources are examined for evidence of a variable component of Faraday rotation. Strong upper limits are set, and these are shown to have profound implications for the physics of these sources. It is suggested that the electron energy spectrum is a relativistic Maxwellian rather than a power law. This may be consistent with the relativistic blast wave models of Blandford and McKee.

COMPACT radio sources which contain appreciable quantities of non-relativistic electrons will exhibit Faraday rotation and Faraday depolarisation at long wavelengths. The rotation at a frequency ν is

$$\chi = \frac{e^3}{2\pi m^2 c^2} \frac{n_c H \cos\theta}{\nu^2} l \text{ rad} \quad (1)$$

where n_c is the density of cold electrons, H is the magnetic field which makes an angle θ with the line of sight, and l is the thickness of the source along the line of sight. When $\chi \sim$ a few radians, the degree of linear polarisation drops to a small value¹.

If a radio source expands conserving magnetic flux, then $\chi \propto$ radius⁻⁴. Thus a variable radio source may exhibit variable Faraday rotation. If observations are made at two or more frequencies then changes in Faraday rotation can in principle be separated from changes in the intrinsic angle of polarisation. In this way, upper limits on the observed Faraday rotation can be used to place stringent upper limits on the density of cold electrons in compact radio sources.

The results of four years of monitoring the flux density and linear polarisation of 82 compact extragalactic radio sources were reported by Altschuler and Wardle², and an analysis of the data was given by Altschuler and Wardle³. Thirty-two of the sources showed distinct variability in their polarisation. Since the observations were made at two frequencies (8,085 MHz and 2,695 MHz), the data can be examined for the presence of Faraday rotation.

An appreciable variable component of polarisation was observed in many sources at 2,695 MHz, suggesting that Faraday rotation is often not large at this frequency. We have used this to derive a crude upper limit on the cold electron density³, and we found that the ratio of the densities of relativistic to cold electrons was typically in the range $n_{rel}/n_c \sim 10^{4 \pm 4}$.

Here the data are examined more carefully and the limits on the density of cold electrons are improved by an order of magnitude. This is shown to have profound implications for the physics of compact radio sources. The data suggest that the energy spectrum of the radiating electrons may be a relativistic Maxwellian rather than a power law. Almost identical conclusions have been reached independently by Jones and O'Dell⁴.

The observational data

In practice it is not a trivial task to isolate effects due to Faraday rotation from the data. There are several reasons for this: the variations in polarisation are rapid and intricate and are often poorly correlated between the two frequencies; there are few well defined simple outbursts; and, perhaps most important, the variable component is superimposed (vectorially) on a constant or slowly varying component of comparable amplitude (see refs 2 and 3).

We cannot determine the polarisation of the underlying component with much confidence in any case, so a simple statistical approach will be used. Of the 32 sources (from ref. 2) that exhibit variable polarisation, 16 are clearly variable in the degree of polarisation at both frequencies. In four cases the variations are distinctly uncorrelated between the two frequencies, and these sources have been dropped from the sample. For the remaining 12 sources the fluctuations in the degree of polarisation are fairly large and reasonably similar at both 8,085 MHz and 2,695 MHz, suggesting that the sources are optically thin much of the time, and that we are looking at the same piece of the source at both frequencies. The 12 sources have been selected without regard to the behaviour of the position angle of polarisation or the total flux density.

0851+20 (OJ287) is particularly important. In March 1974, the polarisation jumped at both frequencies from less than 2.5% to over 12%. Here the source is undoubtedly optically thin. Also, the variable component is so strongly polarised that the effect of any underlying constant component is very small.

If there is an appreciable variable component of Faraday rotation in these sources, we would expect to see changes in the position angle of polarisation at 8,085 MHz that are echoed at 2,695 MHz with an amplitude nine times larger. Inspection of the data in ref. 2 reveals no obvious cases of such behaviour. Usually the observed changes in position angle are either quite well correlated between the two frequencies, or else they are larger at the higher frequency.

Rough upper limits on the amount of Faraday rotation can be placed in the following way. For each day's observations, we take the difference between the position angles at the two frequencies, that is $\Delta\chi = \chi_{2,695} - \chi_{8,085}$. We then compute the mean and standard deviation of $\Delta\chi$ for all the observations of each source. (For 0851+20, we have used only the data between March 1974 and June 1975). The mean, $\langle\Delta\chi\rangle$, represents a constant component of Faraday rotation. This may be attributed to Faraday rotation both in our own Galaxy, and in whatever structure surrounds the variable radio source. The standard deviation, $\sigma\Delta\chi \times 9/8$, is a rough upper limit on the r.m.s. Faraday rotation at 2,695 MHz inside the variable component.

Two corrections should be made to this estimate. First, the average errors in the position angle measurements, $\langle\epsilon_{8,085}\rangle$

and $\langle \epsilon_{2,595} \rangle$, should be subtracted quadratically. Second, the effect of superimposing the polarisation of a constant component is to reduce the observed changes in position angle. This can be compensated for, to first order, by multiplying $\sigma_{\Delta\chi}$ by a factor $(\bar{m} + m_v)/m_v$, where \bar{m} is the average degree of polarisation of the source, and m_v is the r.m.s. amplitude of the variable component, both at 2,695 MHz. The upper limit on the r.m.s. variable Faraday rotation at 2,695 MHz is then:

$$\sigma'_{\Delta\chi} = \frac{9}{8} \left(\frac{\bar{m} + m_v}{m_v} \right)_{2,695 \text{ MHz}} (\sigma_{\Delta\chi}^2 - \langle \epsilon_{8,085} \rangle^2 - \langle \epsilon_{2,695} \rangle^2)^{1/2} \quad (2)$$

The results are presented in Table 1 for the 12 sources in our sample.

It can be seen that in most cases $\sigma'_{\Delta\chi}$ is of the order of half a radian, and in two cases is considerably less. These are, however, probably gross overestimates of the typical variable component of Faraday rotation in these sources. First, in each case (except 0851+20) we have used all the data, averaging over several outbursts. Presumably at certain times the sources may be opaque, leading to fluctuations in position angle that are not correlated at the two frequencies. Second, where there is limited data, or the variable component of polarisation has a large amplitude, then the amplitude of the underlying component may be badly overestimated, and hence too large a correction is applied to the observed values of $\sigma_{\Delta\chi}$ in column (3). Third, in five out of the seven cases where $\sigma_{\Delta\chi}$ is greater than 10° , inspection of the data shows that it is the position angle at the higher frequency that is showing rapid fluctuations, and is, therefore, unlikely to be due to Faraday rotation. In support of the first two points, for 0851+20 where it is certain that the source is optically thin, and where the effect of an underlying constant component is very small, we find $\sigma'_{\Delta\chi} \lesssim 1/8$ radian.

We have found no evidence for variable Faraday rotation in these sources. From the above discussion, the typical amount of variable rotation at 2,695 MHz is most unlikely to be more than a few tenths of a radian. In the calculations that follow, however, we shall use the conservative upper limit of 0.5 radians.

Calculations

The Faraday rotation by cold electrons of density n_c is given by equation (1). The density of relativistic electrons can be estimated from the optically thin flux density. The emission coefficient for synchrotron radiation is given by⁵

$$\epsilon_v = c_5(p)N_0(H \sin\theta)^{(p+1)/2} \left(\frac{v}{2c_1} \right)^{(1-p)/2} \quad (3)$$

where the electron energy spectrum is of the form

$$N(E) dE = N_0 E^{-p} dE, \quad E_1 < E < E_2 \quad (4)$$

The optically thin brightness temperature is

$$T_b = \frac{c^2}{2k\nu^2} \epsilon_\nu l \quad (5)$$

The number density of relativistic electrons is

$$n_{rel} = \int_{E_1}^{E_2} N(E) dE = \frac{N_0}{p-1} (\gamma_1 mc^2)^{1-p} \left[1 - \left(\frac{\gamma_2}{\gamma_1} \right)^{1-p} \right] \quad (6)$$

where $\gamma = E/mc^2$.

Combining these we obtain

Table 1 The observed limits on Faraday rotation, at 2,695 HMz in 12 variable radio sources

Source	$(\Delta\chi)$ ($^\circ$)	$\sigma_{\Delta\chi}$ ($^\circ$)	$\sigma'_{\Delta\chi}$ ($^\circ$)
0300+47	15	3	<15
0605-08	64	13	39
0735+17	5	7	<22
0851+20	23	6	8
1055+01	-42	7	35
1334-12	-50	19	45
1510-08	-7	16	39
1641+39	26	12	81
1749+09	35	34	49
1921-29	-5	7	25
2155-15	21	13	32
2200+42	67	27	33

$$\frac{n_{rel}}{n_c} = \frac{4c_1}{c_5} mce^2 \frac{3-(p+1)/2}{p-1} \frac{kT_b}{\chi \tan\theta} \left(\frac{v}{v_{B1}} \right)^{(p-1)/2} \times \gamma_1^{1-p} \left[1 - \left(\frac{\gamma_2}{\gamma_1} \right)^{1-p} \right] \quad (7)$$

$$\text{where } v_{B1} = \frac{eH}{2\pi mc} \sin\theta.$$

A lower limit on n_{rel}/n_c is obtained by setting $v = \gamma_1^2 v_{B1}$. If $p > 1$, we allow $\gamma_2 \rightarrow \infty$ for simplicity then

$$\frac{n_{rel}}{n_c} \geq \frac{T_b}{\chi \tan\theta} \times \begin{cases} 1.5 \times 10^{-10} \ln \gamma_2/\gamma_1, & \text{if } p = 1.0 \\ 4.9 \times 10^{-10} & p = 1.5 \\ 3.1 \times 10^{-10} & p = 2.0 \end{cases} \quad (8)$$

T_b is typically observed⁶ to be in the range 10^{11} – 10^{12} K. In the previous section we derived upper limits on χ of a few tenths of a radian at 2,695 MHz. Hence for all sources that show variable polarisation at 2,695 MHz, we find $n_{rel} \gg n_c$.

This limit is independent of the magnetic field strength and the source dimensions. This is important since it makes the argument independent of the distance to the source, and also independent of the path length through the source, which may not be observable for example in the case of a thin shell geometry.

Lower limit on the relativistic electron energy spectrum

The limits we have derived on the density of cold electrons are so severe that we must also consider Faraday rotation by the relativistic electrons themselves. This is dominated by the lowest energy electrons and therefore can lead to limits on the presence of low energy relativistic electrons. Faraday rotation by an electron with a Lorentz factor γ is roughly proportional to γ^{-2} . (This is easily understood since in the rest frame of the electron, the radio photon is increased in frequency by a factor $\sim \gamma$.) For a power law electron energy spectrum, the equivalent density of cold electrons is^{4,7}

$$n_c(\text{equ}) = \frac{(p-1)(p+2)}{(p+1)} n_{rel} \frac{\ln \gamma_1}{\gamma_1^2} \quad (9)$$

where γ_1 is the low energy cut off in the electron energy spectrum. Combining (9) with equations (1), (3) and (5) we find

$$\chi = A(p) \frac{\ln \gamma_1}{\gamma_1^2} \left(\frac{\gamma_1}{\gamma_2} \right)^{p-1} kT_b \cot\theta \quad (10)$$

where

$$A(p) = \frac{(p+2)3^{(1-p)/2}e^3}{(p+1)c_6(p)\pi m^2 c^4}$$

If the source becomes optically thick at a frequency close to 2,695 MHz, then $\gamma \sim \gamma_n \sim 10^{-9} T_b$ (ref. 8). Setting $\chi < \frac{1}{2}$ and $\theta = 45^\circ$, we obtain the limits on γ_i listed in Table 2.

Since these are strong lower limits on γ_i , it is clear that the electron energy spectrum falls off rapidly at energies not far below the energy of the electrons radiating at 2,695 MHz. This requires that the acceleration mechanism produces copious electrons with Lorentz factors between 100 and 1,000, but few with Lorentz factors much below 100. This will be discussed later.

Average energy per nucleon

An important parameter for the dynamics of variable sources is the average energy per nucleon, $\Gamma m_H c^2$, where m_H is the mass of the hydrogen atom. A lower limit on Γ occurs when there is no non-relativistic plasma present. In this case

$$\Gamma = \frac{(1+k) \int_{E_i}^{E_j} EN(E) dE}{n_{rel} m_H c^2} \quad (11)$$

where k is the ratio of the energy in the protons to the energy in the electrons. Values of k in the literature range from 0 to $m_H/m \sim 1,836$ depending on the acceleration mechanism under consideration. As an example, with a power law energy spectrum with $p = 1$, and using the limits on γ_i and γ_j in Table 2,

$$\Gamma = (1+k) \frac{m}{m_H} \cdot \frac{\gamma_j}{\ln(\gamma_i/\gamma_j)} \sim 0.2(1+k) \frac{\gamma_j}{\gamma} \left(\frac{T_b}{10^{12}} \right) \quad (12)$$

Hence, unless $k < 5$ and $\gamma_j < 5\gamma$, $\Gamma > 1$ and the material inside a variable radio source is highly relativistic. For larger values of p the conditions are less stringent, but it seems likely that, in general, variable radio sources may contain purely relativistic plasma and that the internal sound speed is close to $c/3$.

This result can be used to argue against certain simple models of variable radio sources based on non-cosmological redshifts. In attempting to understand the apparent 'faster than light' expansion of some sources observed using VLBI techniques, it is sometimes suggested that the radio sources may be closer than is indicated by their redshifts⁹. The double sources that are observed would then be separating at velocities comfortably less than the speed of light, and might simply be similar to the well known giant double radio sources, but on a very much smaller scale.

The results found here, however, are independent of the distance to the sources. If each radio component of a double source expands freely, it will do so at about its internal sound speed, which we have shown to be very probably close to the speed of light. If the two components are separating more slowly than this then they will merge together, and a double source will not be seen at all.

Therefore, as in the case of the giant double radio sources, it is necessary to restrict the velocity of expansion to less than the velocity of separation. There are two obvious ways of doing this. First, one might add non-relativistic matter to reduce the internal sound speed. From the arguments already presented, this can be ruled out. Second, the components may be confined by the ram pressure of the external medium¹⁰. This requires $\rho_{ext} v^2 \sim$ internal energy density $\sim \Gamma c^2 \rho_{int}$, where ρ_{ext} and ρ_{int} are the external and internal densities, and v is the velocity of the component through the surrounding medium. But in this model, $\rho_{ext}/\rho_{int} \sim$ component size/component separation < 1 . Hence we require $v^2 > \Gamma c^2$, which contradicts the original assumption that the motion is non-relativistic.

Table 2 Lower limits on the low energy cut off, γ_i , of the electron energy spectrum

T_b (K)	p	γ_n	γ_i	γ_n/γ_i
10^{12}	1.0	1,000	> 41	< 24
	1.5	1,000	> 100	< 10
	2.0	1,000	> 161	< 6
10^{11}	1.0	100	> 10	< 10
	1.5	100	> 21	< 5
	2.0	100	> 30	< 4

More complicated schemes could doubtless be constructed, but the internal energy densities in these sources are enormous, regardless of their distance. It is, therefore, not very surprising if relativistic motions are observed. If the sources are at smaller distances than indicated by their redshifts, so that the measured motions are non-relativistic, then there may be severe difficulties in constructing models that are consistent with the observed limits on Faraday rotation.

Discussion

We have used the observed limits on Faraday rotation in variable radio sources to argue that (1) the density of any cold electrons present is very much less than the density of relativistic electrons; (2) the energy spectrum of the relativistic electrons cuts off rapidly at Lorentz factors not far below 100, and (3) the average energy per nucleon may well be $> m_H c^2$.

Further information concerning the electron energy spectrum can be obtained from the radio spectra of the outbursts. The rapid rate of decay of individual outbursts, and the relative rates of decay observed simultaneously at 2,695 and 8,085 MHz suggests that the radio spectrum steepens with time³. This would be consistent with a high-energy break in the electron energy spectrum that sweeps through the radio window as the source expands. More direct evidence is given by Andrew *et al.*¹¹ in the case of the giant 1968–69 outburst of 3C354.3. Here the spectrum of the outburst itself can be followed with some confidence, and again it is found that at late times the spectrum steepens drastically.

This high-energy cut off may be caused by radiation losses or may be intrinsic to the electron energy spectrum. But in any case, it seems that the electron energy spectrum is relatively narrow, extending neither to very high energies nor to very low energies. This is an important clue to the nature of the acceleration mechanism.

One possible spectrum of this type is a relativistic Maxwellian, of the form

$$N(E) dE = N_0 E^2 e^{-E/kt} dE \quad (13)$$

Formulae for computing the radiation from a relativistic Maxwellian distribution are given by Pacholczyk⁵. Such a distribution can arise naturally in a plasma at very high temperatures which is in thermodynamic equilibrium. The observed brightness temperatures suggest that plasma temperatures of the order of 10^{12} K are required.

One way of attaining such a high temperature has been discussed in some detail by Blandford and McKee¹². This involves a very high energy shock wave propagating through a thermal plasma at relativistic velocities. The jump conditions imply that in a co-moving frame just behind the shock, the mean Lorentz factor of the nucleons, $\Gamma \sim$ shock Lorentz factor. If the VLBI observations are interpreted in terms of relativistic kinematic effects¹³, then Γ is typically in the range of 1 to 10. If a fraction ϵ of the energy of the shocked particles goes into the electrons, then the electron temperature is

$$T \sim \frac{\epsilon \Gamma m_H c^2}{3k} \sim 3.6 \times 10^{12} \epsilon \Gamma \quad (14)$$

The appropriate value of ϵ is extremely uncertain, but if it is not too small, then electron temperatures of the order of 10^{12} K

can be achieved, and the electron energy spectrum may be close to a relativistic Maxwellian. If at any time the electron temperature is much greater than 10^{12} K, then 'inverse Compton cooling' can rapidly pull the temperature down to 10^{12} K again¹⁴. The radiation from a relativistic blast wave is discussed by Blandford and McKee¹⁵, who consider both power law and relativistic Maxwellian electron energy spectra.

The amount of Faraday rotation expected from a relativistic Maxwellian distribution can be computed easily; from Sazonov (1969), the equivalent density of cold electrons is

$$n_c = - \int_0^\infty \gamma \ln \gamma \frac{\delta}{\delta_\gamma} \left[\frac{1}{\gamma^2} N(\gamma) \right] d\gamma \quad (15)$$

Inserting equation (13), and writing $\gamma_0 = \frac{kT}{mc^2}$

$$n_c \sim \frac{n_{rel}}{2\gamma_0^2} (\ln \gamma_0 + 1 - \xi) \quad (16)$$

where ξ is Euler's constant, and $\gamma_0 \gg 1$.

If we are observing in the optically thin part of the spectrum, but below the peak, that is $\gamma = (v/v_B)^{1/2} < \gamma_0$, then we find

$$\chi = \frac{9.3^{1/3}}{4\pi} \left(\frac{\gamma}{\gamma_0} \right)^{-2/3} \frac{(\ln \gamma_0 + 1 - \xi)}{\gamma_0} \frac{T_b}{T} \cot \theta \quad (17)$$

$$\approx \frac{1}{\gamma_0} \ll 1 \text{ if } T \sim T_b \sim 10^{12} \text{ K}$$

Conclusions

We have shown that the observed lack of Faraday rotation in variable radio sources places severe restriction on the density

of both thermal plasma and low energy relativistic electrons. The fact that $n_{rel}/n_c \gg 1$ can be taken as direct evidence against all models in which the electrons are accelerated by betatron and Fermi processes in a turbulent plasma¹⁶.

It is also clear that the relativistic electron energy distribution does not extend to very low energies. There is weaker evidence from the radio spectra that the distribution does not extend to very high energies either, and may in fact be relatively narrow.

One possible distribution function of this form is a relativistic Maxwellian, such as may be produced behind a relativistic blast wave¹⁸. This automatically gives negligible internal Faraday rotation and if the VLBI observations are interpreted as relativistic kinematic effects, naturally leads to brightness temperatures of 10^{11} – 10^{12} K. The amount of internal Faraday rotation implied by equation (17) is so small, that it may be necessary to consider the decreasing Faraday rotation external to the shock, caused by sweeping up the ambient medium. In some situations this can produce the dominant contribution to $\Delta\chi$.

In a future paper we shall show that relativistic blast wave models can account for several other features of the spectra, flux variations and polarisation of variable radio sources.

This work was supported by NSF grant AST74-18631 A02. I thank Ms E. B. Hessel and Drs D. S. De Young and T. Jones for helpful discussions.

Received 4 July; accepted 15 August 1977.

1. Conway, R. C. *et al. Mon. Not. R. astr. Soc.* **168**, 137–162 (1974).
2. Altschuler, D. R. & Wardle, J. F. C. *Mem. R. astr. Soc.* **82**, 1–67 (1976).
3. Altschuler, D. R. & Wardle, J. F. C. *Mon. Not. R. astr. Soc.* **179**, 153–178 (1977).
4. Jones, T. W. & O'Dell, S. L. *Astr. Astrophys.* (in the press).
5. Pacholczyk, A. G. *Radio Astrophysics* (Freeman, San Francisco, 1970).
6. Kellerman, K. I. *et al. Astrophys. J.* **169**, 1–34 (1971).
7. Sazonov, V. N. *Soviet Astr.* **13**, 369–402 (1969).
8. Jones, T. W., O'Dell, S. L. & Stein, W. A. *Astrophys. J.* **188**, 353–368 (1974).
9. Whitney, A. R. *et al. Science* **173**, 225–230 (1971).
10. DeYoung, D. S. *Astrophys. J.* **167**, 541–551 (1971).
11. Andrew, B. H., MacLeod, J. M. & Wardle, J. F. C. (in preparation).
12. Blandford, R. D. & McKee, C. F. *Phys. Fluids* **19**, 1130–1138 (1976).
13. Cohen, M. H. *Ann. N.Y. Acad. Sci.* **262**, 428–435 (1975).
14. Kellermann, K. I. & Pauliny-Toth, I. I. K. *Astrophys. J. Lett.* **155**, L71–78 (1969).
15. Blandford, R. D. and McKee, C. F., *Mon. Not. R. astr. Soc.* **180**, 343, (1977).
16. Pacholczyk, A. G. & Scott, J. S. *Astrophys. J.* **210**, 311–320 (1976).

Uranium anomaly in Black Sea sediments

Egon T. Degens, Francis Khoo & Walter Michaelis

Geologisch-Paläontologisches Institut der Universität Hamburg, Bundesstrasse 55, 2000 Hamburg 13, West Germany

The upper 90 cm of Black Sea basin sediment with an areal extension of $2.96 \times 10^5 \text{ km}^2$ has an U_3O_8 content of 6.7×10^6 tonnes. Plankton is the prime agent for uranium fixation. Reducing conditions at depth permit uranium to accumulate over the past 5,000 yr. Energetically self-sufficient burning of the top 1-m strata will lead to U_3O_8 concentrations in the order of 100 g per tonne ash.

THE Black Sea is the largest anaerobic water body in the world ocean and measures almost half a million km^3 (ref. 1). Its present environmental state is a consequence of Holocene sea-level rise and associated formation of a stable pycnocline restricting free exchange of molecular oxygen to the deep water². Holocene sediment cores from the euxine abyssal plain show, almost in slow motion, the development from a fully oxygenated fresh water 'Black Lake' to the modern brackish-marine Black Sea³. A typical 1 m sediment section consists from top to bottom of: coccolith ooze, sapropel, and light lutite. The sapropel-lutite boundary, with an assigned age of about 5,000 yr BP

marks the event when the Black Sea became permanently stratified and euxinic conditions established at the sediment-water interface⁴.

Fluctuations in redox potential may cause depletion or enrichment in certain elements both for water and sediment. Here we report on the mechanism of uranium concentration in Black Sea sediment as a function of a changing habitat.

Samples and analytical procedures

The uranium content in abyssal Black Sea mud is almost one order of magnitude larger than in average marine sediment^{5, 6}. Similar concentrations are known from sediments of Norwegian fjords and the Baltic Sea^{7, 8}. It seems, therefore, that restricted environments with a trend to euxinic conditions favour accumulation of uranium.

In the Black Sea abyssal mud the U_3O_8 concentration rarely exceeds 50 p.p.m. and 25 p.p.m. can be taken as a representative mean value. In spite of the high uranium level and the wide areal distribution, the anomaly seems to be, on first sight, of no economic significance. This outlook, however, may change by closer examination of the deposit from a sedimentological and geochemical point of view. This is because the high content

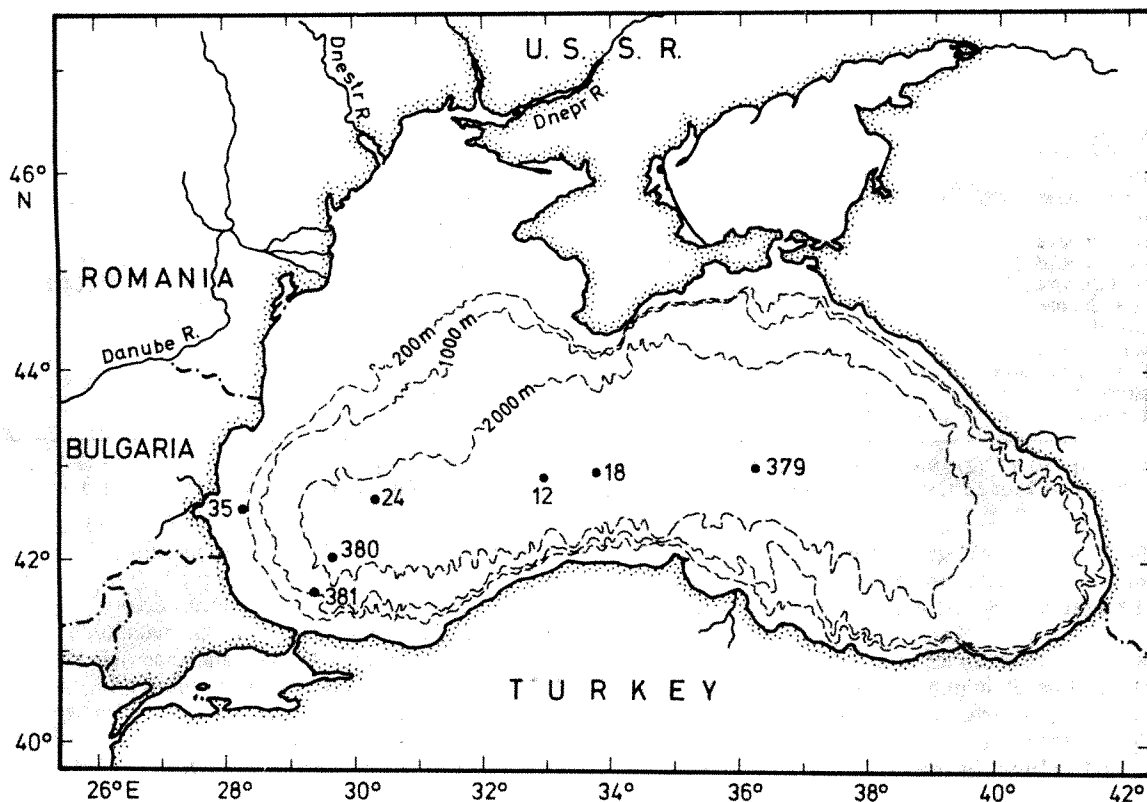


Fig. 1 Bathymetric chart of Black Sea and location of stations.

of organic matter and carbonate may allow for a considerable reduction in sediment mass by burning; furthermore, the material is unconsolidated and thixotropic.

Water and sediment were collected during RV Chain cruise of the Woods Hole Oceanographic Institution in Spring 1975. Location of stations is shown in Fig. 1. The sample collection has been supplemented with Pleistocene sediments obtained during the deep-sea drilling operation of Glomar Challenger, Leg 42 in the Black Sea in May–June 1975 (Fig. 1).

Uranium determinations were carried out spectrophotometrically using methods previously developed^{9,10}. Principally, uranium was extracted with tri-*n*-octylphosphine oxide (TOPO); and 2-(5-bromo-2-pyridylazo)-5-diethyl-aminophenol (bromo-PADAP) was used as a chromogenic reagent. Uranium nitrate was used as spike and syenite rock SY-2 and SY-3 from Canadian certified reference materials project, Mines Branch, Ottawa, were used for intercalibration.

Results

The three stratigraphic units—coccolith, sapropel and lutite—can be distinguished by their carbonate and organic matter content (Table 1). The core section is close to Station 379 (Fig. 1). The carbonate fraction in the lutite unit is principally composed of reworked Cretaceous and Tertiary coccoliths, whereas in the sapropel and coccolith units it is authigenic. The organic matter in the sapropel is principally land-derived, particularly at the base of the unit. Increase in carbonate content towards the top signals a progressively higher input of marine planktonic material.

The U_3O_8 content in the sediment matches closely this stratigraphic development. The freshwater lutite unit deposited in aerobic conditions shows the smallest enrichment, and the brackish-marine coccolith ooze at the bottom of the euxinic abyssal plain the highest one (Table 2; core 18). 'Dilution' of coccolith ooze by detrital clay minerals going from the central basin towards the Bulgarian coast, that is from Station 18 to 35, is reflected in a lowering of the U_3O_8 content in the coccolith ooze (Table 2). On the basis of a few analyses from core material

drilled by Glomar Challenger a similar trend seems to be established. A calcareous mud (Table 2; 379 A) deposited in aerobic conditions has little uranium, whereas the five remaining samples formed in an anaerobic environment show a 10–20-fold enrichment in U_3O_8 . The highest values again are found in the two samples from the central basin (Station 379 A).

Treatment of sample material with HCl or water, or by heat combustion, may cause uranium depletion or enrichment in the

Table 1 The carbonate and organic matter content of the stratigraphic units

Depth (cm)	Unit	CaCO ₃ (%)	Organic C (%)	Organic N (%)
2	Coccolith	41.2	2.86	0.25
5		56.2	3.84	0.33
8		34.9	3.53	0.31
12		60.7	4.31	0.37
15		65.7	5.10	0.44
18		48.2	5.17	0.44
22		14.9	5.91	0.49
25		16.5	7.17	0.60
28	Sapropel	11.0	11.45	0.95
32		16.5	12.23	1.11
35		12.4	13.45	1.18
38		7.8	14.35	1.24
42		8.9	15.73	1.26
45		6.9	14.10	1.26
48		6.9	16.85	1.38
52		3.4	19.90	1.41
55		5.0	18.65	1.37
58		3.4	17.42	1.15
62		3.8	15.35	1.02
65		4.3	15.50	1.01
68		12.6	4.70	0.39
72		6.0	2.07	0.19
75	Lutite	10.2	0.31	0.030
78		10.7	0.45	0.032
82		1.8	2.60	0.24
85		0.54	1.60	0.17
88		9.4	0.81	0.09
92		8.8	0.75	0.07

Table 2 The U_3O_8 content of the stratigraphic units

Core	Sample	Depth	Wt-loss (%) 0–100 °C	Wt-loss (%) 0–1,000 °C	(1)	(2)	U_3O_8 (p.p.m.) (3)	(4)	(5)
18	Coccolith ooze	4 cm	68.7	80.5	55.0	113.9	55.4	73.9	94.3
	Coccolith ooze	21 cm	66.0	77.6	59.7	34.6	9.0	74.7	47.2
	Sapropel (top)	30 cm	65.5	74.6	23.4	17.3	40.4	86.4	17.3
	Sapropel (base)	85 cm	71.0	81.9	15.7	40.1		110.0	55.1
	Lutite	100 cm			2.4				
12	Coccolith ooze	10 cm	61.9		35.4				
24	Coccolith ooze	4 cm	66.5		28.3				
35	Coccolith ooze	30 cm	58.4	68.5	15.0			24.3	
	Coccolith ooze	100 m	38.5		40.1		118.3		
379 A	Sapropel	100 m	24.6		49.5		95.8		
	Calcareous mud	231 m	35.3		2.4				
380 A	Carbonaceous lutite	673 m	25.6		35.4				
381	Diatomaceous marl	837 m	30.5		20.4				
	Diatomaceous mud	237 m	37.0		33.8				

(1) After drying at 110 °C; (2) after drying at 110 °C and treated with 10% cold HCl and washed with H_2O ; (3) after drying at 110 °C and hydrolysed with 6 M HCl for 22 h and washed with H_2O ; (4) after ignition at 1,000 °C; (5) after ignition at 1,000 °C and washed with H_2O .

residue (Table 2). Of special significance is the high U_3O_8 yield of the mineral ash obtained in the combustion experiment, and the release of uranium by extracting the sapropel ash with water.

U_3O_8 concentration diminishes because of: (1) loss of mineral-bound water; (2) loss of sulphur-containing volatiles, (3) loss of salts, (4) calcining of carbonates, and (5) burning of organic matter. U_3O_8 depletion is caused by: acidification, $\text{CaO-H}_2\text{O}$ interactions, and release of water-soluble uranium-organic complexes.

The bulk of the uranium seems to be bound to planktonic matter; land-derived organic debris contains comparatively little uranium. Coccoliths seem to be the prime host for uranium in modern Black Sea sediments, but other planktonic organisms share this affinity. The following tentative model is suggested to account for the enrichment.

Eukaryotic cells contain complex membraneous organelles known as the Golgi apparatus or Golgi body which occupy a central position in the transport system of the cell. For example, they fix and transport metals from within the cell to the outer membrane. This is accomplished by metal-ion coordination to specific proteins and polysaccharides which are subsequently transported to the outside. Biomineralisation is an outgrowth of this process¹¹. In the case of coccoliths, uronic acids and polysaccharide-sulphates¹² are the principal metal-ion fixers. The relationships are revealed in an electron micrograph where the organic template is stained by heavy metals, thus revealing the growth pattern of the coccolith within the Golgi body. In this way, metal ions that are not needed by the organism can be readily neutralised (Fig. 2).

Fig. 2 Heavy-metal stained coccolith, *Umbilicosphaera* sp., revealing growth pattern. Scale bar, 0.2 μm .



Uranium content of Black Sea water ranges from 1 to 7 p.p.b., (parts in 10^9) with the average ~ 3 p.p.b. (refs 13 and 14) which is about the value for standard mean ocean water. Our U_3O_8 values are within the same order of magnitude: aerobic zone (2.4 p.p.b.), interface (5.9 p.p.b.) and anaerobic zone (3.5 p.p.b.). Assuming that coccoliths fix the uranium principally during the life cycle of the organism, a 10,000-fold enrichment is observed.

Marine calcareous material of biological origin is reported to contain at most a few p.p.m. uranium¹⁵. In general, carbonates are less favourable host materials for uranium¹⁶. Thus, the enrichment of U_3O_8 in the Black Sea coccolith unit is unexpected and we are not aware of any systematic study on uranium in coccoliths. In view of the nature of the calcifying matrix in coccoliths, however, we tentatively conclude that the uronic acids and sulphated polysaccharides act as a substrate in the formation of hexavalent uranium complexes.

Non-calcareous plants may operate in the same fashion if their cell walls contain such sugars as they do in diatoms. Studies on a Holocene lake in central Ontario, for example, which is polluted by discharge from a uranium mine shows a 10,000-fold U-enrichment in the diatom-dominated living plankton over the water, that is 210 p.p.m. against 20 p.p.b. The sediments, at a water depth of 10 to 25 m, are principally diatomite and reducing in character; their U-content is between 170 and 380 p.p.m. It thus seems that reducing conditions in the depositional environment are only essential for the preservation of uranium-enriched detritus, and not for the fixation. Following sedimentation, a series of organic molecules may pick up additional increments of uranium as well as other heavy metals from sediment and water. The organic material can become stabilised by heavy-metal complexation to a point that it is finally rendered insoluble to extraction by conventional acid or base treatments.

Mass balance

The Black Sea covers an area of $4.23 \times 10^6 \text{ km}^2$, of which 30% is shelf. The basin proper, therefore, has an areal extent of $2.96 \times 10^6 \text{ km}^2$. The top 1 m sediment has a bulk density of 1.25 g cm^{-3} , which will give a sediment mass of $3.7 \times 10^{17} \text{ g}$. Combustion of total sediment at 1,000 °C will reduce weight of material by 80%; weight of remaining ash is $7.4 \times 10^{16} \text{ g}$. Average U_3O_8 content in ash (Table 2) is $90 \times 10^{-6} \text{ g per g}$ sediment. Total U_3O_8 concentration in sediment ash of top 1 m strata of Black Sea basin is $6.7 \times 10^{12} \text{ g}$ or $6.7 \times 10^6 \text{ tonnes}$.

Sapropel and coccolith ooze have been deposited over the past 5,000 yr. Assuming that uranium is extracted at a constant rate over this time, the basin sediments will annually receive $1.34 \times 10^9 \text{ g U}_3\text{O}_8$. Should this material exclusively be extracted from the aerobic water layer having a mean U_3O_8 content of $3 \times 10^{-6} \text{ g l}^{-1}$ or a total of $1.78 \times 10^{11} \text{ g U}_3\text{O}_8$ for the upper 200 m, about 1% of this amount is annually released to the sediment.

Calorimetric determination of combustion heat

Average Black Sea abyssal mud deposited over the past 5,000 yr contains per 1,000 g sample: 600 g H_2O , 100 g clay, 100 g organic matter, and 200 g $CaCO_3$. Since combustion of sediment at 1,000 °C will substantially increase the U_3O_8 content in the remaining ash, it is of considerable interest to know whether indigenous organic matter can supply sufficient energy for this reaction.

Calcining of carbonates: $CaCO_3 \rightarrow CaO + CO_2$, $E = 47.2$ kcal mol⁻¹ and evaporation of water: $H_2O(aq) \rightarrow H_2O(g)$, $E = 9.73$ kcal mol⁻¹ require a total of 418 kcal per 1,000 g of sediment. This is the minimum amount of energy needed for self-sufficient burning.

Table 3 Combustion heat values for the stratigraphic units compared with common organic compounds

Sample	Combustion heat (Kcal kg ⁻¹ at 25 °C)
Coccolith*	528
Top sapropel*	642
Bottom sapropel*	1,109
Wood†	4,500–4,800
Peat†	5,000–5,800
Lignite†	6,200–7,600
Coal†	7,600–8,750

*Samples were pre-dried at 110 °C.

†See (ref. 18).

Combustion heat was determined for three representative samples from Station 18 (Fig. 1), coccolith ooze, top sapropel, and bottom sapropel. A conventional calorimetric bomb was used and combustion heat values are given in kcal kg⁻¹ at 25 °C. Data are reported in Table 3 together with calorimetric values of common organic compounds.

Combustion heat values for the dry mud samples are considered minimum values, because part of the heat generated

during the experiment has been used for calcining, thermal degradation of clay minerals, and decomposition of sulphides. Still, these values are far in excess of the 518 kcal needed to dry and calcine a 1,000 g wet sediment.

In conclusion, heat combustion of Recent Black Sea mud will release more energy than is required for drying and thermal degradation of mineral matter. The combustion process will yield an ash with a U_3O_8 content close to 100 g tonne⁻¹. Slightly higher values are expected in those parts of the Black Sea basin which are furthest removed from river outlets and turbidite incidents. Such a region could be close to Station 379 (Fig. 1).

Although U_3O_8 values of 100 g tonne⁻¹ are not economically significant now, the situation may change in the years to come should global demand for uranium expand at predicted rates. In view of the high sulphur content of recent Black Sea mud a note of caution should be sounded, since mining of the low-grade ore may introduce environmental hazards.

The work was supported by a grant of the Deutsche Forschungsgemeinschaft. We thank Mr K.-D. Schrader for laboratory assistance.

Received 13 June; accepted 2 August 1977.

1. Degens, E. T. & Ross, D. A. (eds) *The Black Sea—Geology, Chemistry and Biology* (Am. Ass. Petrol. Geol., Tulsa, 1974).
2. Deuser, W. G. *Deep-Sea Res.* 18, 995–1004 (1971).
3. Degens, E. T. *Am. Ass. Petrol. Geol. Mem.* 20, 296–307 (1974).
4. Degens, E. T., Michaelis, W., Mopper, K. & Kempe, S. *Neues Jb. Geol. Paläont., Mh.* (in the press).
5. Rona, E. & Joensuu, O. *Am. Ass. Petrol. Geol. Mem.* 20, 570–572 (1974).
6. Starik, I. E. *et al. Dok. Akad. Nauk SSSR* (English Translation), 139, 704 (1961).
7. Strom, K. M. *Nature* 162, 922 (1948).
8. Koczy, F. F., Tomic, E. & Hecht, F. *Geochim. cosmochim. Acta* 11, 86–102 (1957).
9. Johnson, D. A. & Florence, T. M. *Analyt. chim. Acta* 53, 73–79 (1971).
10. Pakalns, P. *Anal. chim. Acta* 69, 211–215 (1974).
11. Degens, E. T. *Top. Curr. Chem.* 64, 1–112 (1976).
12. de Jong, E. W. thesis Univ. Leiden, (1975).
13. Baturin, G. N., Kochenov, A. V. & Kovaleva, S. A. *Dok. Akad. Nauk. SSSR* (English Transl.) 166, 172–174 (1966).
14. Kolyadin, L. B. *et al. Geochemistry* (English translation), 456–457 (1960).
15. Tatsumoto, M. & Goldberg, E. D. *Geochim. cosmochim. Acta* 17, 201–208 (1959).
16. Bell, K. G. *Geol. Surv. Prof. Pap.* 474-A, A1–A29 (1963).
17. Degens, E. T. & Mopper, K. *Chem. Oceanogr.* 6, 59–113 (1976).
18. *D'Ans-Lax Taschenbuch für Chemiker und Physiker*, 1, 1309 (Springer, Berlin-Heidelberg-New York, 1967).

Ozone concentrations in South-East England during the summer of 1976

A. J. Apling, E. J. Sullivan & M. L. Williams

Department of Industry, Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Herts, UK

D. J. Ball & R. E. Bernard

Scientific Branch, Greater London Council, The County Hall, London SE1, UK

R. G. Derwent & A. E. J. Eggleton

Environmental and Medical Sciences Division, AERE Harwell, Oxfordshire, UK

L. Hampton & R. E. Waller

MRC Environmental Hazards Unit, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1, UK

Surface ozone concentrations at five sites in South-East England were measured during the heat wave of 1976 from 22 June to 12 July. At all sites peak hourly concentrations were the highest ever recorded in the UK and the elevated levels persisted for periods longer than any previously measured. Some discussion of the significance of the results is given together with an analysis of back-track air mass trajectories in the context of oxidant formation in North-West Europe.

We have previously reported measurements of atmospheric ozone in urban and rural areas of southern England during the period 1971–75 (refs 1–5). Although, on occasion, the levels exceeded the US (US EPA) air quality standard of 80 p.p.b. (volume parts per 10⁹, ~ 160 µg m⁻³) maximum 1-h mean⁶, the World Health Organisation (WHO) long-term goal of 60 p.p.b. 120 µg m⁻³ (ref. 7) and the Greater London Council (GLC) guideline of 80 p.p.b. (160 µg m⁻³) (ref. 8), the occurrence of elevated levels was, in most instances, confined to irregularly spaced episodes of only a few days duration. During 1976, the unusual meteorological conditions prevailing between 22 June and 12 July

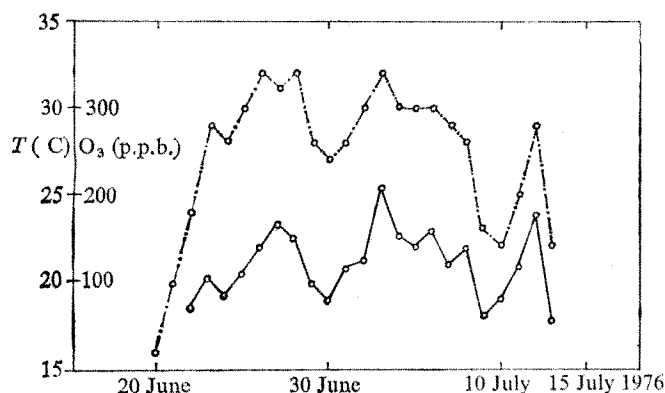


Fig. 1 Ozone and temperature levels during the photochemical episode. \circ --- \circ , temperature at noon, mean of readings at Kew, Gatwick and Heathrow ($^{\circ}\text{C}$). \circ — \circ , highest hourly mean ozone concentration for each day at Stevenage, p.p.b. (parts in 10^9).

resulted in a period particularly favourable for photochemical production of ozone. We present here data showing that the ozone concentrations at all our measuring sites were both higher and more persistent than any we have monitored previously. To illustrate the broad features of the persistence of elevated levels in South-East England during this period we have plotted, in Fig. 1, the highest hourly mean ozone concentration for each day at one of our sites (Stevenage) against the temperature at noon (mean of readings at Kew, Gatwick and Heathrow).

Ozone concentrations are reported from five locations in South-East England, three within the Greater London conurbation and two outside in rural (Harwell) and semi-rural (Stevenage) environments. The Greater London sites were carefully chosen to minimise the effect of local sources of nitric oxide (NO) which is initially a rapid scavenger of ozone. In the centre of London, where this is difficult to achieve, a rooftop site overlooking a courtyard was chosen (Islington, on the City boundary). The suburban sites (Hainault and Teddington) were in mixed open parkland/residential locations. The Warren Spring Laboratory site lies on the western edge of Stevenage New Town with open country to the south and west. Two local sources of NO are a light industrial area to the north and the A1(M) motorway some 40 m to the west. The only significant sources of NO at the Harwell site are likely to be traffic on the A34 road ~1.5 km to the east and vehicles on the AERE site itself. The locations of the sites are shown in Fig. 2, and specific site details in Table 1.

Three sites were equipped with ethene-chemiluminescence⁹ (EC) ozone monitors and two with instruments operating on the ultraviolet absorption principle¹⁰ (UV).

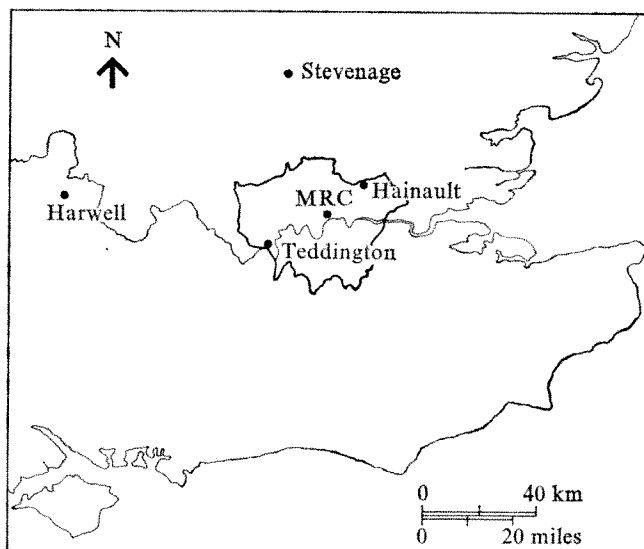


Fig. 2 Sketch map of South-East England showing location of sampling sites.

We have attempted to quantify the similarities and differences between the sites by investigating the correlations between the hourly mean ozone concentrations for each pairing of sites and the results are shown in Table 2. Initially, hourly values from one site were regressed against the concentrations for the same hours at each of the other sites in turn. The results in Table 2 show, that, in general, the Harwell ozone levels are higher than the other sites for which no general pattern can be distinguished. We then investigated, for pairs of sites, the correlations of hourly values displaced relative to one another, that is hour n for site A was correlated with hour $n+m$ of site B. This was done for all pairs of sites for values of m from -11 to +11. It was found that for all pairs of sites not involving Harwell,

Table 1 Site details

Site	Site description	Height above ground (m)	Instrument	Calibration
City (C)	Courtyard in centre of Greater London ~70 m from nearest street	20	EC	KI
Hainault (Ha)	Suburban London. Open country to S and E, otherwise residential	4	UV	KI + gas phase titration
Harwell (H)	Rural, 1.5 km from A34 trunk road	2	EC	KI + optical extinction
Stevenage (S)	Light industry to N, motorway 40 m to W, residential to E, otherwise open country	10	EC	KI
Teddington (T)	Suburban London. Open parkland to S, residential to N	13	UV	KI + gas phase titration

Table 2 Inter-site correlations of ozone levels for 1976 episode

Site versus Site		Lag* (h)	Correlation coefficient	Mean regression†	Significance level of correlation coefficient change with lag (%)
H	S	0	0.78	$S = 0.77H - 7.9$	
H	C	0	0.74	$C = 0.72H - 13.2$	
H	T	0	0.74	$T = 0.72H - 7.7$	
H	Ha	0	0.67	$Ha = 0.73H - 11.3$	
H+1	S	1	0.80	$S = 0.77H - 7.8$	79
H+1	C	1	0.76	$C = 0.71H - 12.7$	77
H+1	T	1	0.81	$T = 0.72H - 7.4$	99
H+2	T	2	0.81	$T = 0.72H - 7.5$	99
H+1	Ha	1	0.72	$Ha = 0.73H - 10.5$	91
H+2	Ha	2	0.73	$Ha = 0.73H - 10.4$	93
S	C	0	0.78	$C = 0.93S - 5.5$	
S	T	0	0.86	$T = 0.94S - 1.5$	
S	Ha	0	0.81	$Ha = 0.94S - 2.8$	
C	T	0	0.81	$T = 1.0C + 5.5$	
C	Ha	0	0.88	$Ha = 1.0C + 2.7$	
T	Ha	0	0.81	$Ha = 1.0T - 2.8$	

H, Harwell; S, Stevenage; C, City; T, Teddington; Ha, Hainault.

*For example, H+1 means hour $n+1$ for Harwell was correlated with hour n for other sites.

†Mean of y on x and x on y .

the correlation coefficient for $m=0$ was highest. In contrast, for correlations of Harwell values against those from all other sites, improved correlation coefficients were obtained with $m = +1$ or $+2$, that is, better correlations were obtained by regressing hourly values from the other sites against later hourly values at Harwell. Two points emerge clearly from this analysis; the results for the Stevenage site are similar in magnitude and diurnal pattern to the Greater London sites and Harwell is significantly different from each of the other sites in both respects. The explanation of both points is in part linked with local emissions of NO and the close proximity of the light industrial area and motorway turning the otherwise rural aspect of the Steven-

age site into a more urban situation while the relatively low NO levels at Harwell lead to less ozone depletion. The important conclusion is that, in common with the Los Angeles area¹⁹ it is the rural areas downwind of the primary pollutant sources which experience the higher ozone levels.

The maximum 1-h mean concentrations of ozone measured at each of the sites exceeded those previously reported by considerable margins (see Table 3).

During the 21-d period 22 June to 12 July the numbers of hours above 100 p.p.b. within Greater London were 85, 86 and 100 at the City, Hainault and Teddington sites and 115 and 158 at Stevenage and Harwell, outside Greater London. The total number of hours above 100 p.p.b.

Fig. 3 Ozone concentrations in p.p.b. (y axis) measured at each site during the photochemical episode. H, Harwell; T, Teddington; Ha, Hainault; C, City; S, Stevenage. *a*, 22 June; *b*, 29 June; *c*, 6 July.

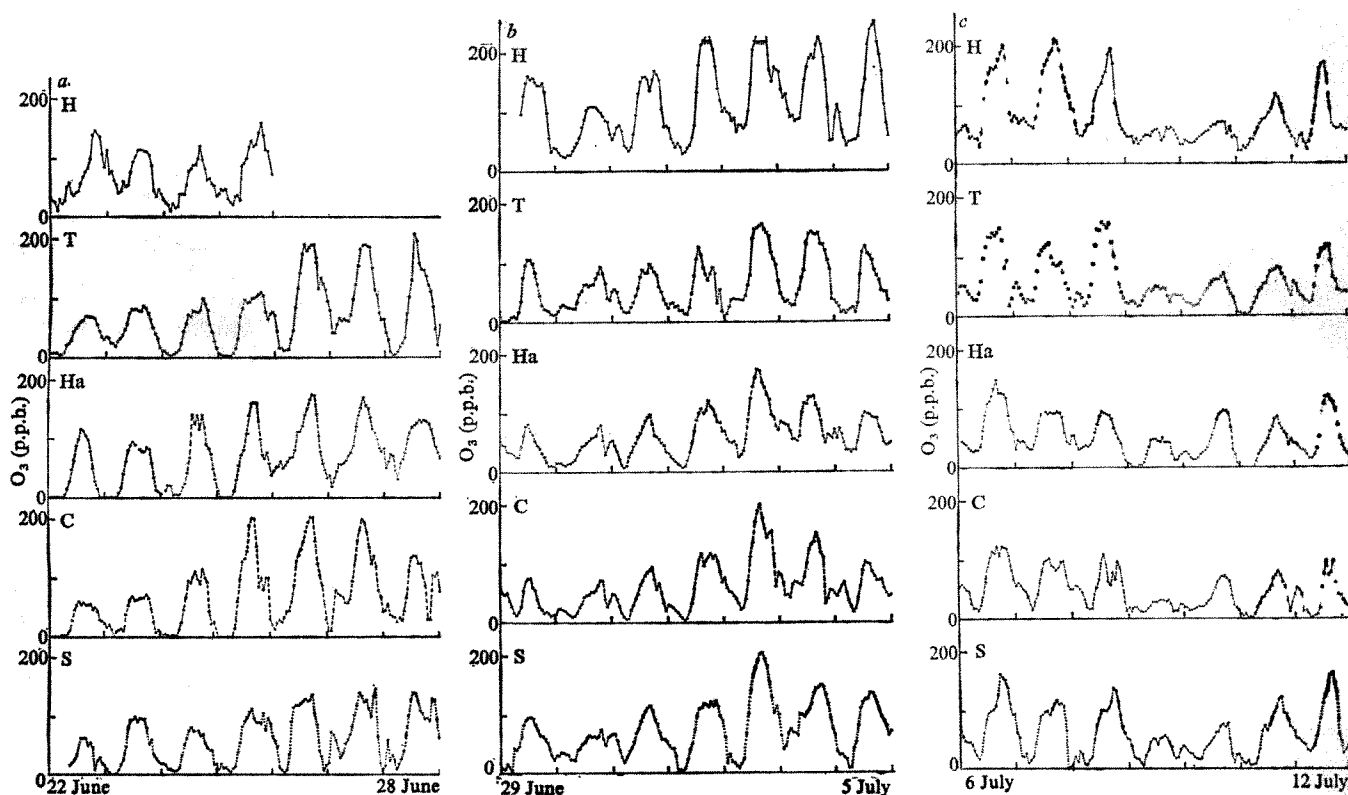


Table 3 Highest hourly mean ozone concentrations observed during 1976 compared with previous measurements

Location	Year	Highest hourly mean, p.p.b.	Ref.
Central London	1972	112	2
	1973	136	3
	1974	164	13
	1975	150	4
	1976	210	This work
London suburbs	1975	130	4
	1976	210	This work
Rural southern England	1971	101	1
	1972	128	14
	1973	141	3
	1974	120	12
	1975	177	12
	1976	258	This work

accumulated during the whole period 1971–75 at the Harwell site was exceeded in this one 1976 episode. For the first time in the UK, urban levels exceeding 200 p.p.b. and rural levels exceeding 250 p.p.b. were measured. At each site there was a continuous period of 5 d or more (Table 4) when the ozone concentration exceeded 100 p.p.b. for at least 8 h each day. Thus the industrial threshold limit value (TLV)¹⁵ was exceeded at all our sites in the outside air in South-East England for the equivalent of a working week. In this analysis the duration of high concentrations at Stevenage was intermediate between that in the urban and rural extremes of London and Harwell. Although these levels were high in comparison with the air quality guides and industrial threshold limit values cited above, it is not possible to evaluate their direct significance in terms of human health. Comparison with the TLV is not necessarily appropriate for such limiting values are intended for use in evaluating health risks for the occupationally exposed. For the general population, which includes the young and the infirm, a further margin of safety is usually regarded as prudent.

The US air quality standard and the WHO long term objective are more stringent than the TLV. Both were exceeded during 1976; but, they are also exceeded in the US and in many other developed nations. There was a sharp increase in mortality in Greater London at the time of this episode¹⁸ but effects due to anything other than the high prevailing temperatures could not be isolated. During early July serious damage was observed on two crops of tobacco plant (Bel-W3) grown specifically to test for oxidant effects at Leeds and at the Fairfield Experimental Horticulture Station, Kirkham, Lancashire (J. T. Fletcher, personal communication). High percentage leaf damage would be anticipated on the basis of models of tobacco plant injury¹⁶ and the ozone concentration reported here. No effects of elevated oxidant levels to any other types of plant were reported during the episode.

Elevated ozone levels in the UK have generally been associated with anticyclones to the east of the UK³ and to this extent the photochemical episode of 22 June to 12 July was typical. In such cases the air mass trajectories over

southern England are easterly and in general cross NW Europe. Typical back-track trajectories are illustrated in Fig. 4. Those for 27 June and 3 July describe the history of the air mass reaching South-East England on two of the days of particularly high ozone concentrations. On 7 July the levels at most sites had begun to decrease and 9 July, the trajectory for which shows a south-westerly air flow over South-East England, was the day on which all sites showed their lowest daily maximum hourly mean ozone concentration throughout the episode.

A closer study of individual back-track trajectories highlights a number of factors which must be borne in mind

**Fig. 4** Back-track air mass trajectories for four selected days during the photochemical episode. The location of the air mass at various times in hours before its arrival at midday in London is indicated on each trajectory. *a*, 27 June; *b*, 3 July; *c*, 7 July; *d*, 9 July 1976.

if an abatement policy for photochemical oxidants were to be considered. First, generation of ozone requires several hours of solar irradiation of the primary pollutant plume even with the more reactive hydrocarbon precursors so that photochemical ozone is usually first detected 50 km or more downwind of the primary source¹⁷. In the densely populated and highly industrialised regions of North-West Europe this makes source identification difficult. Second, photochemical ozone can be transported over hundreds of km from source regions in North-West Europe with the limited deposition at sea surfaces and because ground-based nighttime inversions typical of anticyclonic weather isolate the ozone formed during the day from ground sink surfaces over rural areas³. Third, primary NO emissions, while constituting an important precursor of photochemical ozone are initially a potent sink for ozone in more polluted areas and the emission and dispersion pattern of NO will have a major effect on ozone concentrations measured close to the ground. These factors tend to obscure any simple interpretation of the effect of a particular precursor source region.

As an example, we consider here the trajectory of 3 July (*b*, in Fig. 4). The air mass present over South-East England on 3 July had travelled across southern Poland, a possible

Table 4 Maximum number of consecutive days with ozone > 100 p.p.b. for at least 8 h

Location	No. of days	Longest period
City	5	24 June–28 June
Hainault	5	24 June–28 June
Teddington	6	3 July–8 July
Stevenage	10	1 July–9 July
Harwell	18	22 June–9 July

area of precursor emission, about 45 h previously. Then there was about $\frac{1}{2}$ cloud cover reducing the radiation flux and a temperature of about 25 °C so that photochemical activity was probably limited. Throughout the afternoon of 2 July the air mass traversed central West Germany with clear sky conditions and temperatures of 30 °C. The conditions for precursor injection and photochemical transformation were potentially excellent. By the morning of 3 July the air had reached the industrial areas and refineries of the Thames estuary, also potential sources of precursors, with clear sky conditions still prevailing. At this time the air mass was moving at about 400 km d⁻¹, although surface winds were only 4–10 km h⁻¹. Thus several hours later the air reached central London where maximum ozone values were recorded during the period 1400–1600 BST. The air mass which gave rise to the elevated ozone levels on 3 July, therefore, had 3 d with probable precursor injection and two full days of exposure to strong sunlight and high temperatures during which the ozone was formed. The history of air over London on other days in this episode with high recorded ozone levels was similar.

It has previously been noted that the data for Greater London follow a pattern which is consistent with the mechanism of local generation of ozone, both from the point of view of precursor emissions and the concentration gradient of ozone across London from east to west^{2,4}. That proportion of the total ground level ozone concentration which is produced locally is difficult to estimate solely from ground-level measurements. The complexities of the air mass trajectories presented here and the likely emissions of precursors along their paths together with considerations of precursor reaction times and NO emissions all tend to merge local production into the general pattern of secondary pollutant formation in North-West Europe as a whole.

Our further understanding of this phenomenon and the

role of various primary pollutants clearly requires the co-operation of other European countries, upwind and downwind of the UK, in ozone and precursor measurement programmes. Data on the occurrence of high ozone concentrations in North-West Europe in the period 1971–75 have been assembled in a recent publication²⁰ and we would welcome the further publication and/or exchange of relevant data from those areas which the back-track trajectories indicate to be of importance in oxidant production.

We thank Dr F. B. Smith (Meteorological Office, UK) for help in calculating the trajectories. The measurements carried out at AERE and at Warren Spring Laboratory were part of a programme sponsored by the Department of the Environment.

Received 14 July; accepted 2 August 1977.

1. Atkins, D. H. F., Cox, R. A. & Eggleton, A. E. J. *Nature* **235**, 372–376 (1972).
2. Derwent, R. G. & Stewart, H. N. M. *Nature* **241**, 342–343 (1973).
3. Cox, R. A., Eggleton, A. E. J., Derwent, R. G., Lovelock, J. E. & Pack, D. H. *Nature* **255**, 118–121 (1975).
4. Ball, D. J. *Nature* **263**, 580–582 (1976).
5. Stewart, H. N. M., Sullivan, E. J. & Williams, M. L. *Nature* **263**, 582–584 (1976).
6. *National Primary and Secondary Ambient Air Quality Standards*. Federal Register 36, 84, Part II (US Government Printing Office, Washington DC, 1971).
7. *Technical Report no. 506* (World Health Organisation, Geneva, 1972).
8. Greater London Council Minutes, *London's Air-Guideline Concentrations*, 510–511, 22 July 1975.
9. Hodgeson, J. A. *Int. J. Environ. anal. Chem.* **2**, 113–132 (1972).
10. Nederbragt, G. W., Van der Horst, N. & van Duijn, J. *Nature* **206**, 87 (1965).
11. *Selected Methods for the Measurement of Air Pollutants* (US Public Health Service Publication no. 999-AP-11, May, 1965).
12. Cox, R. A., Derwent, R. G. & Sandalls, F. J. *AERE Report R 8324* (HMSO, London, 1976).
13. Derwent, R. G., McInnes, G., Stewart, H. N. M. & Williams, M. L. *WSL Rep. LR 227(AP)* (Department of Industry, London, 1976).
14. Bell, J. N. & Cox, R. A. *Environ. Pollut.* **8**, 163–170 (1975).
15. *Threshold Limit Values for 1974* (HMSO Technical Data Note 2/74, London, 1974).
16. Larsen, R. I. & Heck, W. W. J. *Air Pollut. Control Ass.* **26**, 325–333 (1976).
17. White, W. H. *et al. Science* **194**, 187–189 (1976).
18. Macfarlane, A. & Waller, R. E. *Nature* **264**, 434–436 (1976).
19. *Photochemical Oxidants in the Ambient Air of the United States* (US Environmental Protection Agency Report no. EPA-600/3-76-017, 1976).
20. Guicherit, R. & van Dop, H. *Atmos. Environ.* **11**, 145–155 (1977).

Palaeoclimates of Central Sahara during the early Holocene

J. Maley

Laboratoire de Palynologie, USTL, 34060 Montpellier, France.

In the Central Sahara lying approximately between 27°N and 18°N, rains were primarily due to tropical depressions in the early Holocene up to about 6500 BP. Then the monsoon rains of Sahelian type dominated up to about 4400 BP.

POLLEN analysis was carried out on Holocene lacustrine deposits¹ sampled every 10–20 or 30 cm in a section of about 7.80 m at Tjéri (13°44'N–16°30'E) near the centre of the great Palaeochad^{2,3} (Fig. 1). Chronology of the Tjéri section was established by two radiocarbon dates on organic material near the base (Fig. 2) and, for some other levels, by correlations with notable events radiocarbon dated elsewhere in the zone of the Palaeochad^{1–3}. The chronology established in the Nile valley (see below) and elsewhere in the southern part of the Sahara gives some valuable correlations¹. Approximate dating of the intermediate levels was established by rates of sedimentation. About 60 yr elapsed for each 10 cm from 9000 to around 7000 BP and about 220 yr from then till about 4000 BP. The change in rates of sedimentation at about 7000 BP could parallel the important change in conditions noted for the Blue Nile at about 7000 BP (ref. 4).

In the dry tropical zone where the Chad basin is situated, the rainfall and its distribution through the year are the most important climatic factors controlling the vegetation and its spatial distribution. Thus there is a direct relationship between vegetation and climatic pattern. In the Chad basin the regular succession of climatic and vegetation zones is a favourable factor for pollen analysis⁵. On the Tjéri section 46 samples were studied and for each about 1,000 to 6,000 pollen grains were counted. The Gramineae, Cyperaceae and *Typha* pollen grains represent about 80 to 90% of each total. Therefore those 3 taxa have been eliminated from the pollen sum and studied apart in order not to distort the percentages of all other pollen grains more typical. Those pollen grains were classified according to the present geographic distribution of the taxa to which they belong and the most typical were placed in four phytogeographical elements covering the four major vegetation zones of the Chad basin, that is, (from S to N) Sudano-guinean element (plants typically growing under 1,500 to 1,000 mm rain per annum), Sudanian element (1,000 to 500 mm), Sahelian element (500 to 100 mm), Montane (Tibesti) element (plants growing at the nearest on the upper Tibesti Plateaux) and also in a group of hygrophilous plants⁵. The relative percentages of the pollen grains for these four elements were used to construct curves which portray the climatic variations occurring in the four zones of the Chad basin¹.

Palaeoclimatic pattern of the Sahelian zone

Comparison of the Sudano-Guinean element and Sahelian element curves showed that the periods of climatic optima (relatively wetter phases) are in general out of phase with each other¹ (Fig. 2). It seems also that during the Holocene, the Sahelian climatic optima have always been synchronous with the warming periods, and the deteriorations with the coolings. Indeed the trends of the Sahelian curve at Tjéri—the amplitudes of variations being different—correlate well with the trends which appear on some curves portraying the evolution of the temperature on the Northern Hemisphere, such as that of Camp Century in Greenland^{6,7}. For the most part of the Holocene, it seems that the Camp Century curve is quite well dated: first, the counting of annual layers was possible with some correction until 8300 BP (ref. 7); second, for the Holocene this curve has good cross-checkings with various eustatic curves⁸⁻¹⁰, with the fluctuations in the

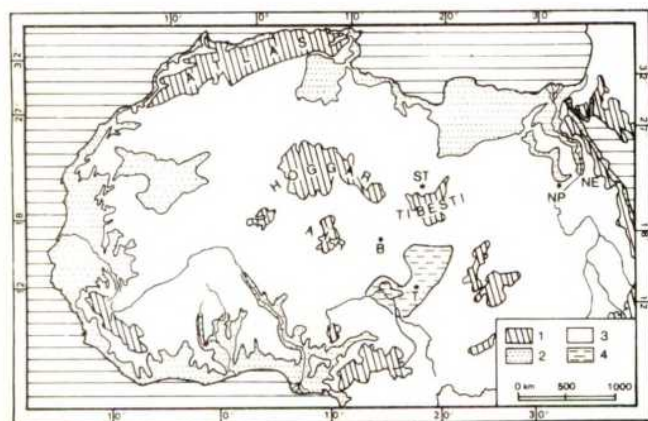


Fig. 1 North Africa. 1, Mountains over 1,000 m. 2, Regions under 200 m. 3, Regions between 1,000 m and 200 m. 4, Holocene Palaeochad, 320 m high. T, Tjéri. B, Bilma. ST, Sérir Tibesti. NP, Nabta Playa. NE, Egyptian Nubia.

atmospheric radiocarbon level^{11,12}, with glacier fluctuations in the Alps¹³, and so on. Comparison of the Sahelian curve with that of Camp Century shows that the lowering periods of the Sahelian curve, which correspond to aridification periods in the Sahel zone, correspond also to cooling periods, and vice versa¹. Study of diatoms^{2,14} and pollen in the same samples provides a direct and important corroboration of this phenomenon. Indeed, from about 8000 up to about 4000 BP, the high percentages of a psychrophile ('cold') diatom *Cymatopleura elliptica* or of a diatom of temperate type *Cyclotella ocellata* occur during the phases of relative lowering or minima of the Sahelian element (Fig. 2). Considering these different correlations, one can also use the curve of Camp Century as a basis for an explanation of the Montane element.

The Montane (Tibesti) element

Pollen spectra of Tjéri also showed small percentages (average 3.1%) of very characteristic taxa originating from more northern regions (*Artemisia* sp., *Pentzia monodiana*, *Erica arborea*, *Ephedra* sp., *Plantago* sp., *Silene* sp.). When many pollen grains are counted on successive samples of the same section, the variation of very low percentages can be used with some confidence—as, for instance, in a pollen study of the west part of the Sahara¹⁵. The pollen grains assigned to the Montane element must surely have been brought to Tjéri by the prevailing wind, that is, the harmattan, blowing from about NE or N. The water transport of pollen grains across the whole actual lake Chad is very limited⁵ and probably was so for the Palaeochad, particularly for pollen grains coming from the Tibesti, because the nearest fluvial source was about 450 km from the station studied. For these pollen grains, the nearest sources at present are some 700 km to the north on the High Plateaux of the Tibesti¹⁶. Further north, other important sources of *Artemisia*,

for instance, are found only on the narrow Mediterranean zone of Libya, about 2,000 km from Tjéri. But the latter sources seem unlikely to be involved, because other pollen types present in the Mediterranean zone of Libya, such as Cupressaceae or *Quercus*—very easily carried in the atmosphere¹⁷—would have been found at Tjéri. In conclusion, the pollen grains of the Montane element originate most probably from the Tibesti.

Comparison of the early Holocene pollen curves for the Sahelian and Montane elements shows (Fig. 3) a rough synchronicity in terms of thousands of years and a good synchronicity for the main aridity maxima. This second point shows that the percentage variability of the Montane element is not governed by variations in intensity of the harmattan, as the aridity maxima coincide with maxima of eolian activity. On the other hand, thanks to the chronology established at Tjéri, it seems that the different pollen maxima of the Montane element show good correlations with the different geological and palaeoecological events radiocarbon dated in the Tibesti (Fig. 2, Table 1). These correlations also increase the reliability of the pollen curve.

The Middle Terrace

In the Tibesti, the early Holocene coincides with the end of the accumulation of the so-called 'Middle Terrace' (MT)¹⁸⁻²³ (Fig. 2). A radiocarbon dating in the lower part of the MT gave a date of 14055 ± 135 BP (ref. 20). It is possible that the base of the MT in the Tibesti began about 17500 BP like the

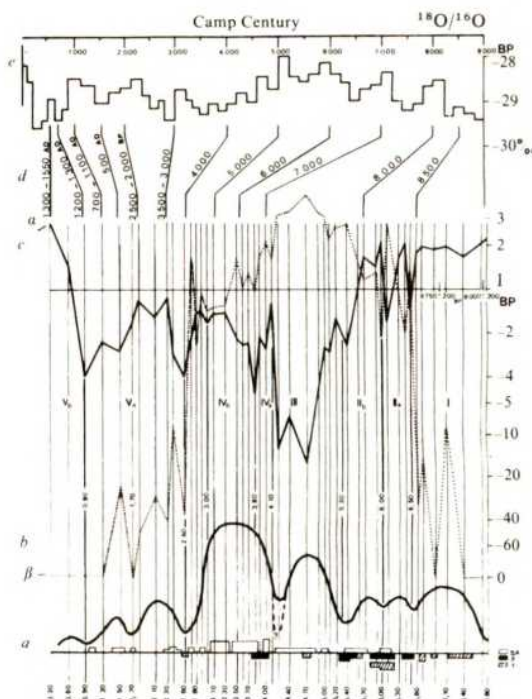


Fig. 2 Comparative evolution for the Tjéri station, from base to top. a, Relative lacustrine levels, after diatom studies^{2,14}. The ? at 4,20 m is the author's interpretation¹ and a brief regression at 3.80 m (around 6600 BP) is also probable. Some diatoms of ecological importance: SA, *Stephanodiscus astrea* var. *minutula*; F, *Cymatopleura elliptica*; T, *Cyclotella ocellata* (relative scale). b, Dotted lines, pollen curve for the Sudano-Guinean element (relative %, mean: 20.2%). β , Present time % (two samples). c, Solid line, pollen curve for the Sahelian element (relative %, mean: 33.9%). α , Present time % (two samples). These two curves (b, c) were constructed using the ratio with the mean (value of 1) carried out on the whole of the levels studied (log scales). On this scale, the value of zero was arbitrarily fixed at -100. d, Chronology reconstituted after various regional correlations. Near the base, two radiocarbon dates: at 7.75 m, 9000 ± 200 BP; at 7 m, 8750 ± 200 BP. The dates are given in BP except for the last two millennia in AD (calendar years from ref. 12). e, Schematic curve of the $^{18}\text{O}/^{16}\text{O}$ ratio for the ice core at Camp Century (Greenland) adapted from M. Ters (ref. 10); from 9000 to 1700 BP after ref. 7 and from 1700 BP to the present after ref. 6. Figure reproduced from ref. 1 with kind permission.

base of the equivalent formation in Nubia (Malki Member of Ineiba Formation)^{24,25}. In general, throughout all the Tibesti, the stratigraphic and sedimentological evolution of the MT, especially the upper part, is constant¹⁸. Some sections with radiocarbon dates facilitate the precise dating of the occurrence of the principal events intervening during the accumulation of the upper part of the MT. For the phases A, B, C, in some craters or depressions throughout the massif, there are lacustrine deposits chronologically equivalent^{18,19,26}. The accumulation of the MT occurred in several phases separated by calcareous crusts, the most recent of which were principally formed about 9200–9300 and 7600–7300 BP (D phase)^{20–22}. By correlation with the Montane element curve, it seems that the last calcareous crust formation corresponds to an arid period (see below). At the end of the D phase, a small erosion occurred before sedimentation started again. This discontinuity is a very characteristic feature. The last phase of bedded deposits (E), sometimes with coarse material, exhibits in some places, before the beginning of phase F, a thin brown palaeosol rich in organic matter^{23,28}, which was dated 6600±140 BP at Mouskorbé (Gif n°3228). In Nubia, a palaeosol with kaolinite (Omda soil) was described and situated approximately at 7000 BP (ref. 24).

At the top of the MT, phase F represents a very clear sedimentological change, that is, a dramatic increasing amount of pebbles and cobbles. This phenomenon, which had started after 6600 BP, is general across the Tibesti and the Central Sahara at the top of formations equivalent to the MT. This sudden influx of pebbles is surely related to a change in climatic regime. Then the erosion of the MT (phase G) to below the actual level of the rivers could occur between about 5500 and 4000 BP. In the Tibesti, during the following H phase approximately dated 4000–2000 BP, a Lower Gravel Terrace was deposited^{18,19}.

The upper part of the MT with its main characteristics or some equivalent lacustrine deposits, can be found in many regions of the Central Sahara—for the MT, in the Hoggar^{18,27}, in Central Aïr³ and in Egyptian Nubia (Sinqari Member, upper part of Ineiba Formation and Shaturma Formation, Member I)²⁴ (Table 1). For the lacustrine deposits, the depression of Nabta Playa²⁸ (altitude about 300 m) about 100 km west of Abu Simbel, exhibits a stratigraphic succession and lacustrine phases with prehistoric occupation sites closely correlated with the events of the Tibesti (Table 1). The Terminal Palaeolithic II (or Epipalaeolithic), though without radiocarbon date, corresponds most probably to the climatic optimum B of the Tibesti (about 8400–8250 BP). On the other hand, near Bilma in the Ténéré (18°40'N–13°E; altitude about 360 m) the early Holocene lacustrine deposits³ can be correlated with phases A, B, C of the Tibesti (Fig. 3). In Adrar Bous (20°18'N–9°E; altitude about 700 m) early Holocene lacustrine deposits are radio-

carbon dated before 7310±120 BP (refs 29, 30). In the middle of Serir Tibesti (23°30'N–17°20'E; altitude about 500 m) lacustrine deposits about 10 m thick are dated of the early Holocene³¹. Thus all these correlations suggest that during the early Holocene the climatic conditions were similar and apparently synchronous throughout all the Central Sahara. Therefore, the local factors seem to have had but a limited action.

One typical section of the MT for the early Holocene was chosen in order to show the correlations with the evolution of the Montane element of Tjéri (Fig. 3). This section was taken by J. Grunert²³ near Yebbi-Bou (20°53'N–18°4'E; altitude about 1,440 m) on the north side of the Tibesti. A radiocarbon-dating on shells at about 2.5 m (8180±70 BP) locates this section in the chronological framework of the Tibesti and the Central Sahara.

Palaeoclimatic interpretation

To try to understand the palaeoclimates of the Tibesti in the early Holocene we can compare the pollen curves for the Montane and Sahelian elements (Fig. 3c, d). The system of representation (the ratio with the mean) facilitates direct comparison for the two curves (see other examples in refs 5, 17). Thus, from one sample to the next we can see completely opposite trends, or similar trends but of very different amplitude. Although in the scale of thousands of years there is a rough synchronicity between the curves for these two elements, the opposite trends shown up by detailed analysis imply that the rains were of different origins in the Sahel zone and the Tibesti.

For palaeoclimatological interpretations it is necessary to use as climatic models meteorological situations and climatic patterns of the present time. For instance, in Aegean regions, the climatic pattern of one winter was used to explain that of the period around 1200 BC (ref. 32); or seasonal change through the year was used to explain some aspects of the past climatic changes³³. Here the present evolution of the Saharian climate through the year is used in an attempt to establish the climatic pattern of this region in the early Holocene.

Climatic model for the present time

At present nearly all the rains falling in the Sahel zone are monsoonal in origin. In summer (July–August) these rains reach the Hoggar and Tibesti mountains^{34–36}. But, over the Central Sahara the heaviest rains fall chiefly during the intermediate seasons of spring (March–June) and autumn (September–December)^{35,37,38}. The study of the cloud formations over the Sahara also confirms the importance of the intermediate seasons³⁹. The rains of inter-

Table 1 Early Holocene events and correlations between Tibesti, Nabta Playa and Egyptian Nubia

Phases	Dates (approximate, in BP)	Tibesti	Nabta Playa (30°40'E–22°25'N) (from ref. 28)	Egyptian Nubia (from ref. 24)
H	4000–2000	Lower Gravel Terrace		
G	5500–4000	Erosion	Erosion (undated)	Erosion
F	6400–5500	Sahelian optimum		Shaturma Formation (Member I)
	~6400	Change of Climatic Régime		
E	7200–6500	Optimum: Palaeosol	Neolithic (II) 6450–7150 BP (6 dates)	Palaeosol (Omda Soil)
D	7700–7200	Erosion	Erosion	Erosion; (?) locally eolian activity, (Seiyala)
C	8100–7700	Optimum	Neolithic (I) 7850–8150 BP (10 dates)	Fluviatile Terrace, Sinqari Member (upper part Ineiba Formation) 10200–8000 BP
	8250–8100	Aridity	Erosion	
B	8400–8250	Optimum	(?) Terminal Palaeolithic (II)	
	8550–8400	Aridity	Erosion	
A	9000–8550	Optimum	Terminal Palaeolithic (I) (partly with A; 2 dates: 8580±80 and 9360±70 BP)	
	(?) 9300–9000	Aridity		

Note that in other places the Neolithic begins in 8065±100 (Tibesti), 8072±100 (Acacus), 8100±130 (Tassili), 8100±130 BP (Hoggar)³⁸.

seasons are linked with the tropical depressions^{34,40-43} also called Sudano-Saharan depressions³⁵ or 'Khamisin' depressions in Eastern Sahara^{44,45}. Rains of this type are often fine and continuous⁴⁶, whereas the monsoon rains are stormy. At present these depressions occur rarely in winter except in Western Sahara (Senegal, Mauritania: 'Heug' rains^{34,35}). On the other hand, these depressions are absent in the height of summer when the ITCZ reaches the Sahara. Schematically the synoptic situations are as follows⁴¹⁻⁴⁶: (1) influx of polar air in the middle or upper troposphere above the Sahara along shallow troughs in the upper westerlies and (2), frequently, ahead of these cold troughs, undulations occur in the ITCZ with brief invasions of humid equatorial air. The undulations of the ITCZ could be due to the action of boreal cold troughs or to surges of monsoon caused by perturbations travelling in the Southern Hemisphere⁴⁶. The depressions created by the cold air aloft favour the advection of the equatorial humid air. In this part of the year the movements of these depressions are north-eastwards or eastwards³⁵. The advection of humid equatorial

an example, two Gramineae, with close taxonomic affinities, *Aristida meccana* and *A. mutabilis*, have geographic distributions which exhibit the same pattern. The first one has an area restricted to the Central Sahara, the second a Sahelian area with some stations in the Central Sahara in zones exposed to the monsoon⁴⁹. I consider that this opposition Sahel-Central Sahara, can only be explained by the hypothesis of different origins for the rains, that is, the direct monsoon and the tropical depressions. Thus this opposition must no doubt be comparable with that which existed over longer periods in the early Holocene.

Application of the present model in the early Holocene

The dominance of tropical depressions over the Tibesti seems to be proved, especially between around 8000 and 6500 BP, by the fact that the fluctuations of the curve for the Montane element approximately follow those of the present tropical depressions over the Sahara through the period of 1 yr (refs

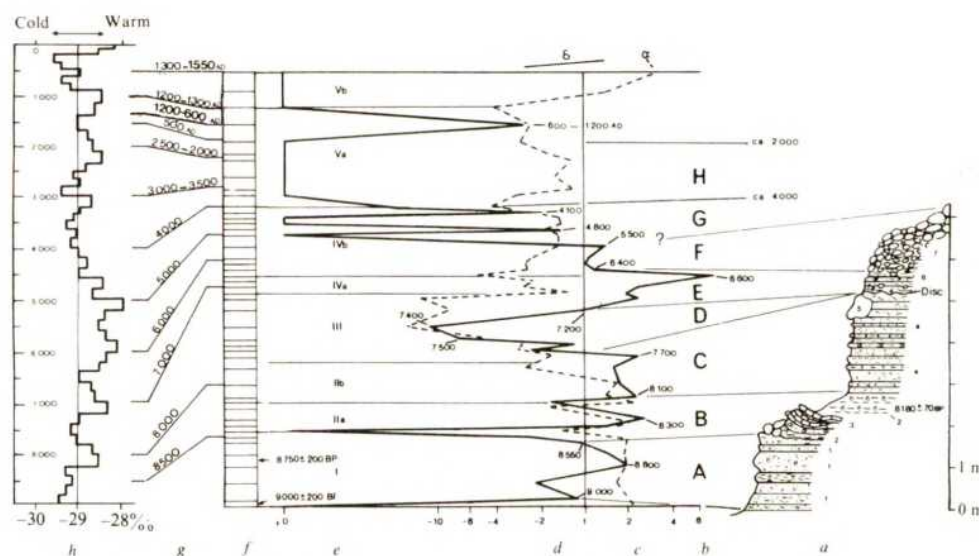


Fig. 3 Comparative evolution of the MT in the Tibesti, of the pollen curves for the Montane and Sahelian elements and of the $^{18}\text{O}/^{16}\text{O}$ ratio at Camp Century. *a*, 'Middle Terrace' (MT), section near Yebbi-Bou, after J. Grunert (23, pr. 20). 1, Indurated layers with coarse sands, bedded towards the base and becoming silty towards the top. 2, Compact silts with shells. Radiocarbon date of 8180 ± 70 BP near 2.50 m. 3, Compact indurated silt layer. 4, Alternating layers of indurated coarse sands and looser silts. 5, Boulder of calcareous tufa. Discontinuity. 6, Finely bedded layers of sand, gravels and some pebbles. 7, Conglomerate of pebbles and cobbles. *b*, Subdivisions of the MT and of the pollen curve for the Montane element of the Tibesti. *c*, Solid line, Tjéri section, pollen curve for the Montane element of the Tibesti (relative %, mean: 3.1 %). *d*, Present time % at Tjéri (two samples). *d*, Dotted line, Tjéri section, pollen curve for the Sahelian element (relative %, mean: 33.9 %). *e*, Present time % at Tjéri (two samples). For the construction of curves *c*, *d* see Fig. 2. *e*, Subdivisions of the pollen curve for the Sahelian element (ref. 1 and Fig. 2). *f*, Positions of the samples and two radiocarbon-dates near the base (see Fig. 2). *g*, Chronological correlations between the $^{18}\text{O}/^{16}\text{O}$ curve and Tjéri samples (see Fig. 2). *h*, See Fig. 2e.

air is essential for the formation of rain from these depressions. At present in winter, the scarcity of these depressions over the Sahara, except the western part with 'Heug' rains, can be explained by the fact that at this time of the year the ITCZ is situated at very low latitudes. But when there is interaction between cold troughs and the ITCZ, the trajectory of depressions remains chiefly over the Sudan and Sahel zones³⁴⁻³⁶.

A study of all rainfall data available since the beginning of the century for Africa north of the equator was carried out⁴⁷. It showed, by the method of spatial correlation of annual rainfalls, that in some years there is a clear opposition between the Sahel and Central Saharian zones. Either the Central Sahara gets heavy rains and the Sahel low or average rainfall, or vice versa. The phenomenon is less clear for Eastern Sahara, due to the lack of sufficient data and to the probability that the trend for the whole Central Sahara is only apparent for longer periods. Thus, for instance, only anomalies over 30 yr have a similar trend through the Mediterranean area⁴⁸. Nevertheless, this climatic opposition is genuine because it had an effect on the distribution, and perhaps the evolution, of some taxa. To give

37, 38, 45), when in the Northern Hemisphere temperatures are rising (spring) or falling (autumn). When temperatures are at their lowest (winter) these depressions are infrequent and when at their highest (summer) they do not occur. Between 8000 and 6600 BP the following correlations appear between the trends of temperatures on the Northern Hemisphere ($^{18}\text{O}/^{16}\text{O}$ curve at Camp Century^{6,7}, see above) and those of the curve for the Montane element (Fig. 3 *h*, *c*).

At around 8000–7700 BP—temperature is falling (autumn climatic model)—the Montane curve is at a maximum. At 7500–7400 BP—temperature and Montane curve are at a minimum (winter climatic model). At 7400–7100 BP—temperature is rising (spring climatic model)—there is a strong positive trend in the Montane curve. At 7100–7000 BP—temperature reaches a maximum (summer climatic model)—there is a slight drop in the Montane curve. At the same time a brief optimum occurs for the Sahelian element¹. At 7000–6600 BP—temperature is falling (autumn climatic model)—there is a strong positive trend in the Montane element curve.

Possibly this type of correlation existed before 8000 BP, but

the data available are not sufficient to allow us to demonstrate it. A consequence of this climatic pattern is that the climatic optima in the Tibesti lasted longer than those in the Sahelian zone, as is clearly evident between 8000 and 7000 BP (Fig. 3c,d). For the optima of the Tibesti, the climate consisted probably of rains fairly well distributed throughout the year, with two principal rainy seasons, one in spring, the other in autumn and with a strong reduction of the evaporation (see the lacustrine deposits). It should also be noted that the disappearance of the 'cold' diatom *Cymatopleura elliptica* in the Palaeochad (2, 14), dated after about 6600 BP at Tjéri (fig. 2) occurs just before the appearance of a warmer climate of Sahelian type (see below). Moreover during some years or periods with large seasonal contrasts (very cold winters and very hot summers), there could have been a combination of rains from tropical depressions and from the monsoon, as can sometimes be observed in present years^{37,42}. This could account for the alternation of finer and coarser layers in phase C of the MT. Finally for the climate of the Tibesti during the arid phases, of which phase D is a good example, we could perhaps imagine tropical depressions in winter and, possibly, with snow³⁵, the rest of the year being almost without rain, which would cause intense evaporation favouring the formation of calcareous crusts. Cyclonic rains from the Polar front in surface are also possible in winter without the intervention of equatorial air. But since the cold air carries little humidity, these rains would in general be relatively light^{34,50}.

Interpretation of the upper part of the MT

From around 6500 to 5500 BP, the relationship with temperature seems to be different (phase F). The trends of the curves for the Montane and Sahelian elements become similar. One may thus suppose that during this time the Tibesti underwent the same regime as the Sahelian zone, which means that almost all rains would be provided by the summer monsoon.

First, the sudden massive appearance of the pebbles and cobbles near the top of the MT is subsequent to 6600 BP, as the palaeosol radiocarbon dated about 6600 BP is itself covered by 40 cm of coarse alluvium²⁸. On the other hand, the spreading of the pebbles requires a very heavy fluvial regime for which rains of Sahelian type can easily be accountable. However one must note that this spreading occurred over the MT without apparent erosion. The erosion period of the MT is not yet well placed in time: perhaps after 5500 BP and before 4000 BP (Fig. 3). In Central Air, the presence of vertisols with calcareous nodules of dates 5680 ± 110 and 5010 ± 110 BP (ref. 3) indicate a wetter climate of Sahelian type. In Adrar Bous a vertisol has been situated between 7300 and 4500 BP (ref. 30). In Egyptian Nubia Member I of the Shaturma Formation²⁴ has a chronology and sedimentology corresponding to the phase F of the Tibesti. The alluviation of Member I ceased before the initiation of a renewed period of Nile dissection which occurred after 5000 BP (ref. 24). The end of Member I and the beginning of the erosion can thus be dated between around 5500 and 5000 BP. Butzer and Hansen²⁴ conclude that these deposits "imply rather effective sheet-flooding with 50 to 100 mm of rainfall at most".

A climate of Sahelian type over the Tibesti and probably also over the Central Sahara is all the more probable as other pollen data at Tjéri indicate a northwards movement of the Sahel zone at that time. The removal of the Sahelian vegetation northwards would explain the flat of the curve for the Sahelian element between around 5500 and 4400 BP (Fig. 2c). This interpretation is corroborated by the curve for the Sudanian element which reached its maximum between around 5900 and 4400 BP (ref. 1), corresponding no doubt also to a slight northwards movement of the Sudanian zone.

Conclusion

First, it seems that for the Central Sahara there are two kinds of wetter periods (optima)—one, more frequent in the Quaternary period, with tropical depressions and temperature relatively

low, and the other with direct monsoon rains and higher temperatures. Second, the annual climatic mechanisms of the present time enable the palaeoclimates of the early Holocene to be coherently explained. The opposition, both seasonal in the present time, and for longer periods during the early Holocene, between the tropical depressions and the monsoon rains can be interpreted by variations in trends of temperature over the Northern Hemisphere and also by variations in general atmospheric circulation. Indeed, the penetrations of the polar troughs aloft, necessary for the formation of tropical depressions, are related to the occurrence of large amplitude waves in the upper westerlies. On the other hand, during hotter years or periods, the polar troughs towards lower latitudes are much less frequent and the circulation of the upper westerlies becomes more zonal. This situation leads to a diminution of tropical depressions, favouring, in contrast, the extension of monsoon rains over the Sahara^{51,52}. But the opposition between these two kinds of rain remains only partial since both are also linked to the activity of the ITCZ. Better knowledge of present events as well as the study of other old periods should allow greater precision in this field.

I thank M. Van Campo, H. H. Lamb, J. Dubief, K. W. Butzer, B. Messerli and P. Guinet for comments, F. Wendorf who provided unpublished data and M. Servant the Tjéri samples, G. Delibrias (Gif) for radiocarbon dating, G. Vignard, M. Skeffington for technical assistance. This work was completed at the Palynological Laboratory of CNRS(ER.25) in Montpellier. Financial and technical support was provided by the Office de la Recherche Scientifique et Technique Outre-Mer (ORSTOM), Paris.

Received 10 January; accepted 29 June 1977.

- ¹ Maley, J. *Suppl. Bull. Ass. fr. Et. Quat.*, Paris 50, 1, 187–197 (edit. by French Com. for 10th INQUA Cong., Birmingham, August 1977).
- ² Servant, M. & Servant, S. *Rev. Géogr. Phys. Géol. Dyn.*, Paris 12, 63–76 (1970).
- ³ Servant, M. thesis, Univ. Paris (1973).
- ⁴ Williams, M. A. J., Clark, J. D., Adamson, D. A. & Gillespie, R. *Bull. Ass. Sénégal. et. Quat. Afr.*, Dakar 46, 75–86 (1975).
- ⁵ Maley, J. *Pollen Spores* 14, 263–307 (1972); *Palaeogeogr.*, *Palaeoclimatol.*, *Palaeoecol.* 14, 193–227 (1973).
- ⁶ Dansgaard, W., Johnsen, S. J., Møller, J. & Langway, C. C., *Science* 166, 377–381 (1969).
- ⁷ Johnsen, S. J., Dansgaard, W., Clausen, H. B. & Langway, C. C. *Nature* 235, 429–434 (1972).
- ⁸ Fairbridge, R. *Quaternaria*, Rome 6, 111–134 (1962).
- ⁹ Möner, N. *Geol. en Mjøn*, 48, 389–399 (1969).
- ¹⁰ Ters, M. *Suppl. Bull. Ass. fr. Et. Quat.*, Paris 36, 114–135 (1973).
- ¹¹ Suess, H. E. *Palaeogeogr. Palaeoclimatol.*, *Palaeoecol.* 10, 199–202 (1971).
- ¹² Michael, H. N. & Ralph, E. K. *Radiocarbon* 16, 198–218 (1974).
- ¹³ Patzelt, G. in *Les Méthodes Quantitatives d'étude des Variations du Climat au Cours du Pleistocène*, 51–59 (CNRS, Paris, 1974).
- ¹⁴ Servant, S. thesis, Univ. Paris (1977).
- ¹⁵ Cour, P. & Duzer, D. *Rev. Géogr. Phys. Géol. Dyn.*, Paris 18, 175–198 (1976).
- ¹⁶ Quézel, P. *La Végétation du Sahara, du Tchad à la Mauritanie*. (Fischer, Stuttgart, 1965).
- ¹⁷ Van Campo, M. in *Problems in Prehistory: North Africa and the Levant* (eds Wendorf, F. & Marks, A. E.) 45–64 (Southern Methodist University Press, Dallas, 1975).
- ¹⁸ Maley, J., Cohen, J., Faure, H., Rognon, P. & Vincent, P. M. *Cahiers Off. Rech. Sci. Techn. O-mér. sér. Géol.*, Paris 2, 127–152 (1970).
- ¹⁹ Hagedorn, H. & Jäkel, D. *Bull. Ass. Sénégal. Et. Quat. Afr.* 23, 25–42 (1969).
- ²⁰ Molle, H. G. *Berl. Geogr. Abh.* 13 (1971).
- ²¹ Jäkel, D. & Schulz, E. Z. *Geomorph.*, *Suppl.* 15, 129–143 (1972).
- ²² Geyh, M. A. & Jäkel, D. Z. *Geomorph.* 18, 82–98 (1974).
- ²³ Grünert, J. *Berl. Geogr. Abh.* 22 (1975).
- ²⁴ Butzer, K. W. & Hansen, C. L. *Desert and River in Nubia* (University of Wisconsin Press, Madison, 1968).
- ²⁵ Maley, J. C. r. *Acad. Sci.*, Paris D 283, 337–340 (1976).
- ²⁶ Messerli, B. *Hochgebirgsforsch.*, Innsbruck 2, 23–86 (1972).
- ²⁷ Rognon, P. *Le Massif de l'Atakor et ses Boredures (Sahara Central)*. (CNRS, Paris) sér. Géol. 9 (1967).
- ²⁸ Wendorf, F., Schild, R., Said, R., Haynes, C. V., Gautier, A. & Kobusiewicz, M. *Science* 193, 103–114 (1976).
- ²⁹ Faure, H. *Quaternaria* 8, 167–175 (1966).
- ³⁰ Clark, J. D., Williams, M. A. J. & Smith, A. B. *Quaternaria* 17, 245–297 (1973).
- ³¹ Pachur, H. J. *Berl. Geogr. Abh.* 17 (1974).
- ³² Bryson, R. A., Lamb, H. H. & Donley, D. L. *Antiquity*, Lond. 48, 46–50 (1974).
- ³³ Kukla, G. J. *Nature* 253, 600–603 (1975).
- ³⁴ Dubief, J. *Essai sur l'hydrologie Superficielle au Sahara* (Gouv. Gén. Alger, 1953).
- ³⁵ Dubief, J. *Mém. Inst. Rech. Sah. Univ. Alger*, 2 (1963).
- ³⁶ Dorize, L. *Rev. Géogr. Phys. Géol. Dyn.*, Paris 16, 393–420 (1974).
- ³⁷ Dubief, J. *Trav. Inst. Rech. Sah. Univ. Alger* 4, 7–23 (1947).
- ³⁸ Mayençon, R. *La Météo.*, Paris 62, 171–180 (1961).
- ³⁹ Winiger, M. *Geographica Bernensia*, Berne 6, 1 (1975).
- ⁴⁰ Dubief, J. & Quézel, P. *La Météo.*, Paris 119, 80–91 (1935).
- ⁴¹ Jalu, R. *La Météo.*, 6(78), 113–127 (1965).
- ⁴² Yacono, D. *Trav. Inst. Rech. Sah. Univ. Alger* 27 (1968).
- ⁴³ Flohn, H. *Bonner Meteor. Abhand.*, 15 (1971).
- ⁴⁴ Elfandy, M. G. Q. J. R. *Meteor. Soc.* 40, 323–335 (1940).
- ⁴⁵ Pedgley, D. E. *Meteor. Mag.*, Lond. 101, 228–244 (1972).
- ⁴⁶ Jalu, R., Bocquillon, M. & Bonnefous, M. *La Météo.* 6(78), 105–112 (1965).
- ⁴⁷ Nicholson, S. E. thesis, Univ. Wisconsin (1976).
- ⁴⁸ Butzer, K. W. *Meteor. Rdsch.* 13, 97–105 (1960).
- ⁴⁹ Bourrelil, P., Gillet, H. & Quézel, P. *Boissiera*, Genève 24, 173–196 (1975).
- ⁵⁰ Jalu, R. *Ann. Géogr.*, Paris 373, 288–296 (1960).
- ⁵¹ Lamb, H. H. *Geogr. J.* 132, 183–212 (1966).
- ⁵² Winstanley, D. *Nature* 245, 190–194 (1973).
- ⁵³ Camps, G., Delibrias, G. & Thommeret, J. *Libya*, Alger 21, 65–89 (1973).

Dispersal in stable habitats

W. D. Hamilton

Imperial College Field Station, Silwood Park, Ascot, Berkshire, UK

Robert M. May

Department of Biology, Princeton University, Princeton, New Jersey 08540

Simple mathematical models show that adaptations for achieving dispersal retain great importance even in uniform and predictable environments. A parent organism is expected to try to enter a high fraction of its propagules into competition for sites away from its own immediate locality even when mortality to such dispersing propagules is extremely high. The models incidentally provide a case where the evolutionarily stable dispersal strategy for individuals is suboptimal for the population as a whole.

In nature, adaptations for dispersal are ubiquitous and are often applied in almost suicidal ventures or in ventures which seem too feeble to be worthwhile (such as dehiscent seeds that fall only a few feet from the parent plant). This behaviour is clearly advantageous if the habitat is unstable or offers many empty, if transient, patches. We discuss here some simple models that help to explain why such dispersal is also advantageous even in stable and saturated habitats.

To begin, we consider a wholly parthenogenetic species in an environment that provides a fixed number of sites, at each of which just one adult can live. In a fixed season at the end of its life each adult produces a certain constant number of offspring, m . A fraction ν of these are programmed to be migrants (for example, insects provided with wings and appropriate instincts, seeds with a pappus for wind dispersal). The remaining fraction $(1-\nu)$ are destined to be sedentary competitors for the home site. The mother's genotype, by means of some maternal influence on each ovum (or testa or fruit), determines the fraction ν . When ready, at about the time of the mother's death, the migrant offspring take off and after mixing with all other migrants, and suffering a mortality such that only a fraction p survive, they are distributed equally to all the sites. There they compete on equal terms with the resident young which, it is supposed, up to this stage suffer no mortality (alternatively, if they do, p is the relative survival of the migrants). In effect, one offspring is chosen at random from among the young present on a site to become the adult at that site in the new generation.

We acknowledge that this simple model probably has few close parallels in the real world. Nevertheless it may usefully force a re-examination of some widely held ideas about migration.

For example, it has been claimed as "intuitively obvious" that in a saturated and time-invariant environment "organisms can never gain any advantage by changing their locations"¹. The schematic illustration presents a counter-example based on the above model

			X		X		
Offspring	o	o	o	o	o	o	o
(after dispersion)	o	o	o	o	o	o	o
	o	o	o	o	o	o	o
	o	o	o	x	o	o	o
Adults	O	O	O	X	O	O	O
Site labels	a	b	c	d	e	f	g

The environment (represented by the eight sites labelled a to h) here is saturated and time-invariant. In the absence of any migrating mutant like X , and with full survival of stay-at-home propagules, the majority genotype O which keeps all propagules at its own site can perfectly maintain the population. But the 'O' strategy is not evolutionarily stable. The genotype O will be replaced by the mutant X , which keeps only one propagule at home, even though it loses two of its remaining four propagules due to mortality in migration (assumed to be 50%; $p = 0.5$): obviously X has a chance of 1/6 of winning each of sites c and f ; meanwhile, at least against so ill-advised a genotype as O , it certainly retains its base at d . Hence X is certain to become the established type. Of course the particular migration probability of X ($\nu = 4/5$) illustrated here will not itself prove to be the evolutionary stable strategy, or "ess"², except for some special value of the survival factor p . Normally other mutations would supervene, after the spread of X , until finally a migration probability that was evolutionarily stable was approximated.

In general, the one or more ess migration probabilities which the model might have can be determined as follows. The population is imagined to contain two types, using migration fractions ν and ν' . An expression is written for the fitness, w' , of one adult of type ν' . This fitness, or expected number of sites to be gained by offspring, will consist of the chance of retaining the home site plus the expectation of sites to be gained elsewhere by migrant offspring. From this we find the value of ν which has the property that $w' \geq 1$ for all $\nu' \neq \nu$ (mean fitness in the model is unity, so that $w = 1$). The value of ν so found, symbolised ν^* , is unbeatable³ in the sense that any genotype with strategy $\nu' \neq \nu$ will have a diminishing frequency in any mixture.

For the simple limiting case where the number of sites and the number of propagules per parent are large, we find by this method that the ess or unbeatable migration probability is

$$\nu^* = 1/(2-p) \quad (1)$$

Because this formula shows no dependence on the composition of the mixture, stable mixtures are not possible, and the ess can be considered safe against both rare mutations and any massive invasion by a different genotype.

A striking conclusion to be drawn from equation (1) is that even when migrant mortality is extremely high (small p), and the environment offers no vacant sites for colonisation, it is still advantageous to commit slightly more than half of the offspring to migration.

Pre-eminent among the artificialities of this model are: (1) insistence on death and replacement of every parent in each generation; (2) absence of vacant sites (stemming from the deterministic description of the propagation processes); (3) pure parthenogenesis. We now indicate how the model may be extended to encompass such effects, paying particular attention to (2).

Death and replacement of parents

There is one simple and often realistic assumption that allows for perennation of the parents, while preserving the result (1). The assumption is that all parents, irrespective of age, have

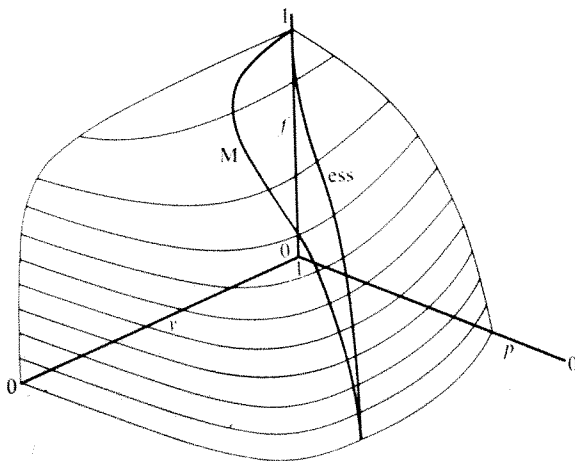


Fig. 1 The surface depicted here gives f (the equilibrium fraction of sites occupied) as a function of v (the fraction of offspring that migrate) and p (the relative survival probability for migrants), under the assumptions delineated in the text, for a mean 'brood size' $m = 3$. The contour lines are for constant values of f , spaced at intervals 0.1 apart (that is $\Delta f = 0.1$). On this surface, the trajectory labelled *ess* shows the dispersal strategy that is evolutionarily stable, $v^*(p)$, as a function of p . The *ess* trajectory is to be contrasted with that labelled *M*, which shows the strategy which maximises site occupancy for given p , $\hat{v}(p)$.

some constant probability q to survive and retain their sites into the next season. This corresponds to the so-called 'Type II survivorship curve', which is known to be not far from true for many organisms. For such organisms, both stay-at-home and migrant offspring have their expectations of inheriting a vacant site devalued by exactly the same factor, namely $1 - q$, which consequently cancels itself out of the analysis. (On the other hand, for organisms whose chance of surviving from one year to the next diminishes with age, the *ess* will be age-dependent, if this is biologically feasible. A likely outcome is a 'bang-bang' strategy⁴, with all propagules dispersed until their parent reaches a certain age, whereupon all propagules stay at home.)

Vacant sites

In our simple model, the evolutionary pressure that commits at least half the offspring to migration, no matter how risky migration is, results from an advantage in arranging competitive interactions as far as possible with unlike genotypes. It can be imagined that in nature such a pressure to take risks could cause a wastage that was damaging to the population's chance of survival. This cannot happen in the simple model because its deterministic assumptions keep all sites occupied; to investigate wastage we need some version that relaxes this assumption (2). Such a version may have some bearing on our confidence in models of theoretical ecology in general, because these often take it for granted that the species discussed already have, or will evolve towards, maximal efficiency of resource utilisation.

To this end, we stochasticise the earlier model by assuming the number of propagules produced at any given site is given by a Poisson distribution, with mean equal to the previously constant value m . It follows that the numbers for emigrant and for stay-at-home offspring, and for immigrants, are all generated by Poisson distributions for which the same symbols and expressions as occurred in the simple model are now to be taken as the means. Note that m is the mean brood size and also the variance in brood size, so that the relative magnitude of statistical fluctuations goes as $m^{-1/2}$. The parameter m thus comes to have an important influence, especially when relatively small; when m is large, the present stochastic model tends to revert to the earlier deterministic one.

Now sites can become vacant. Vacancies begin or continue

whenever a site happens to have no surviving stay-at-home offspring and receives no immigrants. Obviously, some migration is essential if extinction is to be avoided, and from the population point of view there will be some level of migration that keeps extinction at furthest reach⁵.

For any specified combination of m , v and p , the system settles to some stable level of site occupancy f (that is f = fraction of sites occupied), with a zero level signifying that extinction is inevitable. This stable level f is given (for $f > 0$) by the implicit relation

$$f/(1-f) = e^{m(1-v)} [e^{mvpf} - 1] \quad (2)$$

For given m , the site occupancy function f defined by equation (2) may be mapped over the unit square of admissible values of v and p : $f(v, p)$ is found to have the form of a smooth promontory (Fig. 1 for $m = 3$), or, for larger m , a squarish headland (Fig. 2 for $m = 10$). In any such figure, for fixed v the occupancy $f(p)$ falls convexly with decreasing p , and clearly must always hit extinction somewhere short of $p = 0$. The smallest p that still allows the population to exist may be shown to occur when $v = 1/m$, that is when just one migrant is dispatched from each site. The concomitant least value of p is given by $p_{\min} = \exp(1-m)$, which is very small for moderate values of m ; slightly larger values of p carry f up to a 'plateau' at a level of nearly complete occupancy. Conversely, for fixed p , the occupancy f as a function of v at first rises (albeit very slightly for large m) to a maximum as v decreases; then, as v decreases further, this is followed by a steepening fall in f , which goes to zero short of $v = 0$.

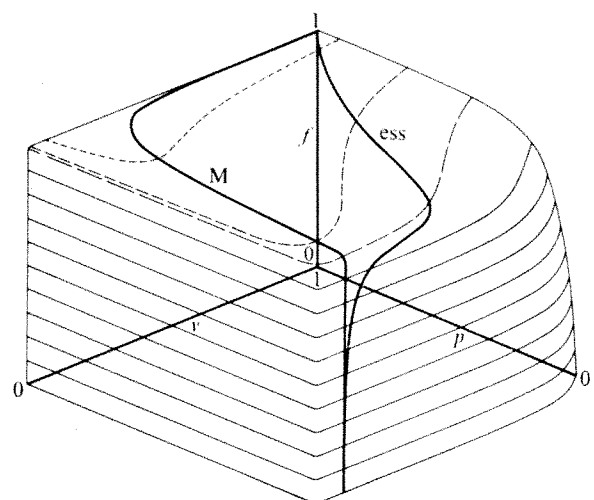
Thus we may trace out the locus of the strategy, \hat{v} , that maximises the site occupancy f for given p ; this locus moves up a 'cliff', and across a 'plateau' to $p = v = 1$ (Figs 1 and 2). This strategy \hat{v} is that which is 'best for the population'.

This locus of the strategy for maximal occupancy, \hat{v} , is to be contrasted with the locus of the *ess* v^* , which is determined by the method described above. The *ess* migration probability, $v = v^*$, is given by

$$\exp[m(u + vpf)] = \frac{v^2p - vpf(u + vpf)}{v^2p + u(u + vpf)(1 - mv)} \quad (3)$$

Here $u = 1 - v$, and f has the value implicitly fixed by equation (2). The derivation of the results (2) and (3) will be set out

Fig. 2 As for Fig. 1, except that now the mean brood size is $m = 10$. Again the solid contour lines depict constant values of f , from $f = 0$ to 0.9 at intervals of 0.1; the increasingly broken contour lines on the 'plateau' are for $f = 0.99$, 0.999 and 0.9999, respectively. For a more full discussion of Figs 1 and 2, see text.



elsewhere (our work with H. N. Comins); unlike the earlier simple result (1), these equations are made more complicated if age-independent or 'Type II' perennation of the parents is introduced.

The locus of the ess migration fraction v^* , so determined, is shown in Figs 1 and 2. We note that ess migration is always higher than maximum-occupancy migration, although they converge at the two extremes (at perfect and at least-possible survival, $p = 1$ and $p = p_{\min}$). This is true for all brood sizes m . As m is increased towards more usual biological values (from $m = 3$ in Fig. 1 to $m = 10$ in Fig. 2), the separation between the ess and maximum-occupancy loci with respect to v also increases strikingly. This separation results mainly from the way the changing shape of the site-occupancy surface $f(v, p)$ controls the course of \hat{v} ; that is $\hat{v}(p)$ is much more affected by changing m than is $v^*(p)$. The separation between the two loci with respect to f , however, remains very small, even for populations that are on the 'cliff face' of the f surface and hence in some danger of extinction.

In other words, the ess v^* can demand far more migration than is 'best for the population' (\hat{v}), but such excess does not, in this model at least, substantially affect population safety nor create many extra vacant sites that would give other species opportunity to evolve as competitors for the niche. Notwithstanding the small difference in occupancy levels, there is usually strong selection to establish v^* rather than \hat{v} . One incidental consequence of the relatively abundant supply of migrants is that some must overflow from the system and occasionally have the luck to found new populations elsewhere. It therefore seems that our models show, after all, a case where a trait is positively adaptive at more than one level: the ess is best for the genotype, suboptimal at the level of an isolated population, and advantageous in carrying the population to new areas.

Sexual organisms

If the organisms are taken to be sexual rather than purely parthenogenetic, with migration probability depending on the genotype of the offspring, lower values of v^* are definitely expected.

This follows from the observation that our models directly treat the genetic success of a mother. A parthenogenetic offspring has an identical genotype, so that whatever maximises inclusive fitness⁶ for the mother maximises it for the offspring. In contrast, the inclusive fitness of a sexual offspring normally differs from that of its mother, and, in some direct relation to the hazards of migration, will find its optimum at a lower migration rate. Hence there is a conflict of interest between parent and offspring^{7,8}. Its outcome will depend on the biological circumstances: as regards applying a pappus to a seed to make it blow away it is clear that the parent has a strong position; as regards making wing buds become wings in an insect it is much more likely that the offspring can have its preference.

In nature, less migration generally means more inbreeding; such inbreeding reduces the genetic contrast between parent and offspring, and thus tends to diminish the difference between parthenogenetic models and sexual models with offspring-determined migration. Various situations can arise. If males mate before migration in a one-per-site situation, inbreeding is total and the model is again effectively as under parthenogenesis. Unfortunately, the small arthropods which almost meet this extreme of inbreeding and which have a dispersal polymorphism (for example, some mites of the genus *Pygmephorus* (ref. 9 and unpublished results of W.D.H.)) are far from meeting the assumption of fixed unitary sites, being rather exploiters of patchy, ephemeral resources. If inbreeding occurs because males sometimes survive along with a sister, then our one-per-site assumption does not hold.

But if male offspring are assumed always to migrate, then outbred sex can be brought in and the one-per-site feature

retained; moreover, this version may approach realism for some of the many insect species that have free-flying males while females are either wholly flightless or polymorphic for flight. For the simple, non-stochastic version of this model with allele semi-dominance, the ess migration probability for the female offspring is indeed lower than for the parthenogenetic model: in place of equation (1), we now have

$$\begin{aligned} v^* &= 0 & \text{for } \frac{1}{2} > p > 0 \\ v^* &= (2p-1)/(4p-1-2p^2) & \text{for } 1 > p > \frac{1}{2} \end{aligned} \quad (4)$$

The fact that $v^* = 0$ for $p < \frac{1}{2}$ suggests that when this model is made stochastic (along the lines followed previously) the ess may imply situations where the population and the species is dangerously liable to extinction. We are pursuing this open question.

(Roff¹⁰ has used computer simulations to study migration in models which not only have sexual reproduction and multiple adults per site with a density-dependent migration structure, but also have temporal variability in site suitability. It is obvious from the facts of nature¹¹, and is confirmed in both Roff's model and several others^{1,12-14}, that erratic habitat suitability (including local extinction) is an extremely important factor in favouring adaptations for dispersal and migration. More relevant to the present discussion, however, is Roff's demonstration that his ess migration probabilities are considerably lower than those which would keep populations highest and most secure from extinction. Although partly attributable to the workings of sexuality and Mendelian inheritance in Roff's models, this effect arises primarily because there are many adults per site, which changes the calculation of inclusive fitness in such a way as to favour the short term, selfish option of not dispersing.)

It would be satisfying to conclude by listing a few biological illustrations of principles suggested by the models. Unfortunately most of the examples which have come to mind are more in the region of jokes than of reality: they range from *Caulobacter crescentus*¹⁵, whose asexual fission always produces one stalked sessile cell and one motile one that swims away, through the inevitable lemmings and on to the non-first sons in Victorian families who joined the army or went to Australia. Both our simplest model, and the refined versions (1) and (2), support what these examples might illustrate, namely a minimum rule that 'at least one must migrate whatever the odds'. All three examples, however, fail to have fixed sites and, except for *Caulobacter*, fail also in regard to asexuality. There are flight dimorphisms in non-sexual insects (for example, aphids in summer: the tiny sub-cortical beetle *Ptinella errabunda*¹⁶) and probably parallels in some weeds (for example, Compositae)^{17,18}, but in being colonist species these fit badly with the assumptions of fixed sites and of no extinction or recreation of habitats on a large scale. But the fact that colonist plants in arid environments, where perennation of the whole plant (as opposed to survival of its seed) may be hardest to achieve, seem especially inclined to produce actually dimorphic or differently shed propagules with one class buried or otherwise retained beside or under the parent¹⁹ (*Sieglingia decumbens* is the best example in Britain) does at least suggest that a plant's perennating body (or its tubers, corms or the like) should be considered its bid to retain the home site. We expect the investment in such aids to perennation, as opposed to investment in flowers and dispersing seed, to show positive correlation with the chance that seed if produced would end up destroyed or in sites that never could support an adult. For example, the more successful a specialised epiphyte or parasite is at getting its propagules to suitable sites, even when these are objects of intense competition, the more it should produce them. A mistletoe should flower more than an epiphytic orchid; and we should not be surprised to find that weedy perennial species put more net assimilation into seed production than do climax perennial species²⁰. This may be so but is not particularly exciting; other explanations are available. It is not easy to test the quantitative conclusion that,

because $v^* > \frac{1}{2}$ for most values of p , we expect to find at least one-half of net assimilation going into some form of growth or propagule production that extends competition beyond the space that the organism already occupies.

The value of our models lies primarily in their approach and method, in their well-determined conclusions, and in the novelty of having made plain a new reason for the ubiquity of dispersal. We have shown that the habitat does not have to be patchy and of erratic suitability; substantial dispersal is to be expected even when the habitat is uniform, constant, and occupied completely.

We thank H. N. Comins, H. S. Horn, T. R. E. Southwood and D. M. Waller for helpful comments. This work was supported in part by the NSF.

Received 9 June; accepted 5 July 1977.

- 1 Cohen, D. *Am. Nat.* **101**, 5 (1967).
- 2 Maynard Smith, J. & Price, G. R. *Nature* **246**, 15 (1973).
- 3 Hamilton, W. D. *Science* **156**, 477 (1967).
- 4 Macevitz, S. & Oster, G. F. *Behav. Ecol. Sociobiol.* **1**, 265 (1976).
- 5 May, R. M. *Stability and Complexity in Model Ecosystems*, Second ed. (Princeton University, Princeton, 1974).
- 6 Hamilton, W. D. *J. theor. Biol.* **7**, 1 (1964).
- 7 Trivers, R. L. *Am. Zool.* **14**, 249 (1974).
- 8 Hamilton, W. D. *J. theor. Biol.* **7**, 17 (1964).
- 9 Moser, J. C. & Cross, E. A. *Ann. ent. Soc. Am.* **68**, 820 (1975).
- 10 Roff, D. A. *Oecologia, Berl.* **19**, 217 (1975).
- 11 Southwood, T. R. E. *Biol. Rev.* **37**, 171 (1962).
- 12 Gadgil, M. *Ecology* **52**, 253 (1971).
- 13 Van Valen, L. *Evolution* **25**, 5 (1971).
- 14 Levins, R. in *Some Mathematical Questions in Biology 2* (American Mathematical Society, Providence, Rhode Island, 1970).
- 15 Kurn, N. & Shapiro, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3303 (1976).
- 16 Taylor, V. A. thesis, Univ. London, (1975).
- 17 Harper, J. L., Lovell, P. H. & Moore, K. G. *A. Rev. Ecol. Syst.* **1**, 327 (1970).
- 18 Stebbins, G. L. *A. Rev. Ecol. Syst.* **2**, 237 (1971).
- 19 Zohary, M. *Plant Life of Palestine, Israel and Jordan* (Ronald, New York, 1962).
- 20 Harper, J. L. & White, J. A. *Rev. Ecol. Syst.* **5**, 419 (1974).

letters to nature

Radio emission from a normal HD26676 star

IN the field of an observation of the cluster Abell 478 made with the Westerbork Synthesis Radio Telescope¹ at 21 cm, we have discovered and report here a weak radio source coincident with the 6.2-mag B8Vn star² HD26676. The source positions derived from both the 21-cm observation, and measurements made later at 6 cm, agree within the errors with the AGK3 position of the star (Table 1). The 21-cm data were obtained during a single 8-h observation, while at 6 cm three measurements, each lasting for 10 h, were made over a period of 6 weeks. There is no significant indication of variability from the three flux densities derived at 6 cm (Table 2). The global 6-cm flux density, as well as the position in Table 1, were obtained by combining the three observations in a single map.

Polarisation (linear and circular) is less than 10% of the total intensity at 21 cm and less than 20% of that at 6 cm. The radio source is unresolved at both wavelengths, which sets an upper limit (in right ascension) of about 4 arcs to the angular size. No radio emission coincides with the reflection nebula associated with the star³ at a level of about 2 mJy per beam (area about 1 arc min²) at 21 cm and about 1 mJy per beam (area about 0.1 arc min²) at 6 cm.

Although the optical and radio positions agree, within the errors, we must consider what the probability of a chance coincidence is. The area enclosed by the 6-cm position errors is about 2×10^{-6} deg². The density of stars brighter than 8 mag (ref. 4) (we assume that anyone finding a radio source close to such a star would be prompted to investigate the possibility of association) is about 1 deg⁻², suggesting an *a priori* probability of 2 in 10^6 that the coincidence occurred by chance. While this is reasonably small, thorough identification work has been

done on nearly 1,000 radio sources observed with Westerbok at 21 cm (see ref. 5 and refs therein), and one other radio position agrees with that of a 13-mag star. Nevertheless, we conclude that the star and radio source are probably related and now consider the consequences of this fact.

Assuming the source did not vary during the 10 months separating our two sets of observations, the flux densities in Table 2 give a spectral index between 6 cm and 21 cm of $\alpha = -0.8 \pm 0.2$ (where flux density is proportional to frequency α). This qualifies HD26676 as a radio star according to the definition of Hjellming *et al.*⁶: if the source has not varied, its radio spectrum clearly indicates that the radiation is not the result of free-free emission from a surrounding nebula. Like several of the other radio stars (such as β Per and β Lyr), HD26676 is of an early spectral type. At a distance of 126 pc (ref. 3) its 21-cm luminosity is 2×10^{10} W Hz⁻¹. The radio power between 1 GHz and 10 GHz (assuming a constant spectral index) is 8×10^{26} erg s⁻¹, a value typical of the 'quiet' state of radio stars.

Table 2 Measured radio flux densities

Wavelength (cm)	Mean date	Flux density (mJy)
6	6.5 February 1977	5.8 ± 1.2
6	21.9 January 1977	3.3 ± 1.1
6	27.9 December 1976	4.3 ± 1.1
6	(combined)	4.1 ± 0.6
21	12.8 February 1976	11 ± 2

In several respects HD26676 differs from other radio stars. Its spectral index is negative, while that of radio stars in their quiet state is usually positive. But there is evidence that the radio flares must be non-thermal⁸ and in several cases, notably Sco X-1 (ref. 9), the spectral index is generally negative. Of course, Sco X-1 is predominant as an X-ray star, and the SAS-3 observation of Abell 478 (ref. 1) enables one to put a limit on any X-ray emission from HD26676. H. W. Schnopper (personal communication) has estimated that the count rate cannot exceed one-third of that from Abell 478. This means that the 2-11-keV luminosity of HD26676 must be less than a few times 10^{31} erg s⁻¹ (epoch 1976.9). This is considerably less than the luminosity of typical X-ray stars such as Sco X-1, Cyg X-1 and Cyg X-3, all of which are also radio sources.

Table 1 Optical and radio positions

Observation	Right ascension (1950.0) (h min s : s)		Declination (1950.0) (° ' " ± ")	
Optical*	04 10	50.11 ± 0.01	10 05	11.54 ± 0.2
6.0 cm	04 10	50.19 ± 0.11	10 05	04 ± 9
21.2 cm	04 10	50.14 ± 0.15	10 05	07 ± 16

*The position given is corrected for proper motion to 1977.0

The most important difference between HD26676 and all other radio stars is the fact that it is not known to be either a binary system or a variable star. Most models of the radio emission make use of mass exchange between stars in the system⁷. For this reason HD26676 could be a critical test case for emission mechanisms. If further observations confirm the radio-optical association and its non-binary character, it would provide an important constraint for future models.

We thank Dr C. Jaschek of the Centre de Données Stellaires, Observatoire de Strasbourg, for references for HD26676. The Westerbork Synthesis Radio Telescope is operated by the Netherlands Foundation for Radio Astronomy with financial support from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

R. G. STROM
D. E. HARRIS

Netherlands Foundation for Radio Astronomy,
Dwingeloo,
The Netherlands

Received 26 July; accepted 15 August 1977.

1. Schnopper, H. W. *et al. Astrophys. J. Lett.* (in the press).
2. Cowley, A. *Astr. J.* 77, 750 (1972).
3. Racine, R. *Astr. J.* 73, 233 (1968).
4. Allen, C. W. *Astrophysical Quantities* (Athlone, London, 1973).
5. De Ruiter, H. R., Willis, A. G. & Arp, H. C. *Astr. Astrophys. Suppl.* 28, 211 (1977).
6. Hjellming, R. M., Blankenship, L. C. & Balick, B. *Nature phys. Sci.* 242, 84 (1973).
7. Jones, T. W. & Woolf, N. J. *Astrophys. J.* 179, 869 (1973).
8. Gibson, D. M. & Hjellming, R. M. *Pub. astr. Soc. Pacific* 86, 652 (1974).
9. Hjellming, R. M. & Wade, C. M. *Astrophys. J. Lett.* 164, L1 (1971).

Enhanced metal depletions and interstellar H₂ abundances

THE OAO 3 Copernicus ultraviolet satellite has provided important new data on the abundances of elements in the interstellar gas in the direction of many O and B-stars along lines of sight of low optical depth¹⁻⁴. This information may make it possible to choose between the following theories of molecular hydrogen formation in interstellar clouds: (1) physical adsorption of H-atoms on to cold dielectric grains and their subsequent recombination and desorption⁵; (2) H₂ recombination on graphite grains⁶; and (3) hydrogen recombination by non-activated chemisorption on transition metal grains⁷. Indirect methods of testing the above theories are essential because there is a glaring lack of experimental data, especially for processes (1) and (2). Also, it does not seem possible that in the near future radio astronomers will be able to measure the abundances of the various hydrogen ions (such as H₂⁺ and H₃⁺) that the above theories predict⁸.

In view of the fact that the catalytic production of H₂ can take place in the laboratory⁹, it probably occurs in the interstellar medium because the temperatures of metal grains are about an order of magnitude hotter than dielectric ones¹⁰. An examination of the depletion of interstellar elements from the gas phase may ultimately be able to discredit or lend credence to this theory. Consider Table 1, which lists the interstellar depletions with respect to solar system abundances ($\delta = \log(N/N_H) - \log(N/N_H)_\odot$) along lines of sight to ζ Oph (ref. 2), o Per (ref. 4), and λ Sco (ref. 3) for certain key elements—carbon, oxygen, silicon, and iron. Towards ζ Oph and o Per, where the

visual extinction $A_v \approx 1.0$ mag, the fraction f of hydrogen that is in molecular form is between 0.5 and 0.7, while towards unreddened λ Sco, $f \sim 10^{-7}$. There seems to be a weak correlation between iron and silicon depletions and H₂ abundances. In contrast there seems to be no noticeable relationship between the amounts of carbon and oxygen depleted from the gas phase and the amount of gaseous H₂ detected. Since it is commonly assumed that process (1)—the physical adsorption of hydrogen—is the primary mechanism for H₂ production, one might have expected to find enhanced carbon and oxygen depletions with the higher abundances of H₂. The reason for this preconceived notion is the assumption that the cold dielectric grains where this process is thought to occur are mainly silicate core particles with heavy mantles of C-N-O 'ices'⁵.

These conclusions are partially reinforced by an examination of depletions in high-velocity clouds. Barlow and Silk¹¹ have shown that there is a systematic depletion of iron and silicon with gas velocities towards unreddened stars: the higher the velocity, the lower the amount of depletion. They make a strong case that this is the result of the sputtering of refractory grains in interstellar shocks. It is interesting to note, however, that the graph of the depletion factors for iron and silicon plotted against the logarithm of gas velocity has the same characteristic shape (but opposite slope) as a plot of $\log N_H$ (cm⁻²) against $\log v$ (km s⁻¹). This effect may be statistical in that Barlow and Silk used data from only seven stars. It is also reasonable to assume that this pattern is not a coincidence and that higher gas velocities always correspond to an increase of iron and silicon in the gas phase and a decrease of atomic hydrogen, as explained by their 'snowplow' model¹¹.

An examination of the data for these seven stars¹¹⁻¹³ shows that although there is a good correlation between the δ s and gas velocities, there is only a very weak correlation between the δ s for iron and silicon and the values of f . For example, for the low-velocity component of μ Col (ref. 12), $\delta(\text{Fe}) = -1.8 \pm 0.2$ and $f \approx 4.5 \times 10^{-5}$, while for the higher-velocity component, $f \approx 6 \times 10^{-7}$. Unfortunately, for HD74455 (ref. 13), $\delta(\text{Fe}) \sim 0.1$ while $f \sim 4 \times 10^{-5}$. Part of the problem is that towards all these unreddened stars, $10^{-4} < f < 10^{-7}$, and a small error in observation or reduction can produce a correspondingly much larger error in f . Also, these stars may have fairly recently undergone heating by interstellar shock fronts, producing large deviations from equilibrium abundances. Thus, studies attempting to correlate $\delta(\text{Fe})$ with H₂ abundances should confine themselves to low-velocity clouds of appreciable ($A_v \approx 1.0$ mag) extinction.

If we do accept the possibility that there is a direct correlation between the amount of H₂ being produced and the amount of iron and silicon in the grains, we are still left with two alternatives. First, some of the H₂ may be formed by means of catalytic reactions on the surfaces of iron grains (process (3)). Although nothing in the above analysis proves this conclusively, nothing contradicts it either. Second, H₂ may be produced by process (1) on cold, silicate grains through the mechanism of physical adsorption. Although it may be possible for reasonably pure iron grains to exist in the interstellar medium by virtue of the fact that metallic iron will condense out of a hot gas before any of its compounds, the same cannot be said for silicon. Studies of the condensation temperatures of compounds and elements¹⁴⁻¹⁶ show that pure silicon is an unlikely possibility. Instead, silicon forms such compounds as CaAl₂Si₂O₈, Ca₂SiO₄, and Mg₂SiO₄¹⁷. Thus, for every atom of silicon that is depleted, there is an average of 3-4 oxygen atoms. As oxygen is about 21 times more abundant cosmically than silicon¹⁸, there is still

Table 1 Interstellar depletions of elements

Star	$E(B-V)$ (mag)	$\delta(C)$	$\delta(O)$	$\delta(Si)$	$\delta(Fe)$
ζ Oph	0.32	-0.87 to -0.54	-0.68 to -0.49	-1.88 to -1.38	-2.05 to -1.89
o Per	0.32	-1.65 to -0.43	-2.70 to -0.59	-2.13 to -1.82	-2.39 to -2.14
λ Sco	0.03	+0.2 to -0.9	-0.7 \pm 0.1	-1.1 \pm 0.1	-1.3 \pm 0.2

plenty of oxygen left to be incorporated into extensive 'ice' mantles and still agree with the interstellar depletions listed in Table 1. Hence, nothing in Table 1 contradicts the possibility of process (1). (As for process (2), nothing can be said at this time since there is a strong possibility that crystalline graphite cannot be produced in the interstellar medium^{19,20}.)

Observations of interstellar depletions may one day be able to decide how H_2 is produced in interstellar clouds. At present all that can be said is that neither process (1) or (3) can be dismissed without further consideration. It is hoped that this paper will stimulate additional research.

RONALD G. TABAK

Youngstown State University,
Department of Physics and Astronomy,
410 Wick Ave,
Youngstown, Ohio 44555

Received 18 July; accepted 19 August 1977.

1. Morton, D. C., Drake, J. F., Jenkins, E. B., Rogerson, J. B., Spitzer, L. & York, D. G. *Astrophys. J.* **181**, L103-L110 (1973).
2. Morton, D. C. *Astrophys. J.* **193**, L35-L39 (1974).
3. York, D. G. *Astrophys. J.* **196**, L103-L106 (1975).
4. Snow, T. P. Jr. *Astrophys. J.* **204**, 759-774 (1976).
5. Hollenbach, D. & Salpeter, E. E. *Astrophys. J.* **163**, 155-164 (1971).
6. Barlow, M. J. & Silk, J. *Astrophys. J.* **207**, 131-140 (1976).
7. Brecher, A. & Arrhenius, G. *Nature phys. Sci.* **230**, 107-109 (1971).
8. De Jong, T. *Astr. Astrophys.* **20**, 263-274 (1972).
9. Bond, G. C. *Catalysis by Metals* (Academic, London, 1962).
10. Tabak, R. G. *Astrophys. Space Sci.* **49**, 41-46 (1977).
11. Barlow, M. J. & Silk, J. *Astrophys. J.* **211**, L83-L87 (1977).
12. Shull, J. M., Hobbs, L. M. & York, D. G. *Astrophys. J.* **211**, L139-L143 (1977).
13. Jenkins, E. J., Silk, J. & Wallerstein, G. *Astrophys. J. Suppl.* **32**, 681-714 (1976).
14. Larimer, J. W. *Geochim. cosmochim. Acta* **31**, 1215-1238 (1967).
15. Anders, E. *Acet. chem. Res.* **1**, 289 (1968).
16. Cameron, A. G. W., Colgate, S. A. & Grossman, L. *Nature* **243**, 204 (1973).
17. Field, G. B. *Astrophys. J.* **187**, 453-459 (1974).
18. Cameron, A. G. W. *Space Sci. Rev.* **15**, 121 (1973).
19. Czyzak, S. J. & Santiago, J. J. *Astrophys. Space Sci.* **23**, 443-458 (1973).
20. Czyzak, S. J., Meese, J. M. & Santiago, J. J. *Astrophys. J.* **207**, 425-431 (1976).

Interstellar grains as possible cold seeds of life

As long as entropy ceases to play any part in equilibria when the temperature approaches absolute zero, all exothermic reactions—up to the formation of the most complex biopolymers—become thermodynamically profitable. In such conditions the reaction rate starts to play a decisive part in the observability of chemical transformations. According to classical views (Arrhenius law) all reactions should completely stop when $T \rightarrow 0$ and therefore the cold synthesis of biologically active substances is absolutely excluded. The phenomenon of a low-temperature limit to chemical reaction rates found recently, however, in the studies of radiation-induced polymerisation of formaldehyde¹⁻³, showing the existence of chemical reactivity even at $T \rightarrow 0$, obviously caused by quantum-mechanical molecular tunnelling, makes it possible to combine complete suppression of the entropy term in equilibria with an observable rate of exothermic chemical reactions, for example, of chain polymerisation (or any other integration of molecules) induced by light or ionising radiation. Thus molecular tunnelling suggests the possibility of a cold pre-history of life¹⁻³.

The possibility of synthesising rather complex molecules under the combination of deep cosmic cold and various radiations of cosmic origin was recently proposed by Wickramasinghe⁴⁻⁶ and Hoyle and Wickramasinghe⁷. Basing their argument on the properties of galactic nebulae in the infrared wavelength region they concluded that formaldehyde undergoes polymerisation in interstellar space with the formation of polyoxymethylene and even of polysaccharides.

As discussed elsewhere⁸, the only possible mechanism of interstellar formation of such complex substances must be molecular tunnelling. From the results given in refs 1-8 one can assume that the grains of interstellar dust clouds serve as cold seeds of life.

Interstellar chemical reactions have attracted the interest of astronomers, physicists, chemists and biologists (see refs 9-15).

Attention has been paid mainly to the gaseous reactions in interstellar clouds as well as to the reactions at the surface of grains. Most data concerning the formation of complex n -atomic molecules for $n \leq 5-9$ were explained by the mechanisms of bimolecular ion-molecule, or radical-radical, or radical-molecule interactions.

All authors, however, agree that the generally accepted mechanisms listed above are insufficient and unsatisfactory for $n \geq 5-9$. So clearly, these mechanisms should not be applied to the formation of polyoxymethylene and more complex compounds.

It seems necessary to consider interstellar grains as possible cold seeds of life, with chemical reactions in the bulk of these grains as a very important stage of prebiotic evolution. The idea of a very significant role of reactions in 'dirty-ice' mantles of grains in the formation of quite complex molecules (up to amino acids) was put forward and defended by Greenberg (see refs 11, 13, 16). He regards as a main process leading to the integration of molecules the recombination of ultraviolet-produced radicals and other active centres assuming that such processes can lead to the explosion of grains once the over-critical concentration of active centres is reached. Besides emphasising that the action of any permanent external ultraviolet source can lead only to saturation in the yield of radicals rather than to their over-critical concentration^{8,17}, one should remember that the recombination of radicals cannot form more complex products than can the other mechanisms mentioned here. I do not discuss here the role of shock wave-induced polymerisation and polycondensation of solids (up to the transformation of amino acids in polypeptides) we have observed¹⁸⁻²⁰.

The cold pre-history of life, of prebiotic evolution in interstellar clouds postulated here is based on several main points. (1) The formation of complex molecules—up to the biopolymers—proceeds in the 'dirty-ice' mantles of interstellar grains in diffuse or dense clouds at very low ($T \sim 10-20$ K) temperatures. The decisive part in such formation is played by exothermic (particularly chain-type) processes of addition and polymerisation proceeding by molecular tunnelling.

(2) Integration of molecules in surface regions of clouds is ultraviolet-initiated. As shown elsewhere⁸, the appearance of long chains ($\nu \gg 1$) of chemical transformations is possible in such conditions only if the duration of an elementary act of continuation of chain $\tau_E \ll R_{act} \tau_{uv}$, where $R_{act} \sim 10^{-3}$ to 10^{-2} is the saturated concentration of active centres, that is, the time spent by each molecule in an active state under the external ultraviolet-irradiation. $\tau_{uv} \sim 100$ yr is the characteristic time between two successive ultraviolet absorptions by any molecule of the grain. Thus for the development of long chains $\tau_E \ll 0.1-1$ yr.

(3) Integration of molecules in the depths of clouds can be initiated only by long-range cosmic protons. As the characteristic time between two successive interactions of such protons with any molecule of the grain $\tau_p \sim 3 \times 10^9$ yr is 10^3-10^4 times longer than the life-time of dust clouds τ_{cl} limited by their gravitational collapse or by cloud-cloud collisions (10^6-10^7 yr)^{4,14} the accumulation of active centres in the depths of clouds does not reach saturation. Therefore, the development of chains of reactions requires the much less stringent condition: $\tau_E < (1/\nu) \tau_{cl}$ and one can expect the formation of more complex molecules, and the occurrence of much more advanced prebiotic evolution in the depths of clouds rather than at their surfaces and in the interstellar gas.

(4) The equilibrium chemical composition of cold interstellar dust can be obtained by relatively simple calculations under the assumption that entropy factors can be neglected and that the whole totality of chemical reactions leads to the formation of the system of monomers with a minimum possible enthalpy (for the given balance of abundances of various elements).

The possibility of existence of some unsaturated H, C, N, O (and also S, P)-containing monomers (with multiple inter-

atomic bonds) in such equilibrium dust would automatically mean also the possibility of exothermic polymerisation of such monomers (like the case of polymerisation of formic acid and methanimine with formation of glycine mentioned in ref. 21), and molecular tunnelling opens the route of such polymerisation valid even at $T \rightarrow 0$.

In this way the possibility of energetically profitable formation of unsaturated monomers at the natural balance of elements' abundances would clearly constitute a serious argument in favour of the hypothesis of cold prebiotic evolution in interstellar grains.

(5) When the cloud collapses and a new hot star is created in its centre, the flattened protoplanetary disk formed from the remnants of the cloud continues to be cold. The temperature does not rise during either the consequent accretion of dust particles with the formation of planetesimals (including comets) or the agglomeration of most of planetesimals into the planets²¹⁻²⁷. This makes it possible that the formation of complex polymer molecules in cold interstellar dust can provide a real cold pre-history of life rather than simple accumulation of negative entropy. The molecules formed can reach such a high degree of complexity that they are even able to display the simplest biological functions as soon as the new star begins to heat its own planets and thus sharply increases the rate and variety of chemical reactions.

I thank Academician A. I. Oparin, Dr V. S. Strel'nitskii and Professor N. C. Wickramasinghe for helpful discussions.

V. I. GOLDANSKII

*Institute of Chemical Physics of the
Academy of Sciences of the USSR,
Vorobjevskoye Shosse 2-b, Moscow 117334, USSR*

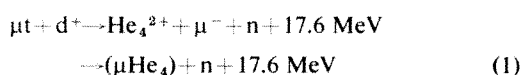
Received 22 July; accepted 31 August 1977.

- Goldanskii, V. I., Frank-Kamenetskii, M. D. & Barkalov, I. M. *Science* **182**, 1344 (1973).
- Goldanskii, V. I. *Russian chem. Rev.* **44**, 1019 (1975).
- Goldanskii, V. I. *Rev. phys. Chem.* **27**, 85 (1976).
- Wickramasinghe, N. C. *Nature* **252**, 452 (1974).
- Wickramasinghe, N. C. *Mon. Not. R. astr. Soc.* **170**, 11 (1975).
- Mendis, D. A. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **33**, L13 (1975).
- Hoyle, F. & Wickramasinghe, N. C. *Nature* **268**, 610-612 (1977).
- Goldanskii, V. I. *Nature* **268**, 612-613 (1977).
- Watson, W. D. *Rev. mod. Phys.* **48**, 513 (1976).
- Herbst, E. & Klempner, W. *Phys. Today* **29**, 32 (1976).
- Greenberg, J. M. *Ned. Tijdschr. Natuurk.* **42**, 117 (1976).
- Millar, T. J. & Williams, D. A. *Mon. Not. R. astr. Soc.* **173**, 527 (1975).
- Greenberg, J. M. in *Molecules in the Galactic Environment* (eds Gordon, M. A. & Snyder, L. E.) 93 (Wiley, New York, 1973).
- Sagan, C. *Nature* **238**, 77 (1972).
- Rank, D. M., Townes, C. H. & Welch, W. J. *Science* **174**, 4041 (1971).
- Greenberg, J. M. *Astrophys. J.* **189**, L81 (1974).
- Jackson, J. L. *J. chem. Phys.* **31**, 154 (1959); **31**, 722 (1959).
- Adadurov, G. A. et al. *Vysokomolek. Soyedin* (Russ.) **7**, 180 (1965).
- Baratova, L. A., Goldanskii, V. I., Kosygin, M. Yu. & Yampolskii, P. A. *Biokhimiya* (Russ.) **35**, 1216 (1970).
- Goldanskii, V. I., Ignatovich, T. N., Kosygin, M. Yu. & Yampolskii P. A. *Dokl. Akad. Nauk. SSSR* (Russ.) **207**, 218 (1972).
- Hoyle, F. & Wickramasinghe, N. C. *Nature* **264**, 45 (1976).
- Shmidt, O. Yu. *Dokl. Akad. Nauk. SSSR* (Russ.) **45**, 245 (1944).
- Gurevich, L. E. & Lebedinskii, A. I. *Izvestiya AN SSSR Ser. Fiz.* (Russ.) **14**, 765 (1950).
- Cameron, A. G. W. & Pine, M. *Icarus* **18**, 377 (1973).
- Cameron, A. G. W. *Icarus* **18**, 407 (1973).
- Cameron, A. G. W. *Icarus* **24**, 128 (1975).
- Goldreich, P. & Ward, W. *Astrophys. J.* **183**, 1051 (1973).

Muon catalysis of hot fusion

THE idea of muon catalysis of cold fusion dates back nearly 50 years¹⁻³, but the death blow to the technique was given by Jackson⁴. It has been revived by Tan⁵ in connection with fusion in inertially confined pellets and his idea is sufficiently important to warrant further investigation. Our calculations suggest that Tan's estimates are unjustifiably optimistic and so there is no possibility of achieving an energy gain.

The basic reactions are



and the corresponding dd and tt reactions which have a rather

lower probability. We assume, following Tan, that the pellet is totally ionised, and for definiteness consider a 0.5-cm radius pellet at a temperature of 10 keV and solid density. The reactions then occur in flight with a total rate of about 10^9 s^{-1} .

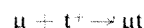
The method avoids the formation of $(\mu\text{He}_4)^+$, which normally terminates the catalysis chain for the following reason: the recoil $(\mu\text{He}_4)^+$ moves off with an energy of 3-4 MeV. Although it is travelling through a dense medium, the usual energy loss mechanism (ionisation of ordinary atoms) is no longer available to it. The only mechanisms available in a plasma are elastic scattering by the Coulomb force and ionisation of the $(\mu\text{He}_4)^+$. Using standard techniques⁶ we find the corresponding rates to be 10^7 s^{-1} and 10^9 s^{-1} (we have rounded off all rates to the nearest order of magnitude). In other words, stripping of the muon is very probable, and it would thus be able to take part in further fusions.

Unfortunately this is irrelevant for the inertial confinement as proposed by Tan. If the confinement time is 10^{-9} s , and the reaction rate 10^9 s^{-1} , on average each muon would catalyse one reaction, hence the occurrence of R_c (the catalytic chain ratio) in equation (5) of ref. 5 is erroneous.

The cost of producing a useful muon (that is, one stopping in the pellet) has been grossly underestimated by Tan. According to Tan e_μ (the energy required to produce one muon) lies between 10^9 and 10^{10} eV , and x_μ (the fraction stopping in the target) between 10^{-4} and 10^{-2} , so that e_μ/x_μ lies between 10^{11} and 10^{14} eV per useful muon. The higher value of e_μ is taken from Jackson who optimistically proposed a ratio of proton beam energy to input electrical power of 0.2 (for TRIUMF, this ratio is ~ 0.01) and assumed that every muon produced was available to catalyse a fusion reaction. In addition, however, only a very small fraction of muons can be focused into a useable beam.

It is more useful in the situation being discussed here to refer to values of e_μ/x_μ that are obtained in today's meson factories, with beams designed to optimise muon stopping rates in small targets. In the standard technique, pions from a target placed in the proton beam are collected in a long ($\sim 10 \text{ m}$) magnetic channel which confines them and their decay muons. The latter emerge from the channel exit in a very crudely focused beam; obviously they cannot be sharply focused as their source is fairly large radially and very long axially. If we take as typical 'state of the art' values, an accelerator input power of $2 \times 10^{25} \text{ eV s}^{-1}$ (TRIUMF: 100 μA at 500 MeV) and a μ stopping rate of $10^5 \text{ g}^{-1} \text{ s}^{-1}$ (SIN: 100 μA at 500 MeV) then for a 0.5-cm radius pellet of density 0.71, e_μ/x_μ is $5.4 \times 10^{21} \text{ eV}$. In fact, as we show below, the stopping rate in an ionised pellet would be very much smaller.

The final objection is the most serious. Whereas it is true that the capture reaction



has a rate of 10^{12} s^{-1} in a solid, it is not true in a plasma, even at solid densities. Using Thomson's classical model⁶, the capture rate is $\sim 10^3 \text{ s}^{-1}$, if we assume the plasma to be totally ionised. In fact, the plasma will be about 99.98% ionised, and the remaining atoms could be reasonably effective in capturing mesons. To estimate this rate, we assume that an atom has the same capture rate in a plasma as would in a solid, and multiply the solid rate by the effective density of atoms. This gives a rate of 10^8 s^{-1} , which in itself is a gross overestimate as capture in a solid occurs only for very slow muons. This is not incompatible with the Saha equation: the only significant population of muons will be in the 1s level, and, by the above arguments, the reaction time for capture into the 1-s level is very much longer than the operative timescale of 10^{-9} s .

The same point may be made in slightly different fashion: Tan assumes that the incident muons have an energy of 4 MeV and are stopped in the first 10^{-3} m of the pellet. In fact, the mean free path will be $\sim 80 \text{ cm}$, so if the pellet is pre-ionised, it will be totally transparent to muons. Further, we note that

the pellet will be transparent to the reaction products (4 MeV α 's and 14 MeV neutrons) so no heating of the pellet will occur.

We thank Dr P. A. Redhead for bringing Tan's paper to our attention, and Dr C. K. Hargrove and Dr H. Mes for discussions.

E. P. HINCKS

Division of Physics,
National Research Council of Canada,
Ottawa, Canada

M. K. SUNDARESAN
P. J. S. WATSON

Physics Department, Carleton University
Ottawa, Canada

Received 9 February; accepted 14 July 1977.

¹ Frank, F. C. *Nature* **160**, 525 (1947).

² Sakharov, A. *Rep. Lebedev. Phys. Inst. (Acad. Sci., USSR)*, 1948).

³ Alvarez, L. W. *et al. Phys. Rev.* **105**, 1127 (1957).

⁴ Jackson, J. D. *Phys. Rev.* **106**, 330 (1957).

⁵ Tan, W. P. S. *Nature* **263**, 656 (1976).

⁶ Mitchner, M. & Kruger, C. H. *Partially Ionised Gases* (Wiley, New York, 1973).

Water-promoted oxidation of carbon monoxide over tin(IV) oxide-supported palladium

CATALYTIC oxidation of CO, both by O₂ and by nitrogen oxides (NO_x), is of considerable relevance to the removal of toxic CO and/or NO_x from exhaust gases. Certain applications such as automobile exhaust control in cold-start conditions, and the removal of CO from tobacco smoke by use of a catalytic filter, demand not only catalytic activity at moderately low temperatures, but also effectiveness in the presence of water vapour. The low-temperature activities of most catalysts are at best (for example precious metals) partially destroyed, or at worst (for example base metal oxides) totally destroyed by poisoning due to water vapour. The results presented here demonstrate that, not only is the low-temperature activity of the Pd-SnO₂ system not poisoned by water vapour, but rather its presence causes a marked enhancement in activity for the oxidation of CO both by O₂ and by NO.

The catalytic activity of SnO₂-supported palladium for the oxidation of CO both by O₂ (refs 1, 2) and by NO (ref. 3) has previously been shown to be considerably higher than that predicted from purely additive or metal dispersion considerations. This synergism has obvious relevance to potential applications where precious metals are supported on oxide carriers, a topical example being the oxide wash-coats used to 'key' precious metals to the honeycomb monoliths developed for the catalytic control of exhaust gases. These previously reported catalytic data for CO oxidation over Pd-SnO₂ were obtained using anhydrous feed gases, and here we report the effects of adding water vapour.

Samples of 1.8% Pd on SnO₂ and 5.8% Pd on SiO₂ were prepared by non-exhaustive ion-exchange sorption, from Pd(NH₃)₄²⁺ solution, on to 36-72 B.S.S. mesh SnO₂ (ref. 4) and SiO₂ (BDH, pretreated with 1 M HNO₃ and exhaustively washed with water) gels respectively. Thermal activation (5 h at 450 °C in air) and reduction (2 h at 150 °C in 30% CO in Ar) were carried out in the catalytic reactors using anhydrous gases. Specific surface areas after this treatment were 40, 70 and 230 m²g⁻¹ for SnO₂, Pd-SnO₂ and Pd-SiO₂ catalysts respectively. With the exception of the CO-O₂ reaction over Pd-SnO₂ which, because of the low weight required was performed at 60 °C in a 2-mm internal diameter stainless steel U-tube, the previously described⁴ glass reactor assembly was used. Water vapour of various concentrations was admitted to the feed gases by bubbling through H₂SO₄ solutions of

known concentrations. Gas chromatographic analyses of the products of the CO-O₂ and CO-NO reactions have been described (refs 5 and 6 respectively). Catalytic rate data for the CO-O₂ reaction are quoted in terms of CO conversion for a feed of 5% CO in air at 100 cm³min⁻¹, and those for the CO-NO reaction in terms of NO conversion (both to N₂O and to N₂) for a feed of 14% NO in CO at 30 cm³min⁻¹.

Typical steady-state (16 h) conversions for the CO-O₂ reaction over the Pd-SnO₂ catalyst (0.25 g) at 60 °C using anhydrous feed gases gave values ranging from 0.7 to 1.2% CO oxidation (four experiments). Introduction of water vapour at a partial pressure of 22 mm Hg caused, after a 4-h conditioning period, an increase in conversion to 10-13%, and this remained constant over a further 24 h period. This order-of-magnitude increase was essentially independent of the water concentration within the partial pressure range of at least 5-22 mm Hg. At p_{H₂O} < 1 mm Hg the enhancement in activity was less than that at higher pressures, but even at p_{H₂O} = 0.1 mm Hg the steady-state activity was still a factor of about 5 higher than the 'anhydrous' rate. Water vapour partial pressures above 22 mm Hg were not investigated because of problems of condensation in the apparatus. The effect of removal of water from the feed gases was to cause only a very slow return to the 'anhydrous' rate. For example, in an experiment in which the 'anhydrous' and 'wet' CO oxidations were 0.7 and 11.7% respectively, removal of the water vapour for 16 h only decreased the conversion to 3.1%. This slow reversibility is presumably attributable to the low rate of dehydration/dehydroxylation of the hydrated surface (see below) at 60 °C.

In contrast to these findings, the steady-state CO oxidation activity of SnO₂ at 170 °C was found to be totally destroyed, and that of the 5.8% Pd-SiO₂ at 140 °C reduced by a factor of >0.2, by the introduction of 22 mm Hg water vapour pressure to the gas feeds.

The presence of water vapour also caused a comparable enhancement in activity for the CO-NO reaction over a 1.0 g bed of the Pd-SnO₂ catalyst at 130 °C. A steady-state (16 h) 'anhydrous' NO conversion of 0% to N₂ and 32% to N₂O was, after the introduction of water vapour at 22 mm Hg partial pressure followed by a 4-h conditioning period, increased to 34% to N₂ and 51% to N₂O. This increased activity remained constant over a further 24 h period although, in contrast to the CO-O₂ reaction at 60 °C, removal of the water vapour resulted in a much more rapid (<16 h) return to the 'anhydrous' rate. This is presumably attributable to a more rapid dehydration/dehydroxylation of the hydrated SnO₂ surface at the higher temperature. In the case of the CO-NO reaction it is apparent that, not only does water vapour increase the overall reaction rate, but it also influences the selectivity towards the preferred (from the pollution control viewpoint) complete reduction of the NO to N₂.

Previous investigations into the CO-O₂ reaction over Pd-SnO₂ have shown strong evidence that the synergistic catalytic effect is attributable to spillover of CO in an activated form from the Pd to the SnO₂ surface³. The results presented here suggest that the role of the water vapour is to increase this spillover rate, as the actual catalytic oxidation rates over both pure SnO₂ and over Pd supported on an 'inert' oxide are decreased by the presence of water vapour. A probable explanation for this effect is that water molecules produce hydroxylated sites on the SnO₂ surface which act as chemical bridges to assist spillover of the Pd-activated CO. It has been observed that adsorbed proton acceptors (reported water and alkanols) can assist spillover of activated hydrogen from precious metals on to oxides⁷. But, the mechanism of Pd-activation of CO before its spillover is not yet clear. In the case of hydrogen spillover the activation process

can be readily explained in terms of dissociative chemisorption to produce hydrogen atoms. Even if dissociation were to occur during CO adsorption on Pd, which is unlikely on the basis of current evidence⁸, it is difficult to envisage how this process could explain the above observations.

GILLIAN CROFT
M. J. FULLER*

International Tin Research Institute,
Fraser Road,
Greenford,
Middlesex, UK

Received 21 July; accepted 6 September 1977.

*Present address: Thorn Lighting Ltd, Great Cambridge Road, Enfield, Middlesex, UK.

1. Bond, G. C., Molloy, L. R. & Fuller, M. J. *Chem. Commun.* 796–797 (1975).
2. Bond, G. C., Fuller, M. J. & Molloy, L. R. *Proc. 6th Int. Congr. Catalysis (London, 1976)* 1, 356–364 (1977).
3. Fuller, M. J. & Warwick, M. E. *Chem. Ind.* 787–788 (1976).
4. Fuller, M. J. & Warwick, M. E. *J. Catal.* 29, 441–450 (1973).
5. Fuller, M. J. & Warwick, M. E. *J. Catal.* 34, 445–453 (1974).
6. Fuller, M. J. & Warwick, M. E. *J. Catal.* 42, 418–424 (1976).
7. Sermon, P. A. & Bond, G. C. *Catal. Rev.* 8, 211–239 (1974).
8. Joyner, R. W. & Roberts, M. W. *Chem. Phys. Lett.* 29, 447–448 (1974).

Windscale effluent in the waters and sediments of the Minch

A SYSTEMATIC intrusion of water of high ^{137}Cs content into the north-eastern North Sea has been reported by the German Hydrographic Office¹. Jefferies *et al.*² later confirmed that a stream of water rich in radioactive caesium from Windscale passes northwards through the Hebridean Channel, around the north of Scotland and into the North Sea. Although the circulation pattern with respect to ^{137}Cs has been significantly refined in recent reports³ and from German data for 1975 (ref. 4) and 1976 (Kautsky, personal communication) little attention has been paid to the status of other radionuclides in this stream. Such a study offers, however, an excellent opportunity of looking at the geochemical discrimination between, for example, ^{137}Cs and the transuranic α -emitting nuclides; it seemed also that an examination of the changes of the ratio of ^{134}Cs to ^{137}Cs during this passage would give a good basis for estimation of the travel time. As a part of the Flex Programme (Fladen Ground Experiment—part of Jonsdap 76 international programme on the interplay of environment and a plankton bloom) in the North Sea in May–June 1976, we collected a series of sediment, water and biota samples for that purpose. We report here our analyses of samples from a large-volume water station and a 21-cm diameter sediment core taken in the Minch, 27 May 1976.

Our sampling procedures are described elsewhere^{5,6} as are our analytical procedures, and their performance in a variety of IAEA- or NBS-organised intercomparison exercises^{7–10}. Table 1 shows the nuclide analyses so far completed on 55-litre unfiltered water samples from four different depths, together with comparable analyses of alternate sections of the sediment core taken at the same time. For water column and sediment, the inventory inferred for each nuclide from the data presented, and the mean ratios of selected nuclide pairs are shown.

It should be noted that both water and sediment collected anywhere in the oceans today may be expected to contain ^{137}Cs , $^{239,240}\text{Pu}$, ^{238}Pu or ^{241}Am , as a result of their dissemination in world-wide fallout. The analysis of these nuclides and the use of their distributions to infer rates of marine processes are major activities of our laboratory^{11,12}. ^{134}Cs , however, was only a trivial component of world-wide fallout, about $0.25 \times 10^6 \text{ Ci}$ produced¹³, mostly before 1958, compared with $34 \times 10^6 \text{ Ci}$ ^{137}Cs ¹⁴. The ^{134}Cs present in environmental samples collected since 1970 is certainly to be attributed to some aspect of the nuclear fuel cycle. In the case of other nuclides, similar inferences can be drawn either from their presence in amounts far exceeding those to be expected from fallout

delivery measurements, or from departures in their relative proportions from the ratios found in fallout.

The data tabulated indicate Windscale effluent as the preponderant source of the nuclides in the water column and as a major contributor of those in the sediment. This is shown by the large amounts of ^{134}Cs , and by its ratio to ^{137}Cs : recent data referring to this phenomenon at Windscale have not been published, but the ratio ^{134}Cs to ^{137}Cs approximated 0.23 in 1970², 0.18 in 1971², 0.17 in 1972², 0.19 in 1973¹⁵, 0.24 in 1974³ and 0.18 in 1975³.

Comparison of the water and sediment inventories of ^{137}Cs , ^{90}Sr and $^{239,240}\text{Pu}$ in the Minch location (Table 2) with delivery estimates from fallout at this latitude¹⁶ strongly support the Windscale conclusion in respect of the source of a major fraction of these inventories. A similar conclusion is reached from comparison of the water ratios $^{137}\text{Cs}/^{90}\text{Sr}$ and $^{238}\text{Pu}/^{239,240}\text{Pu}$ with those to be expected at that latitude from fallout. The $^{238}\text{Pu}/^{239,240}\text{Pu}$ ratio of 0.14 is understandable in terms of the reported discharge value from Windscale of about 0.2 for 1971–73 (ref. 17).

Accepting that the major source of the nuclides measured was the Windscale effluent, a variety of hydrographic and geochemical conclusions can be argued. First, from the change in ratio ^{134}Cs to ^{137}Cs in the water, travel time from the Irish Sea can be estimated. ^{134}Cs has a half life of 2.06 yr compared with 30.1 yr for ^{137}Cs . Making the assumption that the two caesium isotopes are not discriminated in ocean circulation, and less satisfactorily that a ratio of 0.24 characterised the source term, then from the ratio 0.144 observed at the Minch it is concluded that the travel time from the Windscale pipeline to the Minch was about 1.6 yr. If source term ratio had been closer to 0.18, as might be argued from other data^{2,3}, then a travel time closer to 0.7 yr would be concluded. Neither calculation is at first sight compatible with the very slow passage times that were suggested by Jefferies *et al.*² for transit from Windscale to the North Channel. An appreciable fraction of the total travel time may have been spent in the Irish Sea, and this is, in fact, indicated by the mean ratio 0.20 reported³ in plaice and *Nephrops* from the northern Irish Sea in 1974. In the Irish Sea, reduction of the ^{134}Cs to ^{137}Cs ratio by mixing with older, lower ratio water from Windscale could be significant, especially considering the ratios reported for 1971–1973 releases: reduction by mixing with fallout ^{137}Cs free of ^{134}Cs is suggested to be insignificant by the expected fallout ^{137}Cs concentration in coastal water, about 0.2 pCi l^{-1} at most by 1973–1974¹¹ and little more than 0.1 by 1976 (Kautsky, unpublished).

The most likely solution to the dilemma caused by the different transit times derived from this and from Jefferies' work² is that we are seeing the net effect of two (at least) processes. First, the mixing process within the Irish Sea and, second, the outflow from the Irish Sea into the coastal current travelling northwards parallel to the west coast of Scotland. The first process may be very much slower than the second so that the net effect may be controlled more by the mixing times within the Irish Sea. Additional support for this two-component process comes from comparison of our ^{137}Cs concentrations in the Minch in May 1976 with the values about $4\text{--}5 \text{ pCi kg}^{-1}$ for July 1974³. Our values of $4.7\text{--}6.5 \text{ pCi kg}^{-1}$ are not significantly different from the values measured 1.8 yr earlier. As these show little difference, it is arguable that May 1976 samples from the Minch have not begun to be affected by the rise in Windscale ^{137}Cs discharge described by Hetherington³ in 1974. So a net transit time of 2 yr could be composed of a term of 1.8 yr in the Irish Sea and 0.2 yr in the coastal current between North Channel and the Minch. Additional evidence that the coastal current labelled with radionuclides originating from Windscale is moving rapidly, comes from preliminary measurements of the $^{134}\text{Cs}/^{137}\text{Cs}$ ratio in water, also collected in May 1976, in the North Sea between Scotland and Norway. At this location (58° 25.5'N, 0° 03.4'E), the measured ratio of 0.14 is indistinguishable from the ratios measured in the Minch.

Second, from comparison of the inventory of radiocaesium in the sediments of the Minch against the estimated total delivered to the area, with the same relation in respect to fallout, some

Table 1 Radionuclide analyses of water and sediment from the Minch

RV Knorr cruise 54 Leg 6 Station 84: 27 May 1976 at 58 14.6'N:05 49.5'W						
Water station	$^{137}\text{Cs}^*$	$^{134}\text{Cs}^*$	$^{90}\text{Sr}^\dagger$	$^{239,240}\text{Pu}^\ddagger$	$^{238}\text{Pu}^\ddagger$	$^{241}\text{Am}^\S$
Sample depth (m)	(pCi l ⁻¹)	(pCi l ⁻¹)	(pCi l ⁻¹)	(fCi l ⁻¹)	(fCi l ⁻¹)	(fCi l ⁻¹)
Surface	6.13±0.03	0.88±0.05	0.998±0.009	2.6±0.2	0.28±0.09	0.32±0.09
30	6.52±0.05	0.92±0.04	1.00±0.02	2.6±0.1	0.41±0.05	0.09±0.09
60	5.84±0.04	0.86±0.02	0.923±0.009	2.6±0.2	0.46±0.09	0.46±0.09
90	4.66±0.04	0.66±0.01	0.793±0.023	2.2±0.1	0.28±0.05	0.32±0.09
Water depth (100 m)						
Water column inventory (mCi km ⁻²)	579	83.1	92.5	0.25	0.038	0.029
Mean nuclide activity ratios	$^{134}\text{Cs}:^{137}\text{Cs}, 0.144; ^{137}\text{Cs}:^{90}\text{Sr}, 6.2; ^{238}\text{Pu}:^{239,240}\text{Pu}, 0.14; ^{241}\text{Am}:^{239,240}\text{Pu}, 0.12$					

RV Knorr cruise 54 Leg 6 CORE 46: 27 May 1976 at 58 06.1'N:05 51.0'W						
Sediment core	$^{137}\text{Cs}^*$	$^{134}\text{Cs}^*$	$^{239,240}\text{Pu}^\ddagger$	$^{238}\text{Pu}^\ddagger$	$^{241}\text{Am}^\S$	
Sediment section (cm)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)	(pCi kg ⁻¹)
0-1	748±18	65±11	37±1.6	3.4±0.3		12.5±0.5
2-3	536±9	44±2	31±1	2.8±0.2		
4-6	518±14	30±7	25±1.2	1.7±0.2		
8-10	232±9	26±5	17±0.9	0.65±0.09		
12-14	86±4	1.7±1.3	5.7±0.3	0.30±0.05		
16-18			0.4±0.5	0.06±0.02		
Water depth (91 m)						
Sediment column inventory: (mCi km ⁻²)	55.3	4.09	3.1	0.21		
Mean nuclide activity ratios	$^{134}\text{Cs}:^{137}\text{Cs}, 0.074; ^{238}\text{Pu}:^{239,240}\text{Pu}, 0.069$					

Where values are not given the analyses are incomplete.

*By Ge (Li) γ spectrometry after radiochemical separation.

†By anticoincidence β counting after radiochemical separation.

‡By α spectrometry after radiochemical separation.

§By α spectrometry after radiochemical separation; curium isotopes accompany Am in our procedure. No ^{242}Cm was observed, and ^{244}Cm was present only in the amount (about 1 count per day) added as an impurity in our ^{243}Am yield monitor.

conclusions can be drawn about the geochemical behaviour of Windscale radiocaesium. Noshkin and Bowen¹⁶ reported about 7% of fallout ^{137}Cs delivery was found in very shallow-water sediments of Buzzards Bay; using more recent data to interpolate between Buzzards Bay and the 200-m cores from slightly further north, one might predict only 5–6% of fallout in sediment cores under 100 m of water.

Estimating the Windscale-derived delivery of Cs nuclides to the Minch is difficult at best, and made even more so by the fact that Windscale release data for ^{137}Cs have been published only for the years 1957–67¹⁴ and 1972–73¹⁷. Using the data available, and extrapolating to estimate an annual average, ^{137}Cs release of 15,000 Ci for 1968–71, yields a release estimate, through 1973, of 156 kCi, which, divided by the area of the Irish Sea (after Hetherington¹⁷), corresponds to a ^{137}Cs delivery of 6.2 Ci km⁻². The fraction of Windscale ^{137}Cs reaching the Minch can be estimated by comparing the water column inventory (or the total inventory, water plus sediment) in Table 1 (579 or 634 mCi km⁻²) with the mean Area A water column inventory of 2.40 Ci km⁻² in 1974¹⁷. This comparison is consistent with the 2 yr travel time, from Windscale to the Minch, that we inferred above from the ^{134}Cs to ^{137}Cs ratio and ^{137}Cs concentration data. If the shorter, 0.7 yr, travel time were correct, then the inventory for comparison should be that, in the Irish Sea, of late 1975, requiring use of data

not yet available. One arrives, therefore, at the conclusion that the Minch 'sees' about 25% of the ^{137}Cs delivered by Windscale to the Irish Sea, and this should have represented, by 1976, at least 1,550 mCi km⁻² ^{137}Cs delivery to the Minch, and possibly more. The 3.5%, or less, of the inferred Minch delivery of ^{137}Cs found in the sediments may differ significantly from the 5–6% predicted for fallout ^{137}Cs , but we do not believe that the quality of the estimated values justifies this conclusion. If we were to take it seriously, we would argue that it shows either a systematic difference in properties of the Minch sediments, or the effect of isotope exchange at the much higher ^{137}Cs specific activities found in Windscale effluent. Certainly the observations do not suggest the Windscale caesium as either more or less reactive than that from fallout.

Third, from comparison of the ratio of the water column inventories of ^{137}Cs and $^{239,240}\text{Pu}$ with their ratio in the north-eastern Irish Sea conclusions can be drawn about the long-range trajectory of Windscale plutonium in British coastal waters. Calculated inventories of soluble $^{239,240}\text{Pu}$, and of ^{137}Cs , in the waters of the Irish Sea are available¹⁷ for both 1973 and 1974. From these it seems the mean ratio $^{239,240}\text{Pu}$ to ^{137}Cs in July 1974 was 0.094%. This figure is probably representative of the Irish Sea ratio at this time as it was shown not to vary between points within 10 km of the Windscale outfall and points 75–100 km from the

Table 2 Comparison of Minch nuclide inventories and ratios with fallout

	^{137}Cs	Inventories ^{90}Sr (mCi km ⁻²)	$^{239,240}\text{Pu}$	$^{137}\text{Cs}/^{90}\text{Sr}$	Ratios $^{238}\text{Pu}/^{239,240}\text{Pu}$
Water	—	93	—	6.2	0.14
Water plus sediment	630	—	3.3	—	—
Fallout	80	55	1.5	1.0	0.04

outfall. It differs significantly from the ratio, 0.043^{90}_{e} , observed in 1976 in the water column of the Minch. If the 1976 Minch water was the same water mass as characterised the Irish Sea in July 1974, one could conclude from the $^{239,240}\text{Pu}/^{137}\text{Cs}$ ratio change that only 46% of the soluble plutonium that was associated with the ^{137}Cs transported by the coastal current, survived the passage to the Minch. This conclusion, that rather less than half as much of the soluble plutonium avoids sediment uptake during transit from the Irish Sea to the Minch, implies that Windscale 'soluble' plutonium is only partly 'qualitatively similar' to ^{137}Cs . That the details of the separation of these two species may be of predictive importance is indicated by our preliminary data concerning sediments from the Flex area, of the North Sea, where ^{238}Pu to $^{239,240}\text{Pu}$ ratios, as do those of ^{134}Cs to ^{137}Cs , indicate significant delivery of Windscale radionuclides.

Fourth, from comparison of the ratio of ^{241}Am to $^{239,240}\text{Pu}$ observed in the Minch, against that reported in releases from Windscale¹⁸, some conclusions can be drawn about the relative mobilities of these two transuranium elements in marine coastal waters. Hetherington *et al.*¹⁸ have reported the mean ratio ^{241}Am to $^{238,239,240}\text{Pu}$ in Windscale releases to have been 2.56 in 1974, 1.66 in 1973 and 1.40 in 1972; it has also been reported¹⁹ that there were very wide fluctuations in this ratio, month to month, in 1974 releases. The mean ratio observed in the water of the Minch, 0.12, confirms that a very much greater proportion of ^{241}Am than of plutonium is immobilised during travel in the coastal current. A similar conclusion can be drawn from the low $^{241}\text{Am}/^{239,240}\text{Pu}$ ratios recently reported by Murray⁴ in seawater collected in 1975 from around the Orkney Islands. Although reports have consistently shown^{3,15} high ratios of ^{241}Am to plutonium in attached algae like *Porphyra*, it is likely that the major process immobilising ^{241}Am is association with sediments. This is indicated by the ratio of 0.33 observed in the surface sediments of the Minch, compared with the 0.12 observed in the overlying water. Some of the difference in the two ratios may be attributed to ingrowth of ^{241}Am from its parent ^{241}Pu (as discussed in refs 18, 20) but we believe most of the effect should be attributed to separation of americium and plutonium in the water column. We have described elsewhere²¹ differential behaviour of americium against plutonium from fallout in oceanic water columns. We submit that Hetherington *et al.*¹⁸ were unable to see this differential behaviour in the Irish Sea because of the fluctuations in supply ratio, and because trajectories were too short for the differences to be easily measurable.

Analyses of long-lived radionuclides in a water station and sediment core from the Minch have, therefore, confirmed the presence of Windscale-produced ^{90}Sr , ^{134}Cs , ^{137}Cs , ^{238}Pu , $^{239,240}\text{Pu}$ and ^{241}Am . Careful examination of the evidence for changes in the various ratios of these nuclides from those reported in the Windscale release stream has also (1) supported an improved estimate of the northward rate of movement of these nuclides in the coastal current; (2) confirmed that Windscale caesium behaves biogeochemically about as does that from fallout; (3) indicated that the soluble fraction of Windscale plutonium may qualitatively behave like ^{137}Cs , but that only some fraction of it survives travel to the Minch, relative to ^{137}Cs . The size of this fraction, put at about one-half from these measurements, and its possible variation in space and time should be determined; (4) demonstrated a large differential in the behaviour of ^{241}Am and $^{239,240}\text{Pu}$, in that americium seems to sediment out of the coastal current even sooner than does plutonium after introduction from Windscale.

This work has been supported by the US Energy Research and Development Administration. We thank our co-workers for help in sample collection and in radiochemistry.

HUGH D. LIVINGSTON
VAUGHAN T. BOWEN

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts 02543

Received 25 May, accepted 16 August 1977

1. *Univ. Strahlenschutz*, 51, (1971); 52, (1972); 64, (1973).

2. Jelleries, D. F. *et al.* *Mar. Pollut. Bull.* 4, 118-122 (1973).

3. Hetherington, J. A. *Rep. IRL 11* (1976); 12 (1977) (Fisheries Radiobiology Laboratory, Lowestoft).

4. Murray, C. N. & Kautsky, H. *Est. coast. mar. Sci.* 5, 319-328 (1977).

5. Bodman, R. H. *et al.* *J. mar. Res.* 19, 141-148 (1961).

6. Burke, J. C. *Limnol. Oceanogr.* 13, 714-718 (1968).

7. Wong, K. M. *et al.* in *Reference Methods for Marine Radioactivity Studies*, I, 119-127 (IAEA, Vienna, 1970).

8. Livingston, H. D. *et al.* in *Analytical Methods in Oceanography*, 124-138 (ACS, Washington, 1975).

9. Noyce, J. R. *et al.* *Proc. Int. Conf. Environ. Sensing*, Paper 19, 5 (Las Vegas, 1975), (Institute of Electrical and Electronic Engineers, New York, 1976).

10. Lukat, R. *et al.* in *Radioactive Contamination of the Marine Environment*, 3-27 (IAEA, Vienna, 1973).

11. Bowen, V. T. *et al.* *Limnol. Oceanogr.* 19, 670-681 (1974).

12. Bowen, V. T. *High Safety Lab. Environ. Q. HASL*, 291, 1-57, 1-79 (US ERDA, 1975).

13. Persson, R. B. R. *High Phys.* 14, 241-250 (1968).

14. Joseph, A. B. *et al.* in *Radioactivity in the Marine Environment*, 6-42 (NAS-NRC, Washington, 1971).

15. Mitchell, N. T. *Rep. IRL 10* (Fisheries Radiobiology Laboratory, Lowestoft, 1975).

16. Noshkin, V. I. & Bowen, V. T. in *Radioactive Contamination of the Marine Environment*, 671-686 (IAEA, Vienna, 1973).

17. Hetherington, J. A. in *Environmental Toxicity of Aquatic Radionuclides: Models and Mechanisms*, 81-106 (Ann Arbor, Ann Arbor, 1976).

18. Hetherington, J. A. *et al.* in *Transuranium Nuclides in the Environment*, 139-154 (IAEA, Vienna, 1976).

19. Pentreath, R. J. & Lovett, M. B. *Nature* 262, 814-816 (1976).

20. Livingston, H. D. *et al.* *Earthplanet. Sci. Lett.* 25, 361-367 (1975).

21. Livingston, H. D. & Bowen, V. T. in *Environmental Toxicity of Aquatic Radionuclides: Models and Mechanisms*, 107-130 (Ann Arbor, Ann Arbor, 1976).

Surge activity on the Barnes Ice Cap

THERE are conflicting opinions¹⁻⁵ on the occurrence of surges in the major ice sheets, deduced in many cases by indirect means. Where an ice sheet surges directly into the ocean, sea-level rise may be catastrophic. Surges in ice sheets may help to explain the apparent difficulties of accounting for the magnitudes of the decay rates deduced for the Laurentide Ice Sheet in terms of energy requirements for melting ice⁶. That is, large areas of that ice sheet became much thinner than was supposed, a suggestion made on the basis of marine core evidence¹. Pure computer simulations¹⁰ have shown that cyclic surging of the major ice sheets seems to be possible. This report shows that several surges have occurred within the last millennium on the Barnes Ice Cap, a small subpolar ice sheet on Baffin Island, Canada (Fig. 1).

This ice cap is a remnant of the North American Laurentide Ice Sheet¹¹. Surge scars on the ice cap have been tentatively identified¹² and one surge area was subsequently documented in some detail¹³. Now, five distinct surge scars of different ages have been identified with the help of Landsat imagery (Fig. 2). The scars all lie on the south-west side of the ice cap, which is thought to be significant because the south-west margin of the ice cap is, on average, 130 m lower in altitude than the north-east margin and because the orientation of the axis of the ice cap causes about 5% more direct solar radiation to be received on the south-west facing slopes¹⁴. Therefore, the average bulk temperature of the ice on the south-west side should be higher than that of the north-east side. The attainment of basal conditions favourable to surges should thus be expected first on the south-west side.

Except where immediately adjacent to proglacial lakes, the ice margin is frozen to the bed^{15,16}. Measurements (D. F. Classen, unpublished) indicate that ice at the pressure melting point exists at the base in the interior (Fig. 3) and approximate calculations¹³ indicate that this condition could be widespread. Horizontal velocity measurements (Fig. 3) tend to confirm this deduction since considerable basal sliding must be present.

Conn and Bieler Lakes are evidently formed as a result of the ice cap obstructing water flow along the natural slope of land. Generator and Blanchfield lakes have been formed by relatively recent surge ice which has interfered with previous drainage away from the ice cap. The presence of these lakes tends to accelerate ice flows at the margins¹⁵ compared with locations without lakes^{16,17}. Thus, part of surge areas numbers 1 and 3 may be suffering delayed 'recoveries' because of the proglacial lakes.

The general features of the surge scars are: (1) the presence of third and occasionally fourth order meltwater channels. These occur within the large surface depressions characterised by highly irregular topography not related to bedrock topography. The depressions displace the ice divide, and the corresponding bulged margins often extend well beyond the

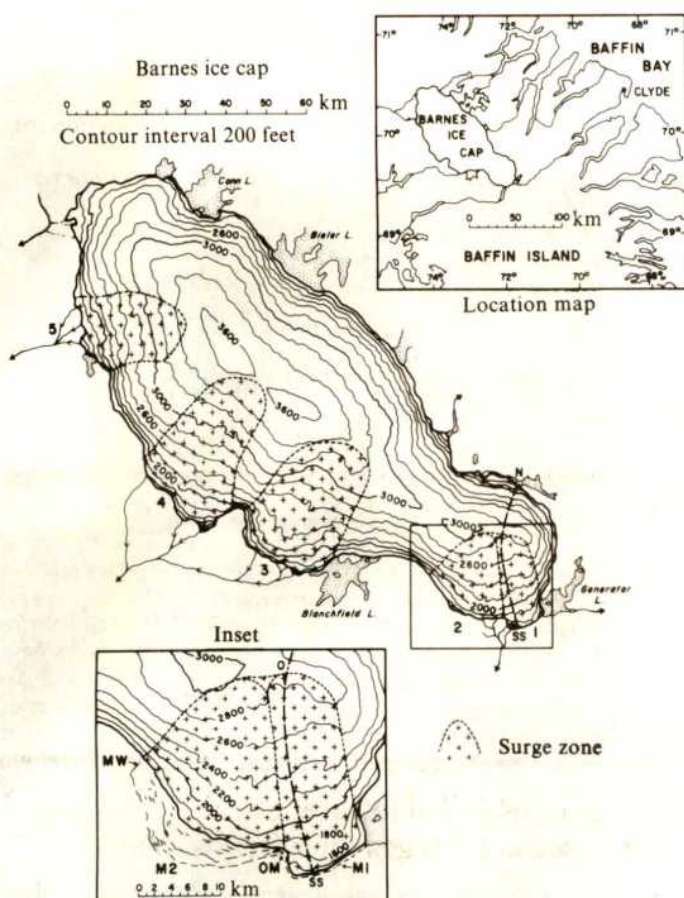


Fig. 1 Map of Barnes Ice Cap showing locations of former ice surges. Inset of south dome area shows location of transect along which data are presented in Fig. 3. Note moraine ridges (MW, M2, OM) associated with surge area no. 2. M1 marks recent moraine adjacent to surge area no. 1.

average line of the ice edge (Fig. 2). (2) Irregular foliation, defined by alternate dark- and light-banded ice (Fig. 2) is developed near the margins¹⁶ and indicates the presence of large shear strains. Measurements¹⁷ in non-surged areas show that the horizontal component of the ice flow vector is roughly parallel to the maximum principal strain rate direction (implying small shear strains) whereas within surge area no. 1, divergence between these quantities reaches 40° (G.H., unpublished). (3) Lakes occur on the ice surface due to trapping of meltwater in local depressions. While many of these lakes are visible on 1961 aerial photographs, some are visible on Landsat imagery

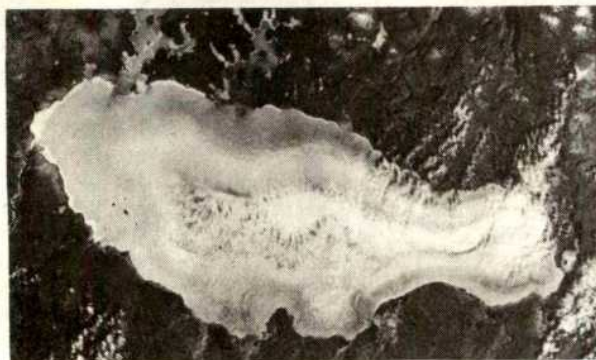


Fig. 2 Landsat image of Barnes Ice Cap (Channel 7, August 1974) showing surge scars. Surge areas 1 and 2 are partly obscured by cloud. Note evidence of intense shear strain in lower part of surge area no. 3 where ice is banded and the existence of lakes in surge area no. 5 (dark patches). The crown of the ice cap is covered by snow (white area) and meltwater streams cause the streak patterns.

(Fig. 2). Several moulines traversing cold ice are present at the margins. (4) Major streams, which flow west from the ice cap, can all be traced to the major surge areas (Fig. 1). (5) Moraines adjacent to the surge lobes tend to be well developed particularly when the surge is recent, since the moraines initially contain a substantial ice core. Also, recently overridden and pushed sediments may be seen near SS in Fig. 1. Ice depth soundings (J. W. Clough, unpublished and refs 18, 19) in surge zones 1,

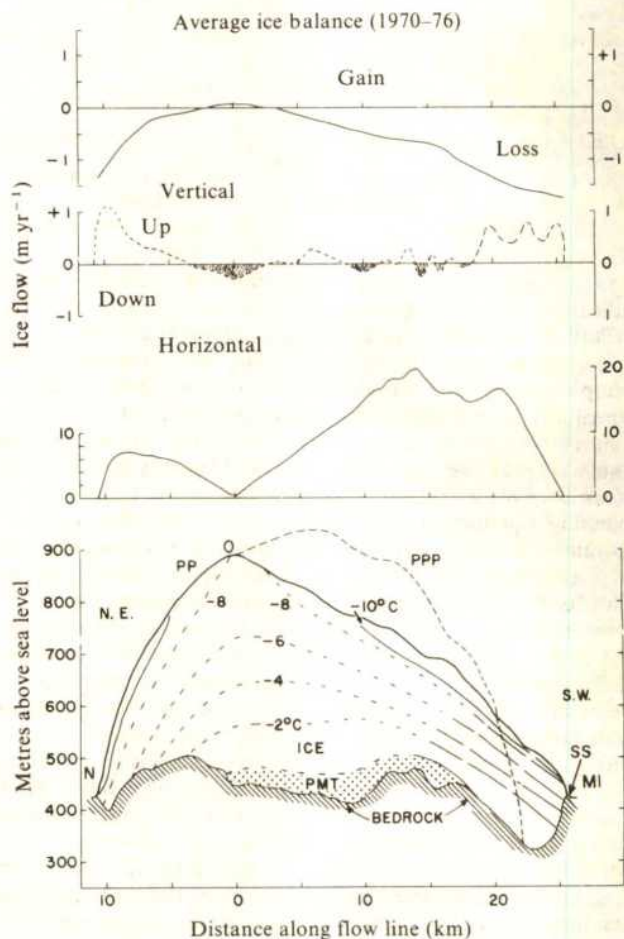


Fig. 3 Cross section through south dome from north-east to south (see Fig. 1) showing ice thickness, temperature, surface flow rates and net mass balance. Note relative irregularity of measured quantities on south (surge) section compared with the same quantities on the north-east section. PP, present profile; PPP, postulated pre-surge profile. Isotherms are shown in short-dashed lines where values have been estimated and long-dashed lines where measurements have been obtained.

2 and 3 show that there, the surface profile does not owe its shape to the basal topography in terms of standard flow theory²⁰. These profiles cannot be fitted to established equilibrium ice-cap profiles²⁰ whereas the contiguous profiles on the north-east side can^{13,17}. Profile PP (Fig. 3) is taken through O-N and O-SS-flowlines (Fig. 1). Plotted isotherms are based on measured values and approximate computation¹⁰. As this is not a steady state situation, the correct solution can only be found by successfully modelling the complete surge or by total measurement. Nevertheless, at least some, and possibly a substantial part, of the base may be at the pressure melting point, switching to cold base at about 18 km down flowline O-SS. Profile PPP is an hypothetical pre-surge parabolic profile which would seem to satisfy mass conservation after allowing for long term mass balance¹⁰. Recent surface net ice balance is also plotted, and comparing this with vertical ice flow values (Fig. 3) it can be seen that, whereas the north-east side is approximately near equilibrium, the south-west side is rapidly wasting. Furthermore,

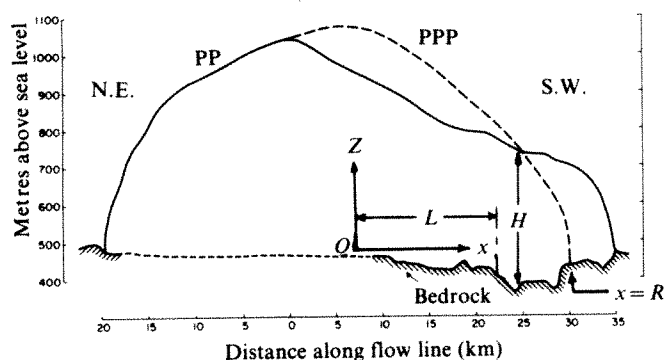


Fig. 4 Cross section through ice cap along two opposing flow lines. The south-west flow line is along the centreline of surge area no. 3. Coordinates and dimensions for equation developed in the text are shown. PP and PPP are as in Fig. 3.

the wavy character of the vertical ice flow on the south-west side indicates a disturbed dynamic flow regime.

This surge could have occurred as recently as 1927 or just before the turn of the century depending on the mass balance¹³, interpretation of the moraine ages²¹, and on the credibility of expedition accounts by Inuit from Clyde (Fig. 1).

Surge no. 2 is associated with a significant frontal moraine system (Fig. 1: inset, Fig. 2 and ref. 21) which may be interpreted in terms of cyclical surge activity with a period of 300–400 yr, spanning a period warmer than today and the 'Little Ice Age'. Morainial geometry indicates that local ice-cap dynamics was involved in their formation. The outer moraine curves sharply towards the ice cap at the north-west end (MW in Fig. 1: inset); at the eastern end (OM) it is truncated by the no. 1 surge lobe. The general retreat of the ice margin (M2) after about AD 500 (J. T. Andrews, personal communication) was punctuated by events at ~AD 900, ~AD 1200, ~AD 1500 and ~AD 1900 (or contemporaneously with surge no. 1)²¹.

Surge no. 3 may have reached a maximum position in about AD 1700 when it caused the formation of Blanchfield Lake in a more expanded form¹⁸. Area no. 4 has similar features to no. 3 and may be of comparable age whereas area no. 5 may be much older. The surface contains low intensity foliation and several large lakes. The inferred cyclic surging of diminishing extent seen for surge area no. 2 may or may not have been present in the other areas, but is a mechanism for maintaining a long-term surge scar.

Study of the surge mechanism is hindered by the lack of data that apply before and during the early stages of the surge. But it seems reasonable to assume that the mechanism described by Schytt²² applies. Figure 4 shows the profile through surge area no. 3 (PP). Let the parabolic pre-surge profile PPP be defined by ice thickness $H(x)$ and radius R of which only an outer portion $R-L$ is frozen to the bedrock. The length L ($< R$) is supposed to grow during a surge build-up. The amount of interior ice at the pressure melting point is thought to be controlled by heating of the upper surface as a result of superimposed ice build-up at the surface over sufficient period of time (10^3 – 10^4 yr). In this way, the onset of surging is related to climate.

As the water layer, represented by L , spreads, more interior ice is able to slide and because of its higher temperature, it will creep faster than the cold marginal ice. A predominantly mechanical mode of failure of the ice dam may follow when

$$\int_0^H \sigma_{xx} dz = \int_L^R \sigma_{xz}^b dx$$

where $\sigma_{xx}(z)$ is the horizontal component of the stress tensor at $x = L$ and $\sigma_{xz}^b(x)$ is the basal shear stress $L < x < R$. The

catastrophic flow which ensues might best be modelled using the finite element method incorporating joint elements (T. Q. Nguyen, unpublished).

A similar process may have occurred in the decaying phases of the Laurentide Ice Sheet in which surges have been inferred from the geological record⁸.

G. HOLDSWORTH

Environment Canada,
Water Resources Branch,
Glaciology Division,
Ottawa, Ontario, Canada

Received 15 August; accepted 1 September 1977.

- Emiliani, C. *et al.* *Science* **189**, 1083 (1975).
- Wright, H. E., Jr. & Stern, J. *Science* **193**, 1968 (1976).
- Farrand, W. R. & Evenson, E. B. *Science* **193**, 1269 (1976).
- Andrews, J. T. *Science* **193**, 1270 (1976).
- Emiliani, C. *Science* **193**, 1270 (1976).
- Hughes, T. *Rev. geophys. Space Phys.* **13**, 502 (1975).
- Weertman, J. *Nature* **260**, 284 (1976).
- Whillans, I. *Nature* **264**, 152 (1976).
- Hare, F. K. *Quat. Res.* **6**, 507 (1976).
- Budd, W. F. & McInnes, B. J. *Univ. Melbourne, Met. Dep. Publ. no. 21* (1976).
- Prest, V. K. *Geol. Survey of Canada, Map no. 1257A* (1969).
- Loken, O. H. *Can. J. Earth Sci.* **6**, 899 (1969).
- Holdsworth, G. *Can. J. Earth Sci.* **10**, 1565 (1973).
- Williams, L. D., Barry, R. G. & Andrews, J. T. *Can. J. Earth Sci.* **11**, 526 (1972).
- Holdsworth, G. *J. Glac.* **12**, 235 (1973).
- Hooke, R. LeB. *Geol. Soc. Am. Bull.* **84**, 3929 (1973).
- Holdsworth, G. *Can. Dep. Envir., Inld Wat. Directorate, Ottawa, Ont. Sci. Ser. No. 52* (1973).
- Jones, S. J. *Can. Dep. Envir., Inld Wat. Directorate, Ottawa, Ont. Sci. Ser. No. 25* (1972).
- O'Neil, R. A. & Jones, S. J. *J. Glac.* **14**, 458 (1975).
- Paterson, W. S. B. *The Physics of Glaciers* **145** (Pergamon, Oxford, 1969).
- Loken, O. H. & Andrews, J. T. *Geog. Bull.* **8**, 341 (1966).
- Schytt, V. *Can. J. Earth Sci.* **6**, 867 (1969).

The rise and fall of intracellular pH of sea urchin eggs after fertilisation

JOHNSON *et al.*¹ have reported an increase in the intracellular pH of sea urchin eggs, seen between 1 and 4 min after insemination, that results from the efflux of H^+ ions from the cells. The rise in pH can be induced by artificial activators of metabolism such as ammonia, or organic amines such as procaine. They have postulated that this increase in cellular pH is responsible for the metabolic derepression of the eggs and the initiation of embryonic development^{1,2}. Our interest in the control of the initiation of DNA synthesis in these synchronously dividing cells³, coupled with their method¹ for estimating the intracellular pH, led us to attempt to determine if fluctuations in pH occur during the cell cycle. Here we confirm the important observation of a rise in pH at fertilisation, and we report that the rise is soon followed by a gradual drop in pH to final values lower than those of the unfertilised condition.

The pH of homogenates of unfertilised sea urchin eggs averaged 6.34 ± 0.1 (41 determinations). By 10 min after insemination the pH rises to an average of 6.76 ± 0.16 (five determinations, Fig. 1). These values are in close agreement with those of 6.48 (unfertilised) and 6.76 (10 min after insemination) reported by Johnson *et al.*¹ with the exception that their average difference between unfertilised and fertilised of 0.28 is smaller than ours of 0.43 units. After a peak rise at 10 min after insemination (Fig. 1), the pH steadily decreases, reaching the value of the unfertilised eggs (6.46) by 70 min and attaining a final value of 6.18 by 140 min, a value that is maintained to at least the 48 h, mid-gastrula stage. No correlation of pH with stage of the cell cycle was found. Identical results were obtained when unfertilised eggs were parthenogenetically activated with the Ca^{2+} ionophore A23187 (ref. 4). It can therefore be concluded that although the initial rise in pH may be obligatory to metabolic activation, maintenance of elevated intracellular pH is not necessary to sustain embryonic development.

Does measurement of the pH of egg homogenates give a valid estimate of intracellular pH? The possibility that

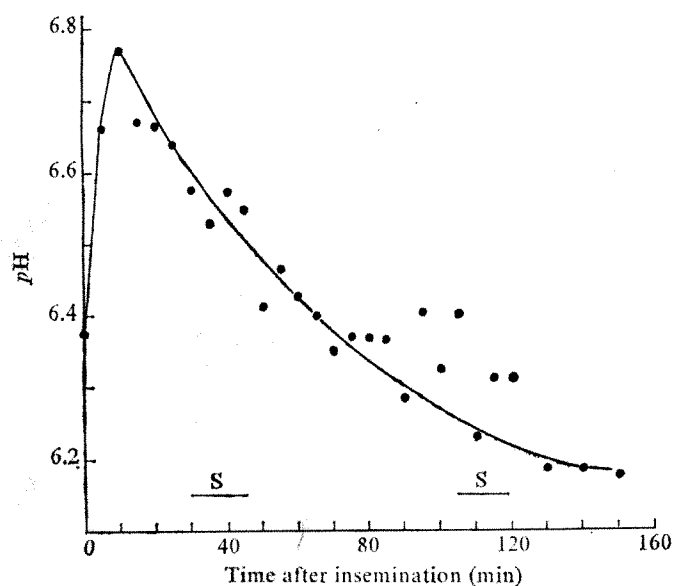


Fig. 1 The pH of sea urchin egg homogenates plotted against time after insemination (average of five separate experiments). Eggs of *Strongylocentrotus purpuratus* were stirred in seawater (pH 7.8) at 1% (v/v) concentration in a 15 °C room in which all media were stored and all pH determinations were accomplished. Aliquots of 50 ml of the culture were sedimented in a 50-ml conical centrifuge tube by hand centrifugation. The seawater was removed and the eggs suspended in 30 ml of bicarbonate-free artificial seawater, pH 7.8 (ref. 1). After resedimentation and removal of the artificial seawater the eggs were resuspended in 5 ml of 0.55 M KCl adjusted to pH 7.0 with HCl and NaOH. The suspension was transferred to a 20-ml Dounce homogeniser and 10 passes were made by hand with a tightly fitting Teflon pestle. Microscopic examination showed that no intact eggs or egg fragments remained. The pH was measured immediately with an expanded scale Corning Model 10 pH meter equipped with a Thomas 4094-L15 universal combination electrode. The timing of the first two periods of DNA synthesis³ is denoted by S on the horizontal axis. The protein concentration of the homogenate was 11.6 mg ml⁻¹. Homogenates containing one half or twice as much protein did not show significant pH differences.

the rise and fall in pH might not be a true estimate of intracellular pH but merely a reflection of compartmentation of H⁺ or OH⁻ ions in organelles such as mitochondria, was investigated by destroying all membrane-enclosed compartments by homogenisation in 0.5% Triton X-100. The results of this experiment (Table 1) show that the rise and fall in pH was also obtained in Triton, suggesting the method might prove to be valid for estimating total cellular pH of these and of other types of cells.

Fertilisation results in an irreversible activation of synchronous cycles of DNA synthesis and cell division³. We previously showed that DNA synthesis in unfertilised sea urchin eggs can be reversibly initiated by treatment with the local anaesthetic procaine hydrochloride⁵. If procaine is removed when the cells are in mid S phase, DNA synthesis abruptly halts. Other workers have shown that procaine treatment causes the efflux of H⁺ ions from these cells which results in a rise in intracellular pH; that is, the weakly basic nature of procaine is not responsible for the observed rise in cellular pH^{1,6}. We wondered if the removal of procaine from eggs would result in a

decrease in intracellular pH which might serve, somehow, as a switch to turn off DNA synthesis. We repeated our earlier experiments of treating unfertilised eggs with 10 mM procaine in seawater for 75 min, then washing out the drug, reculturing in fresh seawater, and determining the pH of egg homogenates. On addition of procaine the intracellular pH rises sharply and remains high for the duration of procaine treatment (Fig. 2). When procaine was washed out and the eggs returned to normal seawater, the intracellular pH rapidly dropped to the value of the untreated eggs, pH 6.46 (Fig. 2).

After their peak rise at 10 min after insemination, fertilised eggs (Fig. 1) required 60 min to return to their unfertilised pH. After procaine removal, however, unfertilised eggs required only 20 min to drop to their original pH (Fig. 2). This difference in rate of pH drop suggests that different mechanisms may underlie the fall in pH fertilised eggs and in unfertilised eggs recovering from procaine treatment. Support for this hypothesis was found in experiments in which fertilised eggs and procaine-activated eggs were treated with the metabolic poison dinitrophenol (DNP). For example, eggs were pretreated with DNP 30 min before fertilisation or procaine addition. Because DNP kills sperm, the eggs to be fertilised were washed from DNP into seawater (5 min washing time) and sperm immediately added. DNP was then added again 2 min after insemination. The normal rise in pH occurred, peaking at 10 min (Fig. 3), but the greater part of the subsequent drop in pH did not occur, and the cells maintained a pH of 6.65 for the duration of the experiment. Identical results were obtained when eggs were parthenogenetically activated by the ionophore A23187 in the continued presence of DNP. In response to procaine, the pH of DNP-treated, unfertilised eggs rose to 6.90 and remained at that value until 75 min when the procaine was washed out with seawater containing DNP. The result was an almost immediate drop in pH back to the original value (Fig. 3). The rise in intracellular

Fig. 2 Intracellular pH and the addition and removal of procaine. Procaine hydrochloride (10 mM, Sigma) was added to a suspension of unfertilised eggs and a rapid rise in the intracellular pH immediately occurred which was sustained for the duration of procaine treatment. At 75 min the procaine was removed (arrow) and the eggs returned to normal seawater⁵. There was a rapid fall in intracellular pH to the untreated value. To be certain that residual procaine was not influencing the pH of homogenates we added 0.5 ml 10 mM procaine to homogenates and found that it did not change the observed pH.

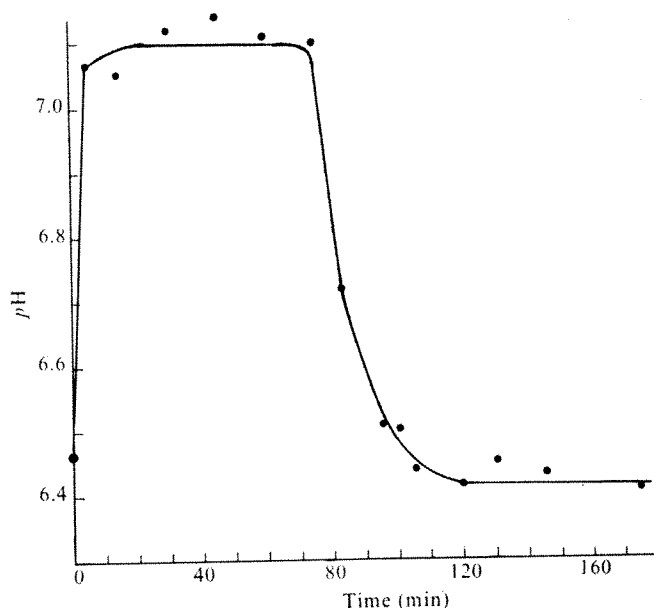


Table 1 The pH of egg homogenates in 0.55 M KCl containing 0.5% Triton X-100

Time after insemination (min)	pH
0	6.39
10	6.70
60	6.58
120	6.43

pH between 0 and 10 min after insemination or after procaine addition in the presence of DNP is evidence that H^+ ion release from mitochondria is probably not responsible for excretion of H^+ from the cells. The subsequent fall in pH in fertilised eggs may depend on the synthesis of ATP (with the reservation that metabolic inhibitors can have nonspecific effects). The drop in pH of the unfertilised eggs immediately after procaine removal in the presence of DNP shows that in these eggs the drop is independent of the production of metabolic energy.

The normal drop in intracellular pH of fertilised eggs (Fig. 1) is the most protracted metabolic change yet reported in the metabolic activation of development², a final value not being reached until 140 min after insemination. This is well after the completion of the first mitosis and the second S phase³. The period of the pH drop corresponds to the period of the activation of the transport systems for amino acids^{7,8} and nucleosides^{8,9} and the increase in the rate of protein synthesis⁷ in these cells. It is tempting to speculate that the development of transport capabilities might be linked to the lowering of intracellular pH. In this context, we have reported that the transport of thymidine into unfertilised eggs treated with procaine is only 2% that of fertilised eggs, whereas DNA synthesis in procaine occurs at roughly 50% the rate of fertilised eggs⁹. The high intracellular pH maintained in procaine may somehow inhibit the development of the transport systems in the cell membranes.

DNA synthesis is reversibly activated by the addition and removal of procaine⁵. The rapid drop in pH after procaine removal and the insensitivity of the drop to respiratory poisons such as DNP show that procaine may activate DNA synthesis by a different mechanism than does fertilisation. Amphipathic drugs such as procaine are thought to affect cells by intercalating into the lipid bilayers of the cell membrane. This can cause the expansion of the membrane which may result in a change in its permeability characteristics¹⁰. Procaine may open channels for H^+ ion excretion which would result in an elevation

of the intracellular pH. After removal of procaine from the external medium, procaine loss from the membrane may re-establish normal permeability, thus allowing the cells to return to a lower pH. The rise and fall in pH on the addition and removal of procaine correlates well with the turning on and turning off of DNA synthesis⁵ indicating that, in these conditions of artificial activation, the control of DNA synthesis may indeed be linked to changes in intracellular pH.

We thank David W. Deamer and Robert J. Gillies for helpful discussions. This work was supported by the USNIH.

Note added in proof: Nishioka and Epel using the homogenisation method, and Shen and Steinhardt using pH microelectrodes have confirmed the transient rise in pH of sea urchin eggs after fertilisation. The pH values obtained by both these groups are slightly higher than those we report here.

ALINA LOPO
VICTOR D. VACQUIER

Department of Zoology,
University of California,
Davis, California 95616

Received 8 June; accepted 29 July 1977.

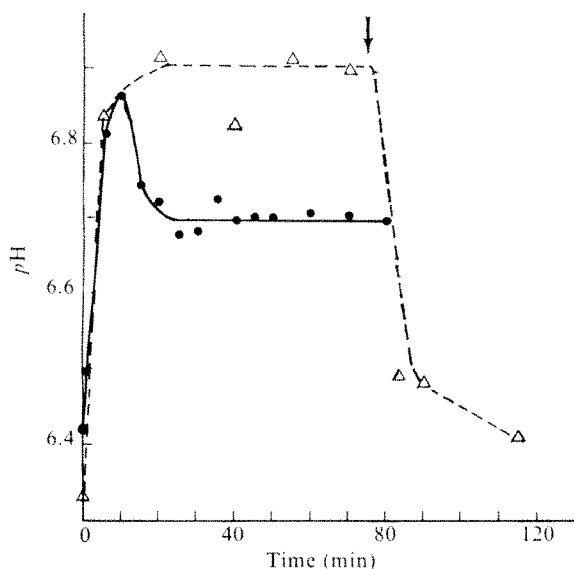
1. Johnson, J. D., Epel, D. & Paul, M. *Nature* **262**, 661-664 (1976).
2. Epel, D. *Am. Zool.* **15**, 507-522 (1975).
3. Hinegardner, R. T., Rao, B. & Feldman, D. E. *Expl. Cell Res.* **36**, 53-61 (1964).
4. Steinhardt, R. A. & Epel, D. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1915-1919 (1974).
5. Vacquier, V. D. & Brandriff, B. *Dev. Biol.* **47**, 12-31 (1975).
6. Paul, M., Johnson, J. D. & Epel, D. *J. exp. Zool.* **197**, 127-133 (1976).
7. Epel, D. *Expl. Cell Res.* **72**, 74-89 (1972).
8. Mitchison, J. M. & Cummins, J. E. *J. Cell Sci.* **1**, 35-47 (1966).
9. Piatigorsky, J. & Whiteley, A. H. *Biochim. biophys. Acta* **108**, 404-418 (1965).
10. Sheetz, M. P. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4457-4461 (1974).

Calcium uptake during mitosis in the myxomycete *Physarum polycephalum*

THE notion that Ca^{2+} is involved in the regulation of the kinetic and contractile events associated with mitosis in eukaryotic cells is attractive. The contractile proteins, actin and myosin, have been identified in nuclei of *Physarum polycephalum*¹ and their interaction is apparently regulated by Ca^{2+} (ref. 2). The formation of spindle microtubules may also be sensitive to Ca^{2+} (ref. 3), although as yet there is little evidence of Ca^{2+} fluctuations during mitosis. Macropasmodia of the myxomycete *P. polycephalum* are particularly suited to the analysis of events during the cell cycle and mitosis. Synchronous mitoses of large numbers of nuclei proceed against a lowered background of contractile events in the surrounding cytoplasm as cytokinesis does not occur and cyclosis or cytoplasmic streaming slows or ceases during the mitotic period⁴. Extranuclear filaments form in telophase⁵, presumably to participate in nuclear division, but earlier nuclear mitotic events should be amplified. We present evidence here that cyclic uptake and release of Ca^{2+} from the extracellular medium occurs during mitosis in *P. polycephalum*, and that these fluctuations correlate with specific structural and kinetic events in the mitotic nuclei.

Plasmodial Ca^{2+} uptake was monitored by adding $^{45}Ca^{2+}$ to the medium, removing samples of plasmodia at 1-min intervals and assaying the radioactivity accumulated. During the interphase period before mitosis, a rapid and regular cyclic uptake and release of $^{45}Ca^{2+}$ is seen (Fig. 1). The frequency of this process is similar to oscillations in light absorption measured in a small plasmodial segment (Fig. 1 inset) which Sachsenmaier *et al.*⁴ correlated with shuttle streaming. Similar observations on cyclical variation in free cytoplasmic Ca^{2+} concentrations during shuttle streaming in *Physarum* have been made by Ridgway and Durham¹⁰. During mitosis a different pattern of Ca^{2+} uptake is observed (Fig. 1). Although some fluctuations in uptake still occur, the regular periodicity detected in interphase is not apparent. In metaphase there is an outflow of more than half of the $^{45}Ca^{2+}$ accumulated during prophase and uptake is substantially reduced for 3-4 min. During

Fig. 3 Effect of dinitrophenol on the fall in pH of fertilised eggs and procaine-activated eggs. Unfertilised eggs were treated for 30 min with 2.5×10^{-3} M DNP in seawater. Eggs to be fertilised were quickly washed from DNP into seawater (the wash required 5 min), sperm was added and 2 min later DNP was reintroduced. The normal rise in pH occurred but the subsequent drop in pH was blocked. Procaine was added directly to the DNP-treated unfertilised eggs and the elevation of pH immediately occurred. At 75 min the procaine was washed out with seawater containing DNP. The intracellular pH rapidly dropped to the value close to that of untreated eggs. ●, Fertilised eggs; △, procaine-treated unfertilised eggs.



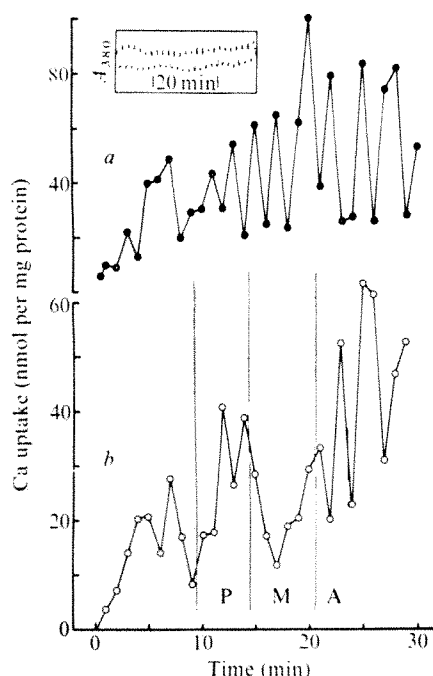


Fig. 1 Time course of $^{45}\text{Ca}^{2+}$ uptake during interphase and mitosis. Macroplasmodia were initiated and cultivated⁶ on filter paper disks at 25 °C using a semi-defined medium⁷ (EDTA, thiamine and biotin were omitted, CaCl_2 was increased to 5.2 mM, and 0.15% yeast extract was included). Transition through the cell cycle was monitored by smearing small fragments in 50% glycerol-ethanol and examining by phase contrast microscopy. $^{45}\text{Ca}^{2+}$ (final concentration $2\ \mu\text{Ci ml}^{-1}$) was added (at 'zero' time) to the growth medium of a macroplasmodium either during interphase before the third mitosis (a) or shortly before it entered the third mitosis (b). $^{45}\text{Ca}^{2+}$ uptake was measured by excising small pieces of plasmodium at intervals after $^{45}\text{Ca}^{2+}$ addition, washing twice by immersion for 10 s in medium containing a 10-fold excess of unlabelled Ca^{2+} , then rinsing briefly in distilled water to remove traces of the medium. The plasmodium was scraped from the filter paper and homogenised in 1 ml of distilled water. Aliquots were taken for liquid scintillation counting and protein assay⁸. The scales are different for the ordinate of the two traces as ^{45}Ca accumulation fluctuates more intensively during interphase. P designates prophase; M, metaphase; and A anaphase as observed by microscopy⁹. The inset shows a tracing of the pattern of A_{380} changes observed in a Varian 635 spectrophotometer when light is directed through a small region of plasmodium growing on an agar-coated piece of Perspex, following the method of Sachsenmaier *et al.*¹. The maximum absorbance change in peaks represents 0.05 units.

anaphase, a large overall accumulation of $^{45}\text{Ca}^{2+}$ once again takes place.

The correlation between Ca^{2+} uptake and mitotic phases is clearer when 5-min pulses with ^{45}Ca are monitored. The results of three such experiments are shown in Fig. 2 and similar patterns were obtained in seven other experiments. Such reproducible cycles in Ca^{2+} uptake were not as evident when the pulse time was shortened to 3 or lengthened to 7.5 min. Peaks in Ca^{2+} uptake are found to coincide with prophase and anaphase while uptake is low during metaphase. The time course of Ca^{2+} uptake during metaphase (Fig. 2 inset) indicates that in the 5-min pulse period, $^{45}\text{Ca}^{2+}$ is initially taken up but is then released back into the medium; in prophase and anaphase, by contrast, uptake is continuous and approximately linear with time. Uptake of Ca^{2+} during interphase is more irregular as the initial 5-min period in Fig. 1 indicates.

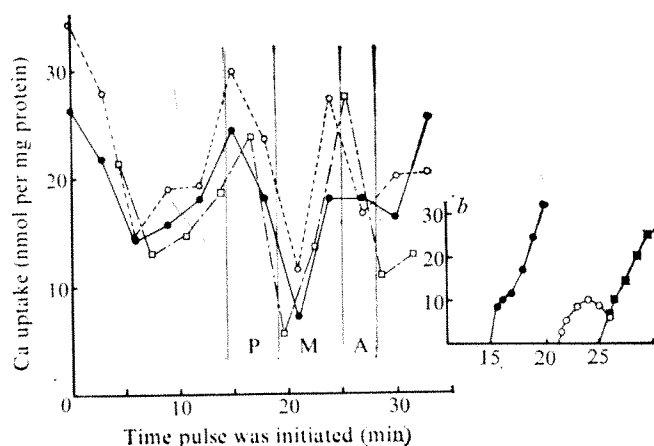
The large amplitude cycles of $^{45}\text{Ca}^{2+}$ uptake are not reflected in fluctuations in the total amount of Ca^{2+} present in the plasmodia. Samples taken during mitosis gave a range of values from 233 to 254 nmol Ca^{2+} per mg protein when measured by atomic absorption spectroscopy, showing no correlation with the pulse experiments. These results show that the cyclical uptake of Ca^{2+} during mitosis may be into particular pool(s)

within the plasmodium, rather than being due to generalised uptake and release of a large fraction of cellular Ca^{2+} and that this pool is not in equilibrium with the major pool of Ca^{2+} present in the plasmodium. Although mitochondria¹¹, cytoplasmic vesicles¹², and endoplasmic reticulum¹³ have been identified as sites for Ca^{2+} sequestration in *Physarum*, we have not so far been able to identify a specific 'mitotic' pool of Ca^{2+} in nuclei, mitochondria or other particulate fractions of the protoplasm.

We have found that Ca^{2+} influx in microplasmodia is largely energy dependent, while efflux is stimulated by KCN. A similar response is observed in the yeast *Schizosaccharomyces pombe*¹⁴. To determine whether the fluctuations observed in Ca^{2+} uptake during mitosis are related to variations in respiratory energy supply, oxygen uptake rates during mitosis were examined. The pattern observed (Fig. 3) is almost identical to that for Ca^{2+} uptake. If the 5-min pulse period for Ca^{2+} uptake is taken into account, the coincidence of the curves could indicate that the changes in O_2 uptake precede those in Ca^{2+} uptake. Whether this relationship is a direct consequence of mitochondrial Ca^{2+} uptake or is related to respiratory energy production is not certain although the effect of caffeine on the motility of plasmodial fragments¹⁵ suggests that a caffeine-sensitive reticulum system is involved in protoplasmic motility. Energy-dependent uptake of Ca^{2+} could involve an active transport system in the plasma membrane, energy-linked endocytosis, or Ca^{2+} sequestration by intracellular organelles, all of which may depend on respiratory energy.

How might the movement of Ca^{2+} into and out of *Physarum* plasmodia be related to the events of mitosis? In heart and smooth muscle cells, an influx of external Ca^{2+} is believed to trigger a release of Ca^{2+} from intracellular stores¹⁶. In sea-urchin eggs, a vesicular system associated with the mitotic apparatus may regulate the local concentration of Ca^{2+} ions¹⁷. It is thus possible that the prophase and anaphase peaks of Ca^{2+} uptake we observe trigger Ca^{2+} release in or near nuclei, thus initiating or in some other way regulating chromosomal movement. During metaphase, when microtubules proliferate and chromosomal movements temporarily cease, a lower rate of influx of

Fig. 2 Uptake of $^{45}\text{Ca}^{2+}$ during mitosis in *P. polycephalum*. During the third synchronous mitosis (24–26 h after initiation), sectors of plasmodium on filter paper (approximately $1 \times 2\text{ cm}$) were removed and placed on a 200- μl drop of medium containing $^{45}\text{Ca}^{2+}$ ($2\ \mu\text{Ci ml}^{-1}$) at the times indicated. After incubation for 5 min at 25 °C the sector was treated as described in Fig. 1 and assayed for radioactivity and protein. a, $^{45}\text{Ca}^{2+}$ uptake observed in three separate experiments (■, ●, ○) during mitosis. b, Time course of $^{45}\text{Ca}^{2+}$ uptake over the 5-min pulse period during prophase (●), metaphase (○), and anaphase (■). Uptake over 5 min during interphase is shown in Fig. 1a. The time course of uptake was reproducible for each phase of the mitotic cycle in three experiments, although data is given here for a single experiment. The zero points for calcium uptake in (b) represent the times when the segments were placed on labelled medium.



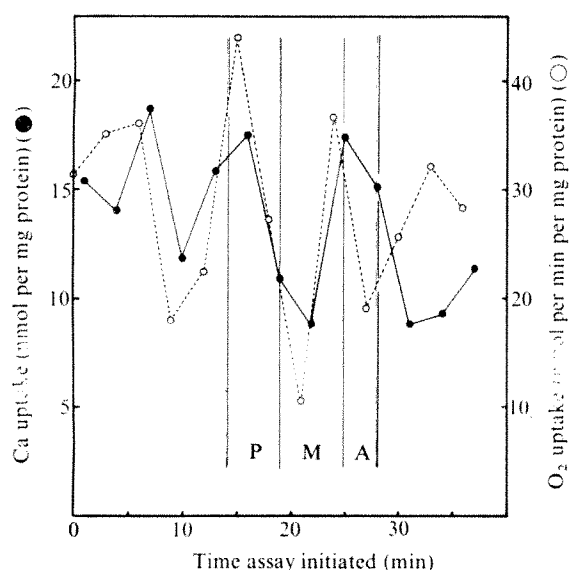


Fig. 3 Comparison of oxygen and $^{45}\text{Ca}^{2+}$ uptake during mitosis. Segments of plasmodium (approximately 1 cm^2) were excised from a macroplasmidium during mitosis and the plasmodial segment was removed from its filter paper support by immersing in growth medium and gently prising it free. Oxygen uptake (\circ) was measured in a Gilson oxygraph with a Clark-type oxygen electrode by placing the plasmodial piece in 3 ml of respiration buffer containing 350 mM mannitol and 10 mM MES buffer at pH 6.0. Each point represents the initial O_2 uptake rate at the time when the segment was placed in the oxygen electrode. Rates were usually linear for at least 1 min. Ca^{2+} uptake (\bullet) was measured in the same plasmodium 1 min later using the 5-min pulse method described in Fig. 2.

Ca^{2+} may be necessary to reduce local Ca^{2+} concentrations so that tubulin polymerisation is promoted³.

This work was supported in part by a grant from the Australian Research Grants Committee.

R. P. HOLMES
P. R. STEWART

Department of Biochemistry,
Faculty of Science,
Australian National University,
Canberra ACT 2600, Australia

Received 27 July; accepted 23 August 1977.

1. Le Sturgeon, W. M., Forer, A., Yang, Y., Bertram, J. S. & Rusch, H. P. *Biochim. biophys. Acta* **379**, 529–552 (1975).
2. Nachmias, V. T. & Asch, A. *Biochemistry* **15**, 4273–4278 (1976).
3. Weisenberg, R. C. *Science* **177**, 1104–1105 (1972).
4. Sachsenmaier, W., Blessing, J., Brauser, B. & Hansen, K. *Protoplasma* **77**, 381–396 (1973).
5. Ryser, U. *Zellforsch. Mikrosk. Anat.* **110**, 108–130 (1970).
6. Guttus, E. & Guttus, S. *Meth. Cell Physiol.* **1**, 43–54 (1964).
7. Carlile, M. J. *Meth. Microbiol.* **4**, 237–265 (1971).
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).
9. Mohberg, J. & Rusch, H. P. *J. Bact.* **97**, 1411–1418 (1969).
10. Ridgway, E. B. & Durham, A. C. H. *J. Cell Biol.* **69**, 223–226 (1976).
11. Nicholls, T. J. *J. Cell Sci.* **10**, 1–14 (1972).
12. Etienne, E. J. *J. Cell Biol.* **54**, 179–184 (1972).
13. Kato, T. & Tonomura, Y. *J. Biochem.* **81**, 207–213 (1977).
14. Boutry, M., Foury, F. & Goffeau, A. *Biochim. biophys. Acta* **464**, 602–612 (1977).
15. Matthews, L. J. *J. Cell Biol.* **72**, 502–505 (1977).
16. Berridge, M. J. *Adv. Cyclic Nucleotide Res.* **6**, 1–98 (1975).
17. Harris, P. *Exp Cell Res.* **94**, 409–425 (1975).

Killed *Listeria monocytogenes* vaccine becomes protective on addition of polyanions

In the interaction between macrophages and intracellular parasites, microbial components which prevent phagosome-lysosome fusion^{1,2} have a decisive role. The resistance to facultative intracellular parasite *Listeria monocytogenes* is a form of cell-mediated immunity³, as it can be passively transferred with viable lymphoid cells⁴. Resistance to *listeria* infection can be induced by sublethal numbers of

Table 1 Resistance to infection following treatment with DS 500 and immunisation with dead *listeria*

No. of bacteria	DS 500 dose (mg per kg body weight)				
	50	25	12.5	5	None
10^9	3/4*	6/6	5/6	4/6	0/6
10^8	6/6	6/6	0/6	0/6	0/6
10^7	0/6	0/6	0/6	0/6	0/6
None (saline)	0/6	0/6	0/6	0/6	0/6

Groups of BALB/c mice were injected i.p. with DS 500 or saline 1 d before i.p. injection of heat-killed (56°C for 60 min) *listeria* or saline. Seven days later all animals were challenged i.p. with 50 LD_{50} *listeria*. The numbers of survivors on the numbers of mice challenged were recorded 2 weeks after challenge.

*In this group two mice died after immunisation.

viable *listeria*^{5,6}. Induction of resistance by non-viable *listeria*, however, is effective only after repeated injections of *listeria* preparations in combination with lipopolysaccharides⁷. Lipopolysaccharide is cytotoxic against macrophages^{8,9}, suggesting that the difference in processing of viable and dead microorganisms by macrophages might explain the induction of resistance by vaccine of live and not of dead bacteria. Impairing macrophage activity might result in a processing of dead *listeria* advantageous for the induction of resistance. We show here that killed *L. monocytogenes* vaccine becomes protective when the polyanions dextran sulphate (DS 500, molecular weight 500,000, Serva) and suramin (Bayer) are added. These polyanions are known to inhibit phagosome-lysosome fusion in macrophages^{2,10}. Moreover, dextran sulphate is a potent adjuvant for both humoral¹¹ and cell-mediated responses¹². The latter might be particularly important in view of the requirement of cell-mediated immunity for resistance to *listeria*³.

BALB/c mice were injected intraperitoneally (i.p.) with graded doses of DS 500 or suramin and 1 d later with different numbers of heat-killed *listeria*. The challenge injection of $50 \times \text{LD}_{50}$ (dose causing 50% killing) was given i.p. 7 d later. Pretreatment with DS 500 allowed induction of resistance with 10^9 and 10^8 dead *listeria* (Table 1). With the latter numbers of bacteria protection could only be induced following high doses of DS 500. The dose of 50 mg DS 500 per kg body weight probably damaged the phagocytic system to such a degree that 10^9 *listeria* were badly tolerated as an immunising dose.

Doses of DS 500 over 50 mg per kg in combination with 10^7 *listeria* did not result in protection. The protection afforded by suramin treatment was slightly less (Table 2). Complete protection was only found in the combination of highest doses suramin and *listeria*. But again one animal did not survive the immunisation.

The doses of suramin required to give protection in addition to injections of dead *listeria* are about 10 times as high

Table 2 Resistance to infection after treatment with suramin and immunisation with dead *listeria*

No. of bacteria	Suramin dose (mg per kg body weight)					
	500	300	150	50	15	None
10^9	5/5*	5/6	5/6	4/5	4/5	0/3
10^8	4/6	2/6	1/6	0/4	0/4	0/3
10^7	0/6	0/6	0/6	ND	ND	ND
None (saline)	0/6	0/6	0/6	0/6	0/3	0/3

Experimental details as for Table 1. ND, Not done.

*In this group one mouse died after immunisation.

as those of DS 500. A similar ratio of activities was found for *in vitro* inhibition of phagosome-lysosome fusion where the optimal inhibiting doses of suramin and DS 500 were respectively 200 and 30 µg (M. J. de Reuver, personal communication). The influence of the interval between DS 500 and immunising injection was studied using 30 mg DS 500 per kg (Table 3). With an interval of 8 h between DS 500 and 10⁹ listeria all mice died after immunisation (data not shown). Reduction of the dose of DS 500 to 15 mg per kg, followed by 10⁹ bacteria resulted in complete protection (Table 3). This suggests that the interval between injections of the toxic products DS 500 and dead listeria should not be too small if high doses of both are used.

Host immunity and survival of intracellular parasites depend on a delicate balance of host-parasite interplay. The ingestion of previously killed intracellular parasites by macrophages leads to rapid and extensive phagosome-lysosome fusion¹³, but no resistance to infection. Resistance to intracellular growing bacteria can in general only be achieved by immunisation with viable bacteria. Impairing macrophage function by DS 500 resulted in increased susceptibility to infection with listeria^{14,15}. These results suggest that although macrophages are host cells for listeria,

Table 3 Effect on resistance to infection of the interval between DS 500 treatment and immunisation with dead listeria

No. of bacteria	DS 500 treatment on				No treatment
	Day -2	Day -1	-8 h	Day -1	
10 ⁹	5/5*	6/6	6/6	5/5*	0/6
10 ⁸	3/6†	6/6	2/6	0/6	0/6
None (saline)	0/6	0/6	0/6	0/6	0/6

Groups of BALB/c mice were injected i.p. with 30 mg DS 500 per kg before or after injection of dead listeria or saline (controls). The groups receiving injections with the 8 h interval were treated with 15 mg DS 500 per kg. Other details as for Table 1.

*In these groups one mouse died after immunisation.

†One mouse died within the 2 weeks recording period after the challenge, but two mice died 1 week later.

the degree of processing of the listeria is decisive for the induction of resistance. The probably incomplete processing of viable listeria by macrophages seems to be a prerequisite for the induction of resistance. The more complete digestion of non-viable listeria does not result in protection.

The finding that lower numbers of non-viable bacteria (10⁸) needed high doses of polyanions to obtain protection supports the hypothesis that the degree of digestion of bacteria should be limited in order to induce resistance. Experiments are in progress to isolate the fraction(s) of listeria responsible for the induction of resistance.

C. VAN DER MEER
F. M. A. HOFHUIS
J. M. N. WILLERS

Department of Immunology,
Laboratory of Microbiology,
Catharijnesingel 59,
Utrecht,
The Netherlands

Received 28 June; accepted 7 August 1977.

- Goren, M. B., Brokl, O. & Schaefer, W. B. *Infect. Immun.* 9, 142-149 (1974).
- Goren, M. B., Hart, P. D'Arcy, Young, M. R. & Armstrong, J. A. *Proc. natn. Acad. Sci. U.S.A.* 73, 2510-2514 (1976).
- Mackness, M. B. *J. exp. Med.* 116, 381-406 (1962).
- Mackness, M. B. *J. exp. Med.* 129, 973-992 (1969).
- Hasenclever, H. F. & Karakawa, W. W. *J. Bact.* 74, 584-586 (1957).
- Kerkhaert, J. A. M., Hofhuis, J. M. A. & Willers, J. M. N. *Immunology* 32, 1027-1032 (1977).
- Rodriguez, G. E., McClatchy, J. K. & Campbell, P. A. *Infect. Immun.* 10, 1163-1169 (1974).
- Kessel, R. W. I. & Braun, W. *Aust. J. exp. Biol. med. Sci.* 43, 511-522 (1965).

- Bradley, S. G. & Watson, D. W. *Proc. Soc. exp. Biol.* 117, 570-572 (1964).
- Hart, P. D'Arcy & Young, M. R. *Nature* 256, 47-49 (1975).
- Diamantstein, T., Wagner, B., Beyse, I., Odenwald, M. V. & Schulz, G. *Eur. J. Immun.* 1, 340-343 (1971).
- McCarthy, R. E., Arnold, L. W. & Babcock, G. F. *Immunology* 32, 963-974 (1977).
- Armstrong, J. A. & Hart, P. D'Arcy *J. exp. Med.* 134, 713-740 (1971).
- Hahn, H. *Infect. Immun.* 10, 1105-1109 (1974).
- Hahn, H. & Bierther, M. *Infect. Immun.* 10, 1110-1119 (1974).

Non-infectious virus induces cytotoxic T lymphocytes and binds to target cells to permit their lysis

MUCH current interest is focused on the involvement of expressed products of the major histocompatibility (*H-2*) gene complex, especially those associated with the K and D regions of *H-2*, in inducing cell-mediated immune responses and in permitting recognition and killing by cytotoxic thymus-derived lymphocytes (CTL). Observations in many laboratories have led to the generalisation that syngeny between stimulator and target cells at the K and/or D region of *H-2* is required for CTL activity to virus-infected cells¹⁻³, minor histocompatibility antigens⁴, the male H-Y antigen⁵ and chemically modified syngeneic cells⁶⁻⁹. This study asks three questions related to the genetic restriction of induction and expression of CTL: (1) can non-infectious virus efficiently induce CTL, (2) can non-infectious virus absorbed to target cells render them susceptible to killing by CTL, and (3) can non-infectious virus absorbed onto or chemically linked to cells induce CTL and does using allogeneic or xenogeneic carrier cells affect this induction?

Two major models have been proposed to explain the involvement of *H-2* antigens and foreign antigens, such as viral antigens, in recognition of target cells by CTL. The 'dual recognition' model proposes recognition of *H-2* and foreign antigens on the target cell surface by two separate receptors on the CTL while the 'adaptor-antigen complex' or 'modified self' model postulates a physical association between *H-2* antigens and the foreign antigen on the cell surface to form hybrid antigens containing elements of self and non-self^{2,10,11}.

Using 6/94 virus, a para-influenza type 1 virus antigenically related to Sendai virus, it has been shown that although only virus-infected cells histocompatible with the effector T cells can serve as susceptible target cells, syngeneic, allogeneic, and even xenogeneic virus-infected cells are capable of inducing virus-specific cytotoxicity¹². But, as the virus used in the previous study retained some infectivity, it was possible that the allogeneic and xenogeneic virus-infected cells used to induce CTL released

Table 1 Characterisation of anti-6/94 effector cells

Treatment	Live 6/94	BPL 6/94
Hold on ice	58	39
4 h at 37 °C	32	31
Nylon column nonadherent	58	48
Guinea pig serum (C)	54	39
Anti-Thy 1.2 + C	4	6
Rabbit α mouse Ig + C	ND	70
Rabbit α 6/94 + C	ND	34

BALB/c mice were injected i.p. with 100 µg of live or BPL-treated 6/94 virus. On day 5, spleen cell suspensions were prepared, treated as indicated and assayed against P815Y target cells incubated for 90 min with BPL-treated 6/94 virus immediately before labelling with Na₂⁵¹CrO₄. Cytotoxicity values are % specific lysis for an 18-h assay and spleen cell: target cell ratio of 50:1. Spontaneous release was 47%. Treatment with anti-Thy 1.2 serum removed 38% of the cells. ND, Not determined.

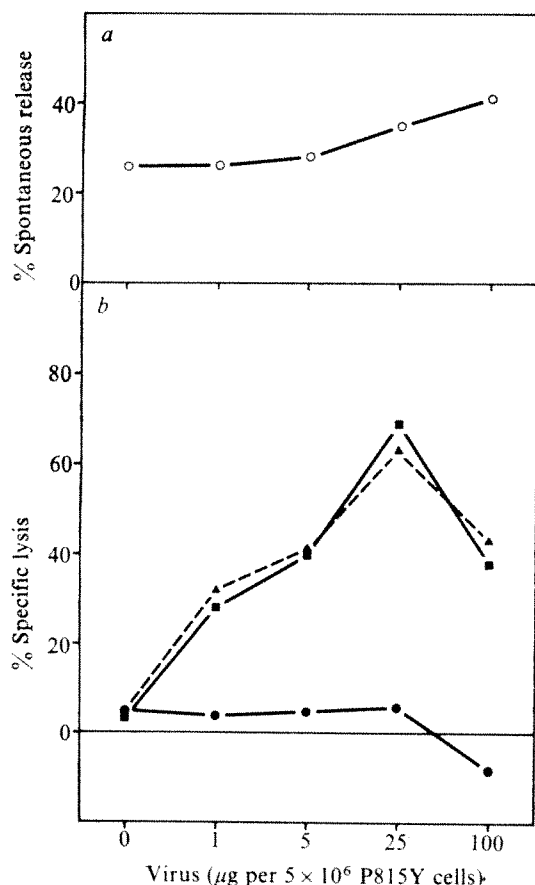


Fig. 1 Induction of anti-viral cytotoxic thymic-derived lymphocytes (CTL) by infectious and non-infectious 6/94 virus with assay on target cells pre-incubated with different amounts of non-infectious 6/94 virus. BALB/c ($H-2^d$) mice were injected i.p. with 100 μg of live 6/94 virus in phosphate buffered saline (Δ), with 100 μg of non-infectious 6/94 virus treated with BPL¹⁸ and verified negative for infectivity by serial egg passage and incubation with susceptible cells in culture (\blacksquare), or uninjected (\bullet). On day 5 spleens were removed and assays were performed for 18 h against P815Y ($H-2^d$) cells incubated with 0, 1, 5, 25 or 100 μg BPL-treated 6/94 virus (5×10^6 cells in 2 ml, 37°C , 90 min, washed and then labelled with ^{51}Cr chromate). All assays were carried out using lymphocyte-target cell ratios of 200:1, 67:1, and 22:1. Only data for 67:1 are shown for simplicity. *a*, Shows spontaneous release in 18 h as a % of total incorporation. *b*, Cytotoxicity in terms of % specific lysis.

virus which then infected host cells; the measured cytotoxicity might then have been induced by virus-infected (syngeneic) host cells.

To compare the immunogenicity of live and non-infectious 6/94 virus, BALB/c mice ($H-2^d$) were injected intraperitoneally (i.p.) with 100 μg of either infectious or β -propiolactone (BPL) inactivated¹⁷ 6/94 virus. Lymphocytes from these mice and uninjected controls were then assayed on day 5 against P815Y cells ($H-2^d$) incubated with 0, 1, 5, 25 or 100 μg of BPL inactivated 6/94 virus for 90 min before labelling with radioactive chromate. Figure 1*b* demonstrates that live and non-infectious 6/94 virus induced the same amount of cytotoxicity and that P815Y cells were made susceptible to killing when incubated with as little as 1 μg of non-infectious virus just before labelling. The rate of spontaneous release is increased as more virus is absorbed to the target cells (Fig. 1*a*), indicating that optimum assay conditions are a compromise between more virus per cell and increasing spontaneous release.

The cytotoxicity induced by both live and non-infectious 6/94 virus was shown to be mediated by CTL (Table 1). The cytotoxicity induced by both live and non-infectious 6/94 virus was not labile at 37°C as is true for 'natural'

killer cells^{13,14}. The activity was largely abolished by anti-Thy 1.2 serum and complement and was not diminished by removal of cells adherent to nylon wool or of cells killed by anti-mouse immunoglobulin and complement; it seems therefore that lysis is mediated by a T cell and not by a B cell or macrophage.

Non-infectious 6/94 virus was absorbed on to (1) P815Y cells which are $H-2^d$ like BALB/c; (2) EL4 cells which are $H-2^b$ and allogeneic to BALB/c, and (3) bovine kidney cells (MDBK) which are xenogeneic to BALB/c; all cells were washed extensively to remove any free virus, and injected i.p. into BALB/c mice.

Table 2 shows that 6/94 virus absorbed to all three cell types induced cytotoxicity against P815Y cells sensitised with either infectious or BPL-treated 6/94 virus. The 6/94 virus, like the well characterised Sendai virus, adsorbs rapidly to the cell surface, fusing its envelope with the cell membrane as a normal event in penetration¹⁵; once this occurs, therefore, virus would not be expected to desorb from the cells used for the injection of mice. To make it even less likely that the effective immunogen is free virus or virus that desorbed from the injected cells and reabsorbed to host cells, the xenogeneic MDBK cell and 6/94 virus were covalently linked using four additions at 10 min intervals of freshly diluted *bis*-diazotized benzidine (BDB) solution¹⁶. The results in Table 2 show that 6/94 virus covalently linked to xenogeneic cells is still able to induce cytotoxicity against 6/94 determinants on P815Y cells.

As was shown in work with CTL induced with 6/94 virus¹², we did not observe significant cell-mediated cytotoxicity of allogeneic EL4 cells or xenogeneic bovine kidney cells adsorbed with 6/94 virus. But, the lack of cytotoxicity by CTL on allogeneic and xenogeneic targets was not due to lack of expression of viral determinants or to an inability of the cells to be lysed, because all these cells were lysed by rabbit anti-6/94 virus antiserum and guinea pig complement (Table 3). This was also true for MDBK cells to which virus had been bound covalently. The P815Y and EL4 cells did not survive the BDB coupling conditions and hence were not tested.

In summary, our results show that non-infectious virus is immunogenic both as free virus and when fused with cells of the same or different $H-2$ as the host. Non-infectious virus covalently linked to xenogeneic cells also was immunogenic. These results make less likely, but *in vivo* experiments can never completely exclude, the possibility that macrophages process free or cell-bound virus and present viral determinants on a host $H-2$ background. Immunogenic determinants in these experiments are contained in the 6/94 virus and do not involve a complex with structures present on the cell carrying the virus. This finding and

Table 2 Induction of CTL with non-infectious 6/94 virus passively adsorbed to or covalently linked with *bis*-diazobenzidine to various cells

Immunising cells	Target cells		
	P815Y	P815Y (BPL 6/94)	P815Y (live 6/94)
None	1	6	9
P815Y (BPL 6/94)	8	39	47
EL4 (BPL 6/94)	5	35	26
MDBK (BPL 6/94)	—7	75	55
MDBK (BDB coupled BPL 6/94)	6	55	51

BALB/c mice were immunised with the indicated cells and assayed on day 5 for 18 h at 150:1 spleen cells: target cell against P815Y incubated with live or BPL-treated 6/94 virus or left without virus. Values are % specific lysis for the mean of triplicate determinations. Standard error of the mean was always less than 2 percentage points or 10% of the mean. Spontaneous release was 31% for P815Y, 54% for P815Y (BPL 6/94) and 48% for P815Y (live 6/94).

Table 3 Lysis of various target cells with anti-6/94 antibody and complement

	Uninfected	+ Live 6/94	+ BPL 6/94	Coupled BPL 6/94
P815Y	25	52	70	—
EL4	2	69	75	—
MDBK	2	42	34	47

For each target, 2×10^4 ^{51}Cr labelled cells in 50 μl were mixed with 50 μl 1:4 or 1:20 dilutions of rabbit anti-6/94 antibody, 50 μl of a 1:9 dilution of guinea pig complement was added. The mixture was incubated for 1 h at 37 °C, and one half the supernatant was counted. Values are % specific lysis for 1:20 initial dilutions of antiserum. Spontaneous release ranged from 3% to 24% for the ten different targets.

the restriction of lysis by CTL of histocompatible target cells displaying virus argues for a view of CTL possessing separate receptors for H-2 and for virus determinants. Thus, while it seems that both receptors must be bound for CTL activity to be expressed, CTL activity is apparently induced by viral determinants alone without involvement of histocompatibility antigens.

We thank Mr David Ficcadenti for technical assistance. This work was supported in part by grants from the US NCI, the National Institute of Communicative and Neurological Diseases and Stroke, and by the National Multiple Sclerosis Society.

JON C. PALMER
LEON J. LEWANDOWSKI
DAVID WATERS

Wistar Institute of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, Pennsylvania 19104

Received 24 January; accepted 20 June 1977.

- 1 Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701–702 (1974).
- 2 Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. *Transplant. Rev.* **29**, 89–124 (1976).
- 3 Blanden, R. V. *et al.* *Nature* **254**, 269–270 (1975).
- 4 Bevan, M. J. *exp. Med.* **142**, 1349–1364 (1975).
- 5 Gordon, R. D., Simpson, E. & Samelson, L. E. *J. exp. Med.* **142**, 1108–1120 (1975).
- 6 Shearer, G. M. *Eur. J. Immun.* **4**, 527–533 (1974).
- 7 Shearer, G. M., Rehn, T. G. & Schmitt-Verhulst, A.-M. *Transplant. Rev.* **29**, 222–248 (1976).
- 8 Rehn, T. G., Shearer, G. M., Koren, H. S. & Inman, J. K. *J. exp. Med.* **143**, 127–142 (1976).
- 9 Pfizenmaier, K., Starzinski-Powitz, A., Rodt, H., Röllinghoff, M. & Wagner, H. *J. exp. Med.* **143**, 999–1004 (1976).
- 10 Schrader, J. W. & Edelman, G. M. *J. exp. Med.* **143**, 601–614 (1976).
- 11 Doherty, P. C., Götze, D., Trinchieri, G. & Zinkernagel, R. M. *Immunogenetics* **3**, 517–524 (1976).
- 12 Lewandowski, L. J., Gerhard, W. U. & Palmer, J. C. *Infect. Immun.* **13**, 712–721 (1976).
- 13 Herberman, R. B., Nunn, M. E., Holden, H. T. & Lavrin, H. *Int. J. Cancer* **16**, 230–239 (1975).
- 14 Kiessling, R., Petranyi, G., Klein, G. & Wigzell, H. *Int. J. Cancer* **17**, 275–281 (1976).
- 15 Watkins, J. F. *Methods Virol.* **5**, 1–32 (1971).
- 16 Likhite, V. & Schow, A. in *Methods in Immunology and Immunochemistry* **1** (eds Williams, C. A. & Chase, M. W.) 164–167 (Academic, New York, 1967).
- 17 Neff, J. M. & Enders, J. F. *Proc. Soc. exp. Biol. Med.* **127**, 260–267 (1968).

Effects of a nonapeptide FTS on lymphocyte differentiations *in vitro*

BACH *et al.*^{1,2} have reported the amino acid sequence of a putative thymic hormone isolated from pig serum³, a nonapeptide called FTS (*facteur thymique serique*). Natural and synthetic FTS showed high activity in the rosette test used as a bioassay^{1,2}. Bioassays for thymic hormones⁴, however, can be spuriously triggered by many substances of non-thymic origin⁵, a finding probably related to mediation by a cyclic AMP second signal⁶. Early steps in B-cell differentiation are also mediated by cyclic AMP (refs 6–9) and comparison of induction of T cells and B cells from committed precursor cells *in vitro* (the dual induction assay) enables a distinction to be made between selective and non-selective inducing agents^{7,8}. This is exemplified by induction studies in the mouse with

thymopoietin^{10,11} and ubiquitin^{7,12,13}. Bovine thymopoietin selectively induces T-cell differentiation and actually inhibits B-cell differentiation⁹. By contrast, ubiquitin is non-selective, inducing both T- and B-cell differentiation⁷. Ubiquitin at high concentrations does not induce differentiation whereas these high concentrations do not impair induction by thymopoietin or other agents^{8,14}. Another feature of induction by ubiquitin is its inhibition by the β -adrenoceptor-blocking drug propranolol, which is without effect on induction by thymopoietin⁷. We have evaluated synthetic FTS in the dual induction system in the chicken to determine whether it showed inductive selectivity appropriate to a thymic hormone, and have found that it induced non-selective differentiation of both T cells and B cells and closely resembled ubiquitin in its activity.

The nonapeptide, $\text{NH}_2\text{-Gln-Ala-Lys-Ser-Gln-Gly-Gly-Ser-Asn-COOH}$, was synthesised using solid-phase methodology (Peninsula, San Carlos, California). The purified peptide showed a single spot in four thin-layer chromatography systems and on high voltage electrophoresis at pH 6.5. After acid hydrolysis, the amino acid composition showed appropriate molar ratios. Ubiquitin was isolated as described previously⁷. The dual induction assay for T-cell and B-cell differentiation in fractionated bone marrow cells from newly hatched chickens was carried out as described previously⁸. Synthetic FTS induced both T-cell and B-cell differentiation (Fig. 1) with high dose inhibition at the higher concentrations ($100 \mu\text{g ml}^{-1}$). B-cell induction was only obtained consistently at a concentration of $1 \mu\text{g ml}^{-1}$, whereas T-cell induction was obtained over the broader concentration range of 1 ng ml^{-1} to $1 \mu\text{g ml}^{-1}$. More detailed information about the mechanism of induction by FTS was obtained from inhibition studies, the inhibiting agent being added at the same time as the inducing agent (Table 1). Inductive concentrations of FTS ($1 \mu\text{g ml}^{-1}$) were inhibited by inhibitory concentrations of ubiquitin ($100 \mu\text{g ml}^{-1}$), and inductive concentrations of ubiquitin ($0.5 \mu\text{g ml}^{-1}$) were inhibited by inhibitory concentrations of FTS ($100 \mu\text{g ml}^{-1}$). Inductive concentrations of both FTS and ubiquitin were inhibited by propranolol 10^{-5} M , which did not affect induction by cyclic AMP.

Fig. 1 Relationship between concentration of synthetic FTS and induction of Bu-1⁺ (a) or Th-1⁺ cells (b) in fractionated bone marrow from newly hatched chickens. Bone marrow cells from newly hatched chickens were fractionated by ultracentrifugation on a discontinuous bovine serum albumin gradient, and cells from the lighter layers were incubated with or without various concentrations of FTS. After incubation for 2.5 h, cells were washed and tested in the cytotoxicity assay using anti-Bu-1 and anti-Th-1 alloantisera and a mixed avian-mammalian complement system as described previously⁸. FTS induced both Bu-1⁺ and Th-1⁺ cells and showed high-dose inhibition (six experiments, each line represents one experiment).

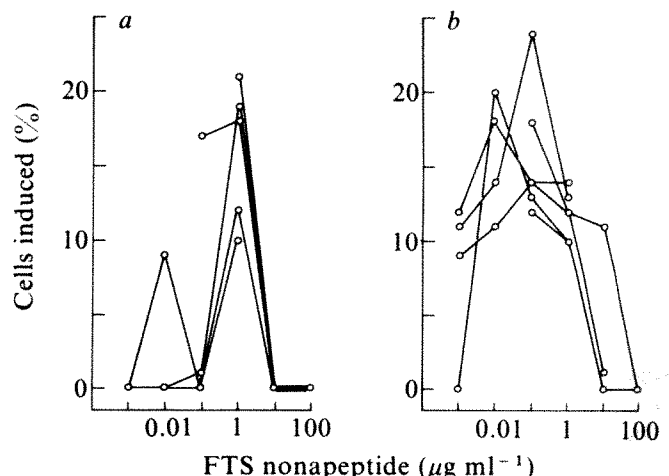


Table 1 Inhibitor studies with synthetic FTS and ubiquitin in the dual induction assay using chicken cells

Inducing agent	Inhibiting agent	Cells induced (%)	
		B cells (Bu-1 ⁺)	T cells (Th-1 ⁺)
FTS 1 µg ml ⁻¹	—	11,16	11,19
FTS 1 µg ml ⁻¹	Ubiquitin 100 µg ml ⁻¹	1,0	2,0
—	Ubiquitin 100 µg ml ⁻¹	1,0	0,0
Ubiquitin 0.5 µg ml ⁻¹	—	9,16	20,16
Ubiquitin 0.5 µg ml ⁻¹	FTS 100 µg ml ⁻¹	0,0	0,0
—	FTS 100 µg ml ⁻¹	0,0	0,0
FTS 1 µg ml ⁻¹	—	15,22	18,20
FTS 1 µg ml ⁻¹	Propranolol 10 ⁻⁵ M	0,0	0,0
Ubiquitin 0.5 µg ml ⁻¹	—	19,11	15,17
Ubiquitin 0.5 µg ml ⁻¹	Propranolol 10 ⁻⁵ M	0,0	0,0
Cyclic AMP 0.2 × 10 ⁻³ M	—	18,8	21,17
Cyclic AMP 0.2 × 10 ⁻³ M	Propranolol 10 ⁻⁵ M	19,13	11,14

High concentrations of FTS or ubiquitin inhibited inductive concentrations of the other. Inductive concentrations of either were inhibited by propranolol.

Each figure represents the mean of duplicate or triplicate tubes in a single experiment.

The amino acid sequence of FTS (refs 1, 2) differs from that of thymopoietin^{10,11}, and the biological activity of these two peptides in the dual induction assay was also quite different. Bovine thymopoietin was inactive in the dual induction assay in the chicken⁸. Unlike bovine thymopoietin⁷, porcine FTS was both active and non-selective in the dual induction assay using chicken cells and was inhibited by propranolol. By contrast, our findings show a close similarity between the activity of FTS and that of ubiquitin, in that both caused non-selective induction in the dual induction assay; induction by both was inhibited by the β -adrenoceptor-blocking agent, propranolol; and in that high concentrations of each inhibited induction by inductive concentrations of the other. This finding is especially noteworthy because the primary structure of FTS does not closely resemble that of ubiquitin^{12,13}; the similarity of tertiary structure of the active sites implied by the similarities of activity of these two peptides must be generated by folding of chains of dissimilar primary amino acid sequence. Evolutionary convergence rather than modification of a common ancestral gene would be the likely cause of this relationship¹⁴.

We conclude that FTS is a biologically active molecule that induces lymphocyte differentiation, and that the biological activity of FTS mimics ubiquitin, a polypeptide which induces lymphocyte differentiation non-selectively by reason of having an adrenomimetic active site. Our observations indicate that FTS is probably not a thymic hormone, but they do confirm that this circulating non-peptide can induce lymphocyte differentiation. These findings do not detract from interest in this factor. Rather, they raise important questions concerning its origin in the body and a possible physiological role in regulating lymphocyte differentiation and maturation.

This work was supported by the US Public Health Service. We thank Dr E. A. Boyse for reviewing the manuscript and Dr D. H. Schlesinger for the amino acid analysis. A.B. is a graduate student with the Biology Department, GSAS, New York University.

ANN BRAND
DOUGLAS G. GILMOUR

Department of Microbiology,
New York University Medical Center,
New York, New York 10016

GIDEON GOLDSTEIN*

Memorial Sloan-Kettering Cancer Center,
New York, New York 10021

Received 20 June; accepted 8 August 1977.

*Present address: Ortho Pharmaceutical Corporation, Raritan, New Jersey 08869.

1. Bach, J. F., Dardenne, M., Pleau, J. M. & Rosa, J. C. *hébd. Acad. Sci. Paris* **283**, 1605–1607 (1977).

2. Bach, J. F., Dardenne, M. & Pleau, J. M. *Nature* **266**, 55–58 (1977).

3. Bach, J. F., Dardenne, M., Pleau, J. M. & Bach, M. A. *Ann. N.Y. Acad. Sci.* **249**, 186–210 (1975).
4. Komuro, K. & Boyse, E. A. *Lancet* **i**, 740–743 (1973).
5. Scheid, M. P. *et al. J. exp. Med.* **138**, 1027–1032 (1973).
6. Scheid, M. P., Goldstein, G., Hammerling, U. & Boyse, E. A. *Ann. N.Y. Acad. Sci.* **249**, 531–540 (1975).
7. Goldstein, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 11–15 (1975).
8. Brand, A., Gilmour, D. G. & Goldstein, G. *Science* **193**, 319–321 (1976).
9. Hammerling, U. *et al. Eur. J. Immun.* **6**, 868–872 (1976).
10. Schlesinger, D. H. & Goldstein, G. *Cell* **5**, 361–365 (1975).
11. Schlesinger, D. H., Goldstein, G., Scheid, M. P. & Boyse, E. A. *Cell* **5**, 367–370 (1975).
12. Schlesinger, D. H. & Goldstein, G. *Nature* **255**, 423–424 (1975).
13. Schlesinger, D. H., Goldstein, G., Scheid, M., Boyse, E. A. & Tregear, G. *Fedn. Proc.* **34**, 551 (1975).
14. Twomey, J. J., Goldstein, G., Lewis, V. M., Bealmeier, P. M. & Good, R. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2541–2546 (1977).
15. Barker, W. C. & Dayhoff, M. O. in *Atlas of Protein Sequence and Structure* (ed. Dayhoff, M. O.) 108–109 (National Biomedical Research Foundation, Washington, DC, 1972).

Expression of MuLV GP71-like antigen in normal mouse spleen cells induced by antigenic stimulation

ALL mouse strains investigated possess cellular DNA sequences which are homologous to the genomes of endogenous C-type particles¹. Also, major envelope glycoproteins of the oncoviruses², such as GP71, are found on the surface of normal cells and they display a considerable polymorphism. An antigen serologically indistinguishable from AKR murine leukaemia virus (MuLV) GP71 is present in normal bone marrow cells of all mouse strains investigated but not in normal spleen cells³. Allogeneic⁴ and mitogenic stimulation^{5,6} of spleen cells have been reported to result in C-type particle synthesis in some but not all mouse strains. We report here that both T and B lymphocytes of mice express a GP71-like antigen on their surfaces if antigenically stimulated.

Goat⁷ or rabbit⁸ antisera prepared against isolated GP71 of Friend leukaemia virus (FLV) were applied to the mouse lymphocytes. In some experiments, a goat antiserum prepared against dissociated feline leukaemia viruses (FeLV) was included⁹. These antisera possess antibodies to type-, group- and interspecies-specific determinants of MuLV GP71 (ref. 10) and thus can react with the major glycoprotein moieties of all C-type murine oncoviruses. The antisera and the normal control sera were thoroughly absorbed with fresh suspension of liver and spleen cells of the mouse strains used in a particular experiment. Fresh rabbit sera served as source of complement. Mishell–Dutton-type cultures of mouse spleen cells activated by sheep red blood cells (SRBC) were used to examine the effect of anti-GP71 antisera on the primary immune response *in vitro*¹¹. The immune responses were assessed by the haemolytic plaque test of Jerne¹² as modified by Mishell and Dutton¹¹. The number of plaque-forming cells (PFC) routinely increases after 3 d of culture and usually reaches an optimum on day 5.

Table 1 shows that the addition of anti-GP71 antiserum to spleen cultures of F₁ hybrid mice (C57B1/6 × DBA/2 = B₆D₂) on day 3 of culture significantly reduced the number of PFC

discovered on day 5 dependent on the presence of active complement. Since the anti-SRBC response is T-cell dependent¹³, either T or B lymphocytes or both may be affected by the treatment. To determine whether or not antibody-producing B cells can thus be affected, cultured cells were treated directly before the plaque assay when all of the antibody-producing B cells were already present.

While the controls treated with normal goat serum (NGS) and complement (C) contained 4,147 PFC per 10^6 recovered cells, treatment with anti-FLV GP71 antiserum and C resulted in a drastic reduction (125 PFC per 10^6 cells = 3%). Plaque-forming cells were similarly reduced by treatment with anti-FeLV antiserum and complement (65 PFC per 10^6 cells = 1.6%). This suggests that the induced antigen is in fact viral GP71 and not simply another membrane structure displaying a chance cross-reactivity. Anti FLV GP71 antiserum reacts predominantly through group-specific determinants on the GP71 molecules of all mouse oncoviruses while anti-FeLV antiserum can only detect their interspecies-specific determinants. Thus, antibody-producing B lymphocytes of $B_6D_2F_1$ mice express sufficient GP71-like antigens on their surface to be eliminated by corresponding antisera and complement.

Examination of a plaque centre cell revealed actual C-type particle synthesis by these antibody-producing cells. Immunological induction of GP71-like viral antigens is, however, also possible in lymphocytes of NIH-Swiss mice. These mice only possess a xenotropic provirus¹⁴.

The following experiments were designed to find out whether or not T-helper cells also express oncoviral antigens on immunological stimulation. For this purpose, C57B1/6 mice were primed and boosted with dinitrophenyl (DNP)-KLH *in vitro* using the method of North and Askonas¹⁵. In secondary spleen cultures derived from those mice and restimulated with DNP-KLH, a considerable IgG immune response is obtained (Table 2). The removal of T-helper cells by treatment with anti-Thy 1 and complement before the culture prevents the immune response which, however, can be reconstituted by addition of specifically antigen-activated T cells¹⁶ (Table 2). These reconstituting T-helper cells, however, are sensitive to anti-FLV GP71 antiserum and complement, indicating that, like immunologically activated B lymphocytes, they also express the GP71-like antigen. This is of particular interest since concanavalin A stimulation of C57B1/6 spleen cells leads to the induced expression of GP71-like antigens without C-type particles synthesis in contrast to DBA/2J or $B_6D_2F_1$ cultures where both occurs (manuscript in preparation).

Our results suggest that both T and B lymphocytes of mice, if antigenically stimulated, either *in vivo* or *in vitro*, display GP71-like antigens on their surfaces where they are accessible to antibodies.

To demonstrate more directly that unstimulated lymphocytes do not express these antigens, the following experiment was performed. Spleen cells of mice primed and boosted with DNP-KLH were treated with normal goat or with goat anti-FLV GP71 antisera and complement before culture. One set of cultures

Table 2 Effect of anti-FLV GP71 antiserum and complement on primed spleen cells of C57B1/6 mice

Treatment before culture	PFC per 10^6 cells (anti-DNP IgG) day 5	% Control
None (control)	4,716	100
T-helpers reduced (TR)	245	5
TR + untreated helpers	4,820	102
TR + treated helpers (NGS + C')	2,498	53
TR + treated helpers (GP71 + C')	789	16

Mice were primed i.p. with 200 μ g of dinitrophenyl (DNP)-KLH (312 DNP groups per 10^6 molecular weight) and 10^9 *Bordetella pertussis* organisms. The mice were boosted with 20 μ g DNP-KLH i.p. 7 d before killing. Spleen cells were cultured directly with DNP-KLH or after treatment with mouse anti-Thy 1 and complement¹⁶. T-helper cells were obtained from C57B1/6 mice primed with KLH and boosted 3 d before use. In reconstitution experiments, 1×10^6 Thy 1 + C'-treated cells were cultured with 4×10^6 helper cells in 0.5 ml of medium. Treatment of helper cells with anti-FLV GP71: 3×10^7 cells, 30 min on ice with 0.5 ml of 1:5 diluted serum. After washing, cells were incubated with 0.5 ml of 1:5 diluted rabbit complement and washed twice.

received DNP-KLH, another SRBC. The secondary immune response to DNP-KLH *in vitro* was practically abrogated by the antiserum treatment (Fig. 1). Although the anti-SRBC response was also reduced, it still was about 100-times higher on a percentage of control basis than the anti-DNP response. This suggests a remarkable specificity of the treatment which indeed seems to predominantly effect antigen-activated lymphocytes (DNP-specific) while unstimulated cell clones (SRBC-specific) are preserved. In principle, an antigen-specific immune suppression seems to be possible in these experimental conditions by applying appropriate antisera against oncoviral antigens such as GP71. Also, using type-specific antisera, it will now be possible to characterise the type of endogenous viruses, the antigens of which

Fig. 1 Specificity of anti-GP71 antisera for antigen-activated lymphocytes. DBA/2 mice were used. The condition of *in vitro* priming and boosting, and antiserum treatment before culture were as described in Table 2. One set of cultures received KLH-activated helper cells and DNP-KLH as antigen, the other received SRBC and TRF on day 2 for reconstitution of T-helper function¹⁷. Samples a-d all underwent T-cell reduction. a, NGS + C' + helper; b, anti-GP71 + C' + helper; c, NGS + C' + helper; d, anti-GP71 + C' + helper. The value for b is 0.3%.

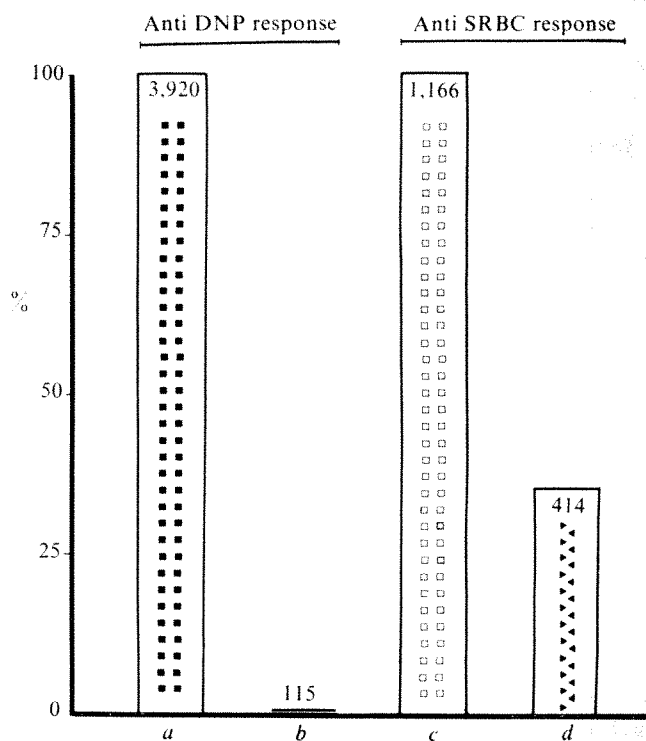


Table 1 Effect of anti-GP71 antiserum and complement on anti-SRBC response *in vitro*

Additions on day 3	PFC per 10^6 cells, day 5 (anti-SRBC IgM)	% Control
None (control)	1,377	100
NGS + complement	1,033	75
Anti GP71 + complement	197	14
Anti GP71 + inactivated complement	1,522	110
Inactivated anti GP71	1,640	119
Inactivated anti GP71 + complement	88	6

5×10^6 spleen cells of $B_6D_2F_1$ mice in 0.5 ml of medium containing 5% FCS were cultured together with 2.5×10^6 SRBC¹¹. On day 3, cultures received 0.1 ml of the respective sera diluted 1:5 and 0.05 ml of undiluted rabbit complement or 0.05 ml of medium. 1.5 ml of the diluted sera had been absorbed for 3 h on ice with suspended cells equivalent to one liver and one spleen of $B_6D_2F_1$ mice. Heat inactivation was performed 30 min incubation at 60 °C.

can be expressed on lymphocyte surfaces of the various mouse strains.

These findings are compatible with the notion that the expression of endogenous viral antigens may generally have a role in cellular proliferation and/or differentiation. Using cells of the immune system might prove quite helpful in studying these questions. All immune reactions are characterised by proliferation and differentiation of the responding lymphocytes. Both events can be induced consecutively and *in vitro* by distinct signals¹⁷. The appearance of endogenous viral antigens in dependence of one or both events can thus be studied in detail.

We thank Dr Werner Schäfer (Max-Planck-Institute for Virus Research, Tübingen) for a gift of antisera and for useful discussions, and Ms R. Weck and Mr A. Zant for technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

E. WECKER
A. SCHIMPL
T. HÜNG

*Institut für Virologie und Immunbiologie,
der Universität Würzburg
Versbacher Landstrasse 7
D-8700 Würzburg, FRG*

Received 13 June; accepted 19 August 1977.

- Callahan, R., Benaviste, R. R., Lieber, M. M. & Todaro, G. J. *J. Virol.* **14**, 1394–1403 (1974).
- Fidler, J. H., Jensen, F. C., Bryant, M. L. & Lerner, R. A. *Nature* **267**, 23–28 (1977).
- McClintock, P. R., Ihle, J. N. & Joseph, D. R. *J. exp. Med.* **146**, 422–434 (1977).
- Hirsch, M. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **69**, 1069–1072 (1972).
- Moroni, C. & Schumann, G. *Nature* **254**, 60–61 (1975).
- Moroni, C., Schumann, G., Robert-Guroff, M., Suter, E. & Martin, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 535–538 (1975).
- Schäfer, W., Schwarz, H., Thiel, H. J., Wecker, E. & Bolognesi, D. P. *Virology* **75**, 401–418 (1976).
- Moening, V., Frank, H., Hunsmann, G., Schneider, I. & Schäfer, W. *Virology* **61**, 100–111 (1974).
- de Noronha, F., Baggs, R., Schäfer, W. & Bolognesi, D. P. *Nature* **267**, 54–56 (1977).
- Hunsmann, G., Moening, V., Pister, L., Seifert, E. & Schäfer, W. *Virology* **62**, 307–318 (1974).
- Mishell, R. J. & Dutton, R. W. *J. exp. Med.* **126**, 423–442 (1967).
- Jerne, N. K., Nordin, A. A. & Henry, C. in *Cell Bound Antibodies* (eds Amos, B. & Koprowski, H.) 109–116 (Wistar Institute, Philadelphia, 1963).
- Schimpl, A. & Wecker, E. *Nature* **226**, 1258–1259 (1970).
- Chattopadhyay, S. D., Lowy, D. R., Teich, N. N., Levine, A. S. & Row, W. P. *Proc. natn. Acad. Sci. U.S.A.* **71**, 167–171 (1974).
- North, J. R. & Askonas, B. A. *Eur. J. Immun.* **6**, 8–15 (1976).
- Hüning, T., Schimpl, A. & Wecker, E. *J. exp. Med.* **145**, 1216–1227 (1977).
- Schimpl, A. & Wecker, E. *Transplant. Rev.* **23**, 176–188 (1975).

Are endogenous C-type viruses involved in the immune system?

DNA sequences coding for infectious C-type viruses (oncornaviruses) are present in the genome of normal cells of various species, those best characterised being chicken, mouse, cat and baboon¹. Mammalian endogenous viruses, in general, are restricted for growth in their autologous species but replicate in autologous species and are termed xenotropic². In mice and some other species a further class of endogenous viruses has evolved which is restricted in heterologous cells but replicates in homologous cells, they are termed ecotropic³. Endogenous viral genes are inherited in the germ line and have co-evolved, in general, with non-viral host genes as indicated by evolutionary data⁴. One explanation for the retention of these genes is that they are involved in physiological functions as proposed by Temin in his provirus hypothesis⁵. We have shown previously that B-cell proliferation induced by certain B-cell mitogens is frequently associated with expression of endogenous virus^{6–10}. This has been confirmed by other workers who reported a xenotropic host range of the mitogen-induced virus¹¹. In contrast to other virus induction methods, mitogen stimulation closely resembles a physiological process, that of antigen stimulation followed by lymphocyte proliferation. Whereas B cells can be induced by mitogens to release virus, we have evidence that T cells are refractory to induction when using T-cell mitogens as well as 5-bromo-2'-deoxyuridine⁸. These results suggest that the expression of endogenous xenotropic C-type viral genes

may be physiologically required for B cells to participate in the immune response. As a test of this hypothesis we examined the effect of antisera directed against xenotropic endogenous C-type virus on the humoral immune response of mice. We show here that such sera are immunosuppressive.

To obtain an antiviral serum with no activity against non-viral mouse cellular components, we immunised rabbits with mouse xenotropic C-type virus produced in a rabbit cell line (SIRC). This rabbit line had been infected with endogenous C-type virus induced from BALB/c spleen cells by the B-cell mitogen lipopolysaccharide and was used as virus source 8 months after infection. For absorption controls, the same virus was purified from the culture fluid of a similarly infected mink cell line (CCL-64). The sera used in these studies were obtained from two rabbits following multiple intramuscular injections each of approximately 0.2 mg viral protein emulsified in complete Freund's adjuvant. Sera taken from the same animals before immunisation were used as controls. Sera was complement inactivated by heating at 56 °C for 30 min. The immune sera displayed complement-dependent cytotoxicity on virus-infected SIRC cells and showed no activity on uninfected SIRC cells. Pre-immune sera were not cytotoxic.

We examined the effect of antiviral sera on the humoral immune response to sheep red blood cells (SRBC) as assayed in the Mishell–Dutton *in vitro* system¹². In this assay B cells which secrete antibody against SRBC are quantitated by their plaque-forming ability. A typical experiment is presented in Table 1 which shows that antiserum but not control serum reduces the number of plaque-forming cells by 70%.

To confirm the viral specificity of the immunosuppressive effect of our antisera, absorption experiments were performed with equal quantities of various viruses as well as with SRBC. Table 2 shows that absorption of the antiserum with xenotropic virus fully removed the immunosuppressive activity. Xenotropic BALB/c virus, grown in mink or rabbit cells, as well as xenotropic AKR virus, were similarly effective in absorption. Some absorption occurred also with ecotropic AKR virus, while tobacco mosaic virus, a completely unrelated virus, showed no absorption. Since rabbit antisera frequently contain activity against Forssman antigen, we tested whether absorption with red cells would remove the immunosuppressive activity. As shown in Table

Table 1 *In vitro* immunosuppressive activity of antiserum against mitogen-induced xenotropic C-type virus

Serum (final dilution)	PFC per 10 ⁵ spleen cells*	% Inhibition
—	3,900	—
Control serum (1:100)	3,900	0
Antiserum (1:100)	1,200	70
(1:400)	1,900	51
(1:1,600)	1,700	56
(1:6,400)	3,800	3

The effect of the antiserum against xenotropic C-type virus on the induction of an antibody response to sheep red blood cells was investigated in cultures of BALB/c spleen cells from 8–15-week-old mice by a modified Mishell–Dutton technique¹². 8×10^6 spleen cells were cultured in 35×10 -mm disposable tissue culture Petri dishes (Falcon) containing 1 ml RPMI 1640 medium (Microbiological Associates), supplemented with 8% foetal bovine serum, 1% horse serum and antibiotics. At the onset of the culture period 10 μ l control serum or antiserum was added. The cultures were rocked during the 5-d culture period in an incubator containing 10% CO₂, 83% N₂ and 7% O₂. They were stimulated with 4×10^6 sheep red blood cells. Direct plaque-forming cells (PFC) were assayed by the local haemolysis technique in liquid medium¹³. The cell recovery of the various cultures did not differ significantly.

*Mean values from triplicate cultures.

Table 2 Specificity controls by absorption experiments

Expt no.	Antiserum added	Antiserum absorbed with	PFC per 10 ⁷ spleen cells	% Inhibition
1	—	—	5,200	—
	+	—	800	85
	+	x-BALB/c*	5,200	0
	+	x-AKR†	4,200	19
	+	e-AKR‡	2,200	58
	+	SRBC	1,200	77
	+	TMV§	1,400	73
2	—	—	2,700	—
	+	—	500	82
	+	x-BALB/c	2,300	15
	+	e-AKR‡	1,300	52
	+	TMV§	500	82
3	—	—	5,800	—
	+	—	600	90
	+	x-BALB/c*	5,600	3
	+	TMV§	1,000	83
	+	SRBC	800	86
	+	Rabbit RBC	600	90

The antiserum was absorbed with either virus or red blood cells. Virus absorption: 400 µg of density-gradient purified virus was pelleted by centrifugation and incubated with 30 µl of sera diluted 1:10 for 60 min in ice. Serum was recovered by centrifugation and 10 µl of the absorbed serum was added to 1 ml cultures. Absorption with red blood cells: 1 part packed cell volume was incubated with 2 parts of sera diluted 1:2 for 30 min on ice. Serum was recovered by centrifugation and subjected to two further absorption procedures. Finally, the serum was clarified by centrifugation in a Sorvall SS 34 rotor at 5,000 r.p.m. for 10 min. Cultures received 10 µl of the absorbed sera or 10 µl of the non-absorbed serum 1:10 diluted. Culture conditions as Table 1.

*Mitogen-induced endogenous xenotropic BALB/c virus, grown in mink CCL-64 cells.

†Mitogen-induced endogenous xenotropic AKR virus, grown in mink CCL-64 cells.

‡Endogenous ecotropic AKR virus grown in mouse NIH-3T3 cells.

§Tobacco mosaic virus.

As *, but virus grown in rabbit SIRC cells.

2, this was not found to be the case. Furthermore, no absorption occurred when the serum was incubated with rabbit SIRC cells (data not shown).

The immunosuppression was also shown in experiments carried out *in vivo*. Four days after immunisation with SRBC those mice which had simultaneously received an intravenous injection of 0.2 ml antiviral serum showed a reduced number of plaque-forming cells, inhibition ranging from 75 to 84% (Table 3). Those mice injected with control serum showed no reduction of plaque-forming cells.

In further experiments the immunosuppressive activity of these sera was also demonstrated with a second antigen, horse red blood cells, which is serologically non-cross-reactive with SRBC. The immunosuppressive activity was found to be due to immunoglobulin since incubating the sera with goat anti-rabbit Ig-serum removed the activity (data not shown).

The possibility that antibody directed against a non-viral cellular antigen is responsible for the observed immunosuppressive effect is highly unlikely for several reasons. As the rabbit antisera did not react with normal rabbit SIRC cells by cytotoxicity or by fluorescence, a contaminating antigen of rabbit origin is unlikely. A mouse cellular antigen is ruled out as the only mouse antigens present in the infected rabbit cell line are viral gene products of the integrated mouse virus (barring transduction of non-viral mouse genes by the virus). Most importantly, virus grown in a third species, mink, also fully removed the activity by absorption.

There seem to be three possible explanations of the mechanism by which our antisera exert this immunosuppressive effect. First, the effect is due to complement-dependent cytotoxicity directed against lymphocytes

Table 3 *In vivo* immunosuppressive activity of antiserum against mitogen-induced xenotropic C-type virus

Expt no.	Serum (dilution) injected	PFC per 10 ⁷ spleen cells*	% Inhibition
1	Control serum	38,400 ± 1,700	—
	Antiserum	9,450 ± 540	75
2	None	28,900 ± 1,400	—
	Antiserum	5,950 ± 470	79
	1:2	4,430 ± 520	84
	1:4	5,480 ± 350	81
	1:8	8,480 ± 480	71
	1:16	11,030 ± 650	61

BALB/c mice aged 8–15 weeks were immunised by one i.p. injection of 5×10^8 SRBC. Antiserum or control serum (0.2 ml per mouse) was injected intravenously immediately after antigen administration. The animals were killed after 4 d and direct plaque forming cells (PFC) were assayed from spleen cells.

*Average of quadruplicate values from spleens of two individual mice. Values are given ± s.e.m.

participating in the anti-SRBC response. This seems unlikely, as the sera were not cytotoxic for spleen cells in a cytotoxic test using rabbit complement. Cytotoxicity on the level of the plaque-forming cells or its precursors also seems unlikely since the immunosuppressive activity was only observed when the antiserum was injected within 2 d after SRBC injection. Later injections during the 4-d immunisation period had no effect. A second possibility is that viral antigen is in close proximity to a membrane structure necessary for the immune response, for example, a receptor. Binding of antibody would then lead to steric hindrance of the receptor function. Finally, viral antigen itself may be required on the membrane. Here it could, for example, play a receptor-like role in cell-cell interaction. Blocking of the antigen therefore would lead to immunosuppression. While this last explanation is consistent with our working hypothesis, it is clear that further studies are necessary if a functional link between endogenous C-type viruses and the immune system is to be established.

These results show that antibody directed against endogenous xenotropic C-type viral antigens represents a new and interesting principle of immunosuppression. Work to identify the target cells of the antisera, the nature of the viral antigen and the mechanism of the immunosuppressive effect is in progress.

C. MORONI

Friedrich Miescher-Institut,
P.O. Box 273,
CH-4002 Basel, Switzerland

G. SCHUMANN

Research Department,
Pharmaceuticals Division,
CIBA-Geigy Limited,
CH-4002 Basel, Switzerland

Received 22 June; accepted 22 August 1977.

1. Toozé, J. (ed.) *The Molecular Biology of Tumour Viruses* (Cold Spring Harbor Laboratory, 1973).
2. Levy, J. A. *Science* **182**, 1151–1153 (1973).
3. Pincus, T., Hartley, J. W. & Rowe, W. P. *J. exp. Med.* **133**, 1219–1233 (1971).
4. Benveniste, R. E. & Todaro, G. J. *Nature* **252**, 170–173 (1974).
5. Temin, H. M. *J. natn. Cancer Inst.* **46**, 3–7 (1971).
6. Moroni, C. & Schumann, G. *Nature* **254**, 60–61 (1975).
7. Moroni, C., Schumann, G., Robert-Guroff, M., Suter, E. R. & Martin, D. *Proc. natn. Acad. Sci. U.S.A.* **72**, 535–538 (1975).
8. Schumann, G. & Moroni, C. *J. Immun.* **116**, 1145–1150 (1976).
9. Moroni, C. & Schumann, G. *Virology* **73**, 17–22 (1976).
10. Schumann, G. & Moroni, C. *Virology* **79**, 81–87 (1977).
11. Phillips, S. M., Stephenson, J. R., Greenberger, J. S., Lane, P. E. & Aaronson, S. A. *J. Immun.* **116**, 1123–1128 (1976).
12. Mishell, R. I. & Dutton, R. W. *J. exp. Med.* **126**, 423–442 (1967).
13. Cunningham, A. J. *Nature* **207**, 1106–1107 (1965).

Development of improved cholera vaccine based on subunit toxoid

THE cholera vaccines now available consist of killed vibrios, and give rise to only limited protection of short duration¹. The finding that the excessive diarrhoea in cholera is due to the action on the small intestine epithelium of an exotoxin produced by the vibrios^{2,3} has focused attention on the potential of toxoid for improved immunoprophylaxis. The development of a suitable toxoid has met with great difficulties, however. Formalin toxoid, although reasonably antigenic, proved unsatisfactory because of reversion to toxicity⁴. Glutaraldehyde toxoid was stable but poorly immunogenic which explains its low efficacy in a recent field trial⁵. We describe here the development and properties of a subunit cholera toxoid, the nature of which eliminates the risk of reversion to toxicity. The high protective immunogenicity observed in experimental animals gives promise that addition of this toxoid to the conventional vaccine will result in a significantly improved immunoprophylactic agent against cholera.

Different *Vibrio cholerae* strains, irrespective of their serotype, produce an immunologically identical exo-enterotoxin⁶. The toxin contains two types of non-covalently linked subunits, one heavy (H or A; molecular weight (MW) about 28,000) and five or six light ones (L or B, MW about

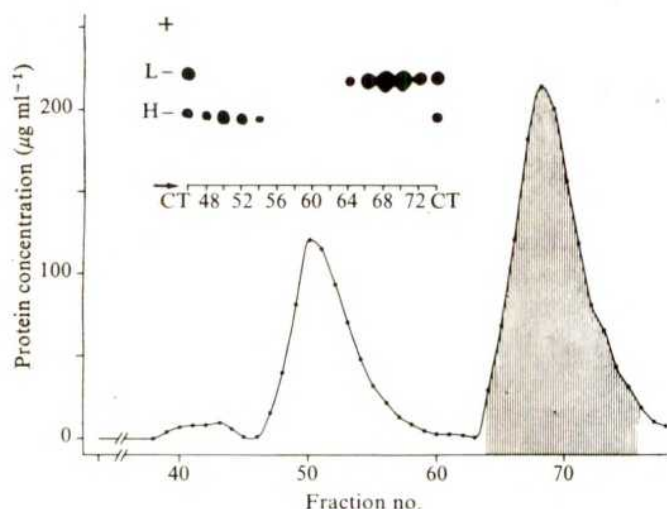


Fig. 1 Preparation of L-subunit toxoid from purified cholera toxin. The toxin (about 10 mg in 1–2 ml) was diluted with 20% concentrated formic acid to give a 5% final concentration of the acid. After incubation for 1 h, the sample was filtered (at room temperature) through a 2.5 × 87.5-cm Sephadex G-100 column equilibrated as well as eluted (25 ml h⁻¹) with 5% formic acid. Consecutive 4-ml fractions were collected and their light absorbance at 280 nm determined. The diagram shows the protein elution profile from a typical experiment, in which 8.5 mg toxin was processed. Two distinct peaks emerge, the first representing isolated subunit H and the second, bigger one subunit L. The amounts of applied toxin and eluted subunits were estimated from the absorbance values using the $A_{280}^{1\%}$ coefficients¹ 11.4 for the toxin, 15.1 for subunit H and 9.56 for subunit L fractions. The fractions hatched in the diagram were pooled, dialysed against PBS (0.05 M phosphate, 0.14 M sodium chloride, pH 7.2) at 4°C for 48 h, and then used as L-subunit toxoid. The inserted photograph illustrates the results obtained on testing samples of individual gel filtration fractions by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). The samples were lyophilised, redissolved in 2% SDS–8M urea solution, incubated at 53°C for 20 min and then processed by SDS-PAGE⁷. Every second fraction from number 48 to 72 was tested. In addition, cholera toxin (CT) was included in two positions as a reference for the migration of subunits H and L. The arrow indicates start line and + the anode direction.

Table 1 Protective immunity and serum antibody titres in rabbits after immunisation with L-subunit and glutaraldehyde toxoids

Dose of toxoid (µg)	No. of animals	Protection factor Live vibrios	Crude toxin	Serum antitoxin titre
L-subunit toxoid				
30	7	18	5.0	400,000
15	4	10	2.5	130,000
10	8	3.2	1.8	100,000
7.5	5	1.3	1.4	25,000
Glutaraldehyde toxoid				
30	4	5.0	1.6	20,000
15	4	0.7	1.1	10,000

New Zealand rabbits weighing 1.2–1.5 kg at the onset of immunisation were used. The L-subunit toxoid was prepared as described in Fig. 1. The glutaraldehyde toxoid²¹ (Wyeth; lot 20101) was provided by Dr C Miller of NIH. The antigens were injected s.c. without adjuvant in two 0.5 ml portions above the right and left posterior legs twice with a 2-week interval. A similar number of control rabbits as in the immunised group were injected with phosphate-buffered saline (PBS). Five days after the booster injection the immunised and control animals were tested for susceptibility to experimental cholera. In each animal 21 about 5-cm long segments of the small intestine were ligated. Into each loop was injected graded doses (in 1-ml volumes) of live cholera vibrios (strain 569B) or crude cholera toxin (*V. cholerae* 569B culture filtrate, NIH lot 4493G). Five doses of bacteria (10⁵–10⁹) and crude toxin (0.1–10 mg) were tested in duplicate at randomly chosen positions. The remaining loop was injected with 1 ml PBS and served as a negative control. Fluid accumulation in the intestinal segments was measured after 15–18 h as ml fluid per cm intestine and ED₅₀ (dose of bacteria or toxin producing half-maximal intestinal filling) determined for each group of animals²⁰. The protective effect of immunisation—the protection factor—was estimated as the ratio between the group ED₅₀ values for immunised and concurrently tested control animals. Serum antitoxin titres were determined by the enzyme-linked immunosorbent assay (ELISA)²² on serum samples, pooled in each group, collected on the day of the intestinal challenge.

11,000 each)^{7–12}. The L subunits are responsible for the binding of toxin to the cell membrane but not for the subsequent activation of adenylate cyclase. Conversely, the H subunit is essential for toxicity but, because of its inability to bind to cells, it is inactive in the absence of L subunits^{13,14}. The two types of cholera toxin subunits are immunologically unrelated¹³. The subunits also differ markedly in immunogenicity so that immunisation with intact toxin gives rise to antisera which always react strongly with the L subunit but only irregularly and at low titre with subunit H. Furthermore, isolated anti-H subunit antibodies have very low cholera toxin-neutralising capacity in contrast to antibodies reacting with subunit L¹⁵. Immunological protection by means of cholera antitoxin thus probably results from prevention of toxin binding to intestinal cell receptors rather than from interference with the 'active site' on the effector subunit. We have therefore directed our efforts to the preparation of pure L subunits and reaggregation of these subunits. Theoretically this should yield an almost ideal toxoid for immunisation, since the elimination of subunit H excludes the risk of reversion to toxicity without causing any significant loss of protective capacity.

The method for preparation of L-subunit toxoid was based on the fact that the two types of subunit in cholera toxin can be isolated by gel filtration of toxin in acidic buffer⁷. Highly purified toxin, prepared essentially according to Finkelstein *et al.*¹⁶, was treated with formic acid and the dissociated subunits separated on Sephadex G-100 (Fig. 1). This resulted in an almost quantitative isolation of the toxin L subunits in pure form. In five different experiments the yield of L was 90–93% and contamination with subunit H could be excluded at a level of 0.2% (w/w) using sensitive immunological methods. As determined with the rabbit skin¹⁷ and mouse thymocyte cyclic AMP accumula-

Table 2 Increased duration of protection by immunisation with toxoid or toxoid-enriched cholera vaccine

	Protection factor				Serum antibody titres			
	Day 5		Day 21		Day 5		Day 21	
	Live vibrios	Crude toxin	Live vibrios	Crude toxin	LPS	Toxin	LPS	Toxin
Cholera vaccine (4×10^9 killed vibrios)	10	1.1	2.0	1.0	120,000	<500	300,000	<500
L-subunit toxoid (10 μ g)	3.2	1.8	13	3.0	<500	100,000	<500	200,000
Cholera vaccine + L-subunit toxoid	80	2.1	90	3.0	100,000	400,000	500,000	600,000

Groups of 8–12 rabbits were immunised according to the schedule described in Table 1. Twelve concurrently PBS-injected rabbits served as controls. Half of the animals in each group were challenged with live vibrios or crude toxin in alternating loops 5 d and the others 21 d after the booster immunisation. The protective effect of immunisation (the protection factor) and the serum antibody titres against *V. cholerae* lipopolysaccharide (LPS) and enterotoxin were determined as in Table 1.

tion assays¹⁸, all batches of L-subunit toxoid had less than 0.1% of the toxicity of intact toxin.

Radial immunodiffusion tests (Mancini) suggested that the L subunits in the toxoid, with retention of their full reactivity with antibody, were aggregated to a complex with a diffusion rate similar to that of native toxin. This aggregation was demonstrated directly by thin-layer gel filtration in Sephadex G-200 superfine¹⁹. The L-subunit toxoid migrated as a single component with a rate faster than that of subunit H and there was no detectable toxoid protein filtering at the rate which would be expected for the individual L subunits.

The toxoid was usually kept frozen in aliquots at -30°C , and storage for more than 18 months did not result in detectable changes according to the immunological, physicochemical and biological criteria mentioned above. In the presence of 0.01% merthiolate the toxoid has been stable at $4^\circ\text{--}8^\circ\text{C}$ for more than 6 months.

The immunogenicity of the L-subunit toxoid was studied in rabbits and compared with that of the recently field tested glutaraldehyde toxoid. Two subcutaneous (s.c.) immunisations with 10 μ g of L-subunit toxoid in each injection induced significant protection (Table 1). The effect in-

creased with increasing amounts, so that 30 μ g gave an approximately 20-fold increased resistance to live vibrio challenge and 5-fold protection against challenge with toxin. The glutaraldehyde toxoid was much less effective and also gave rise to lower serum antibody titres than the L-subunit toxoid (Table 1). Adsorption to aluminium phosphate did not significantly enhance the protective immunogenicity of the L-subunit toxoid as tested with 10 μ g of toxoid on 0.034 mmol of carrier. No side-effects of immunisation were observed in any instance.

V. cholerae enterotoxin and cell wall lipopolysaccharide (LPS) has been shown to induce a synergistic protective immunity against experimental cholera infection²⁰. In agreement with this, combinations of L-subunit toxoid and conventional cholera vaccine or purified LPS were found to induce a level of protection against intestinal challenge with live vibrios which equalled or exceeded the product of the immunity attained with the individual antigens (Fig. 2). Challenge in alternate loops with crude toxin (not shown) revealed that the somatic antigens neither gave rise to any antitoxic immunity nor enhanced the efficacy of the toxoid component in protection against toxin. Furthermore, the serum antibody titres induced by the antigens in the mixture

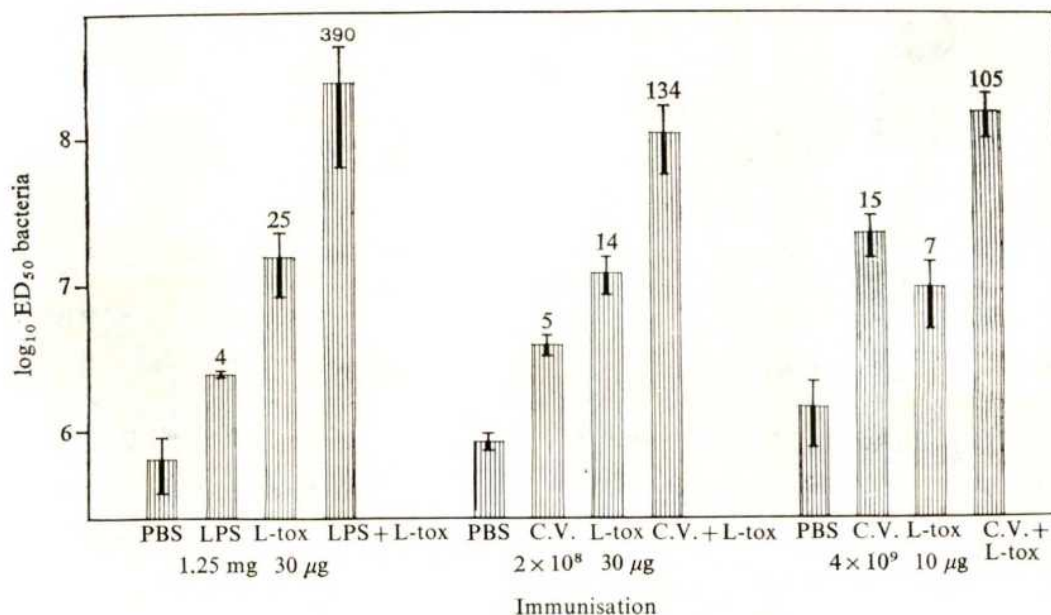


Fig. 2 Synergistic protective effect of immunisation with combinations of *V. cholerae* somatic antigen and L-subunit toxoid. Three experiments are presented, each comprising four groups of four to eight rabbits. One group received PBS injections only, and the others were immunised according to the schedule described in Table 1 with, respectively, somatic antigen (killed *Vibrio cholerae* vaccine (C.V.) in two doses, or purified *V. cholerae* lipopolysaccharide (LPS)), L-subunit toxoid (L-tox.), or the somatic antigen and toxoid in mixture. Five days after immunisation intestinal challenge with graded doses of live vibrios was performed as described in Table 1 and ED₅₀ was determined for each animal. The means \pm s.e.m. of the individual ED₅₀ values of bacteria are shown for each group of rabbits. The figures on top of the bars are the ratios between the mean ED₅₀ for immunised animals and the mean ED₅₀ for the PBS-injected control rabbits, that is, the fold-increase protection achieved by immunisation. The cholera vaccine (National Bacteriological Laboratory, Stockholm) contained equal numbers of phenol-killed Inaba and Ogawa vibrios. The LPS was purified from *V. cholerae* 569B by means of hot phenol-water extraction and ultracentrifugation²¹.

did not differ significantly from those produced by the respective antigens alone. These data indicate that the synergistic protective effect obtained by immunisation with toxoid and somatic antigen in combination is not due to enhancement of the antitoxic and antibacterial immune responses in themselves. Instead, it probably results from the interference of the immunity with two separate events of pathogenic importance, that is, the mucosal adhesion of cholera vibrios and binding of enterotoxin to specific membrane receptors¹⁸.

As in humans, the conventional vaccine gave rise only to short-term protective immunity in rabbits. The immunity against challenge with live vibrios decreased markedly between day 5 and 21 after the booster injection, in spite of a slight increase in the serum anti-LPS antibody titre (Table 2). In contrast, a combination of L-subunit toxoid and vaccine gave rise to immunity which rather increased during this period. This was probably due to a sufficient rise in the antitoxic immunity induced by the toxoid component to compensate for the rapid decrease in antibacterial immunity, as immunity induced by L-subunit toxoid alone increased substantially from day 5 to 21 (Table 2).

In conclusion, a subunit cholera toxoid having no risk of reverting to toxicity has been developed. Vaccination with a mixture of this toxoid and killed vibrios should result in more effective, longer lasting immunity against cholera than that given by immunoprophylactic agents used previously. Studies in progress suggest that inexpensive large-scale production may be possible by preparing the subunit toxoid from crude toxin preparations.

We thank Dr Charles Wadsworth for performing the thin-layer gel filtration analyses. This work was supported by the Swedish Medical Research Council and the WHO.

J. HOLMGREN
ANN-MARI SVENNERHOLM
I. LÖNNROTH

Institute of Medical Microbiology,
University of Göteborg,
Sweden

MARGARETA FALL-PERSSON
B. MARKMAN
H. LUNDBECK

National Bacteriological Laboratory,
Stockholm, Sweden

Received 21 June; accepted 1 August 1977.

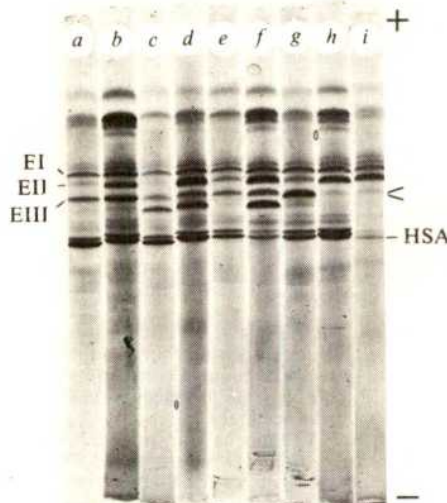
- 1 Finkelstein, R. A. *Crit. Rev. Microbiol.* **2**, 553-623 (1973).
- 2 De, S. N. *Nature* **183**, 1533-1534 (1959).
- 3 Dutta, N. K., Panse, M. V. & Kulkarni, D. R. *J. Bact.* **78**, 594-595 (1959).
- 4 Northrup, R. S. & Chisari, F. V. *J. Infect. Dis.* **125**, 672-673 (1972).
- 5 Curlin, G., Levine, R., Aziz, K. M. A., Rahman, M. A. S. M. & Verwey, W. F. *Proc. 11th U.S.-Japan Cholera Conf.* New Orleans November 1975, 314-329 (US Department of Health, Education and Welfare, Washington, 1975).
- 6 Holmgren, J., Lönnroth, I. & Ouchterlony, O. *Infect. Immun.* **3**, 747-755 (1971).
- 7 Lönnroth, I. & Holmgren, J. *J. gen. Microbiol.* **76**, 417-427 (1973).
- 8 Cuatrecasas, P., Parikh, I. & Hollenberg, M. D. *Biochemistry* **12**, 4253-4263 (1973).
- 9 van Heyningen, S. *Science* **183**, 656-657 (1974).
- 10 Holmgren, J. & Lönnroth, I. *J. gen. Microbiol.* **86**, 49-65 (1975).
- 11 Lai, C. Y., Mendez, E. & Chang, D. J. *Infect. Dis.* **133**, Suppl. S23-S30 (1976).
- 12 Kurosky, A., Markel, D. E., Peterson, J. W. & Fitch, W. M. *Science* **195**, 299-300 (1977).
- 13 Holmgren, J. *Proc. 9th U.S.-Japan Cholera Conf.* Grand Canyon October 1973. *US Department of State Publ.* 8762, 196-213 (1973).
- 14 Holmgren, J., Lindholm, L. & Lönnroth, I. *J. exp. Med.* **139**, 801-819 (1974).
- 15 Holmgren, J. & Svennerholm, A. M. *J. Infect. Dis.* **136**, Suppl. August (1977).
- 16 Finkelstein, R. A., Fujita, K. & LoSpalluto, J. J. *J. Immun.* **107**, 1043-1051 (1971).
- 17 Craig, J. *Nature* **207**, 614-616 (1965).
- 18 Lönnroth, I. & Lönnroth, C. *Expl. Cell. Res.* **104**, 15-24 (1977).
- 19 Hanson, L. A., Holmgren, J. & Wadsworth, C. *Int. Arch. Allergy appl. Immun.* **40**, 806-819 (1971).
- 20 Svennerholm, A. M. & Holmgren, J. *Infect. Immun.* **13**, 735-740 (1976).
- 21 Rappaport, R. S., Bonde, G., McCann, T., Rubin, B. A. & Tint, H. *Infect. Immun.* **9**, 304-317 (1974).
- 22 Holmgren, J. & Svennerholm, A. M. *Infect. Immun.* **7**, 759-763 (1973).
- 23 Orskov, F., Orskov, I., Jann, K., Müller-Seitz, E. & Westphal, O. *Acta pathol. microbiol. Scand.* **71**, 339-358 (1976).

Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man

PRIMARY dysbetalipoproteinaemia (broad- β disease, hyperlipoproteinaemia type III) is a familial disorder of plasma lipoprotein metabolism associated with xanthomatosis and early onset of severe atherosclerotic vascular disease¹⁻⁴. A defect in the catabolism of triglyceride-rich lipoproteins probably underlies the accumulation of atypical cholesterol-rich lipoproteins (very low density β lipoproteins, ' β -VLDL') in the S_{12-400} density fraction⁵⁻⁷. The disorder is assumed to be rare⁸ and we have provided evidence for an autosomal recessive mode of inheritance^{9,10}. Patients show a variant of one major protein component (Apo E) of VLDL^{11,12}. Apo E is a glycoprotein of molecular weight $\sim 39,000$ that splits into three main bands designated E-I ($pI \sim 5.3$), E-II ($pI \sim 5.4$) and E-III ($pI \sim 5.55$) in isoelectric focusing¹³. The Apo E variant is characterised by a deficiency of Apo E-III in triglyceride-rich lipoproteins. We report here that Apo E shows a genetic polymorphism determined by two alleles *Apo Eⁿ* and *Apo E^d*. About 1% of the German population is homozygous for the allele *Apo E^d* and exhibits Apo E-III deficiency. All individuals of this genotype have a primary dysbetalipoproteinaemia but not necessarily hyperlipidaemia. This is therefore the most frequent monogenic dyslipoproteinaemia known in man.

A simple method for the detection of Apo E patterns without ultracentrifugation that allows the selective recognition of patients with dysbetalipoproteinaemia in a hyperlipidaemic group (refs 9, 11 and unpublished work of G.U., M.H., G. Mühlfellner, N. Pruin and K. H. Vogelberg) has been applied to evaluate Apo E patterns in 490 blood donors from Marburg/Lahn. VLDL was precipitated from 1 ml of serum by addition of 50 μ l of a 5% aqueous heparin solution and 50 μ l 2 M $MgCl_2$. Crude VLDL was isolated by low-speed centrifugation, resuspended in 0.5 ml 0.02 M Tris-HCl, 0.1 M NaCl, 0.017 M sodium citrate pH 7.7 and reprecipitated by addition of 25 μ l 2 M $MgCl_2$. VLDL was extracted with chloroform-methanol, 2:1 (v/v). Apo VLDL was solubilised

Fig. 1 Analytical isoelectric focusing (pH 3.5-10 in polyacrylamide gels containing 6 M urea) of nine individual apo-VLDL preparations demonstrating the different Apo E phenotypes Apo E-N (a, c, g), Apo E-ND (b, d, e, f) and Apo E-D (h, i). The additional cathodic band ($pI \sim 5.75$) in gels c, d, f represents a further independent genetic marker (Apo E-IV(+)-variant) that had a frequency of 26.9% in the population sample studied and is transmitted as an autosomal dominant trait¹⁷ (see also Fig. 3).



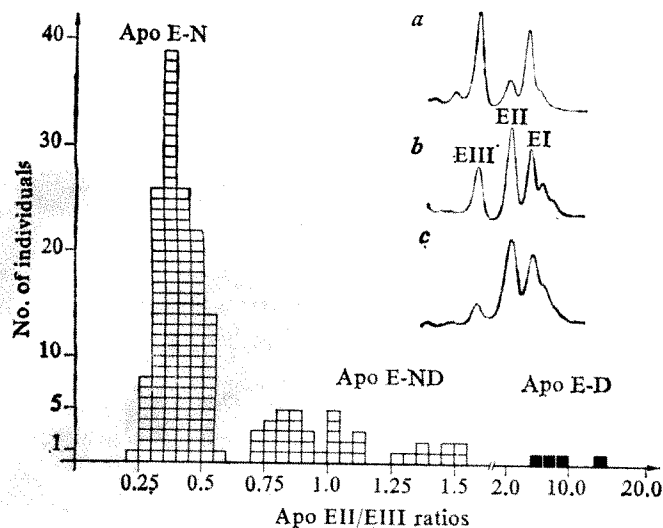


Fig. 2 Distribution of Apo E patterns according to Apo E-II/E-III ratios (densitometric areas) in individual apo VLDL samples ($n=181$) that have been evaluated by scanning densitometry. Each square represents one individual. Proband with features of dysbetalipoproteinaemia are marked in black. Insert: representative densitometric scans of stained focusing gels demonstrating the three common Apo E phenotypes Apo E-N (a), Apo E-ND (b) and Apo E-D (c). Only the Apo E region is shown. The pI values of the individual bands determined by flat bed isoelectric focusing in granulated gels were ~ 5.3 (E-I), ~ 5.5 (E-II) and ~ 5.55 (E-III). (U. Beisiegel and G.U., unpublished) and differed slightly from those determined in the analytical polyacrylamide gels¹³.

in 200 μ l 0.02 M Tris-HCl, 8 M urea, pH 8.2 and subjected to isoelectric focusing in a pH gradient from 3.5–10 in polyacrylamide gels containing 6 M urea. Protein bands were stained with coomassie brilliant blue and evaluated by scanning densitometry.

Three phenotypes, Apo E-N, Apo E-ND and Apo E-D (E-III deficiency pattern) were differentiated on the basis of the Apo E-II/E-III ratios (Figs 1 and 2). The ratio was 0.40 ± 0.07 for phenotype Apo E-N, 1.02 ± 0.25 for phenotype Apo E-ND and 9.07 ± 4.48 for phenotype Apo E-D. No overlap was observed between the distinct groups in this study.

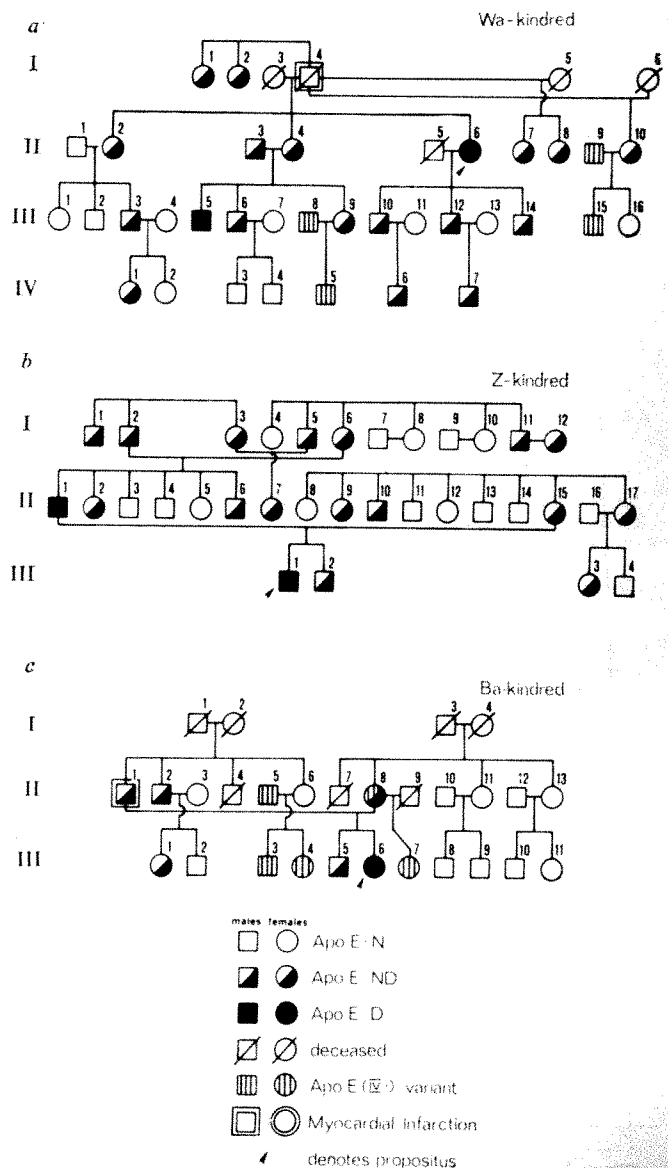
In control experiments VLDL was isolated by conventional preparative ultracentrifugation from individuals of phenotypes Apo E-D ($n=5$) and Apo E-ND ($n=51$) and from a control group of the same size. All individuals were in a fasting state. In all cases the previous diagnosis was confirmed. The demonstration of Apo E phenotypes is therefore independent of the method used for isolation of VLDL. The frequencies of the three common Apo E phenotypes were 83.3% for Apo E-N, 15.7% for Apo E-ND and 1.0% for Apo E-D.

Apo E patterns were determined in three kindreds of probands of phenotype Apo E-D (Fig. 3). The segregation of Apo E phenotypes among children from different parental matings is consistent with a simple Mendelian mode of transmission where two alleles determine the three phenotypes Apo E-N, Apo E-ND and Apo E-D. Considered together, the population and family data show that, in man, genetic polymorphism of apolipoprotein E is governed by two common autosomal alleles *Apo Eⁿ* (frequency 0.9112) and *Apo E^d* (frequency 0.0888). The observed frequencies of the three phenotypes are in good agreement with those expected according to Hardy-Weinberg's law. No significant differences between females ($n=144$; $Apo E^n=0.9201$) and males ($n=346$; $Apo E^n=0.9075$) were observed.

The frequency of the allele *Apo E^d*, however, was unexpectedly high. We therefore investigated whether the

homozygous probands of phenotype Apo E-D detected had dysbetalipoproteinaemia. Analysis of the composition of the main lipoprotein fractions of all five probands in comparison with a control group of 94 subjects of phenotypes Apo E-N ($n=43$) and Apo E-ND ($n=51$) showed that they in fact represented a biochemically distinct group with abnormalities in their lipoprotein spectrum characteristic of dysbetalipoproteinaemia. These were (1) an abnormal quantitative distribution of lipoproteins (demonstrated by density gradient centrifugation) with reduced levels of LDL and elevation of VLDL and also intermediary density lipoprotein levels (Fig. 4); (2) a markedly reduced or absent LDL band in polyacrylamide gel electrophoresis (Fig. 5); (3) presence of ' β -VLDL' in the $d < 1.006$ g ml⁻¹ fraction in the ultracentrifuge (Fig. 5) and (4), an increase in the relative and absolute concentrations of cholesterol in VLDL (Table 1). Thus all probands had dysbetalipoproteinaemia according to the 'floating- β test' and also according to at least one of the lipid-chemical definitions recently proposed for the disorder^{14,16}. However, none had hypercholesterolaemia and only three had mild hypertriglyceridaemia: total serum cholesterol levels

Fig. 3 Pedigrees of three kindreds demonstrating the segregation of Apo E phenotypes. Propositi were a A.W. (Wa-kindred); b, B.Z. (Z-kindred) and c, M.B. (Ba-kindred) (see Table 1).



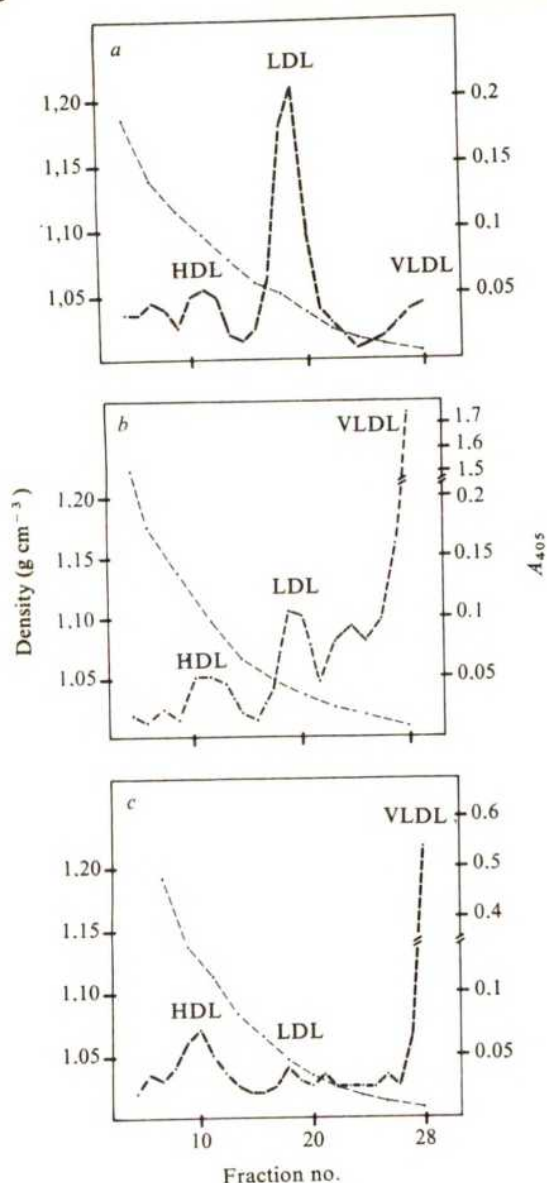


Fig. 4 Profiles of lipoprotein cholesterol after density gradient centrifugation of 4 ml of serum from a normolipidaemic control subject (a), a hyperlipidaemic proband of phenotype Apo E-D (b), and a normocholesterolaemic proband of phenotype Apo E-D (c). Centrifugation was performed in a SW-41 rotor at 40,000 r.p.m. for 24 h at 4 °C. The gradient was from 1.006 g ml⁻¹ to 1.21 g ml⁻¹ KBr and prepared according to Redgrave *et al.*¹⁸. Fractions were collected from below and cholesterol determined with the enzymatic method of Roeschlau¹⁹. — — —, Absorbance at 405 nm of 3,5-diacetyl-1, 4-dihydrolutidine the endproduct in the enzymatic test for cholesterol. — — —, Density.

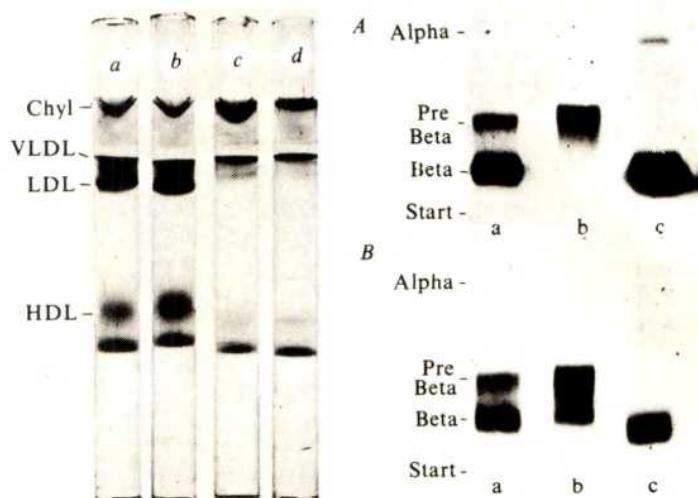


Fig. 5 Left: Discontinuous electrophoresis in polyacrylamide gels of 3.75% monomer concentration of sera prestained with sudan black B²⁰ from two controls of phenotype Apo E-N (a, b) and two probands of phenotype Apo E-D (c, d). The poor staining of the HDL-band in gels c and d indicated a decrease in the concentration also of this component but was not seen in all individuals of phenotype Apo E-D. Chyl, Chylomicrons. Right: Agarose gel electrophoresis of sera (a), VLDL (b) and fraction d > 1.006 g ml⁻¹ (c) from a control subject (A) and a proband of phenotype Apo E-D (B). Staining sudan black B.

were abnormally low in the three probands under age 30 yr. From a study of subjects with primary hypocholesterolaemia where about 10% were of phenotype Apo E-D (compared with 1% in the general population observed in this study) we have additional evidence that young individuals of phenotype Apo E-D represent a novel genetic form of hypocholesterolaemia with hypobetalipoproteinaemia (G.U., H. Kaffarnik, and N. Pruin, in preparation). According to the data presented here the gene frequency for dysbetalipoproteinaemia (including hypo-, normo- and hypercholesterolaemic subjects) is 0.089 in the German population sample studied. This high frequency explains the occurrence of vertical transmission of dysbetalipoproteinaemia^{2,4,8} as pseudo-dominance (see Z-kindred in Fig. 2 and refs 9, 10).

In contrast to the high frequency of dysbetalipoproteinaemia in the population, individuals of phenotype Apo E-D that have gross hyperlipidaemia and xanthomatosis seem to be rare. Studies in progress in our laboratory indicate that additional mutant genes for other forms of familial dyslipoproteinaemia (familial combined hyperlipidaemia, familial hypertriglyceridaemia, familial hypercholesterolaemia) are operating in those patients that have

Table 1 Clinical data on the five probands of phenotype Apo E-D

Subject	Age/sex	Serum		VLDL*		VLDL _{Ch/TG}	Ch _{VLDL} /TG _{serum}	β-VLDL
		Cholesterol (mg per 100 ml)	Triglycerides (mg per 100 ml)	Cholesterol (mg per 100 ml)	Triglycerides (mg per 100 ml)			
B.Z.	24/M	89	77	23	61	0.38	0.3	+
B.Th.	26/M	128	157	64	83	0.77	0.41	+
M.B.	28/F	113	78	42	45	0.94	0.54	+
G.W.	60/M	196	141	41	66	0.61	0.27	+
A.W.	65/F	177	175	42	74	0.53	0.24	+
Mean ± s.d.		141 ± 45	128 ± 47	42 ± 15	66 ± 14	0.65 ± 0.22	0.35 ± 0.12	
Controls ± s.d.		191 ± 31	102 ± 34	17 ± 6	59 ± 39	0.32 ± 0.15	0.17 ± 0.07	

A group of six age- and sex-matched blood donors of phenotype Apo E-N served as controls.

*VLDL was isolated from 200–250 ml of serum to get reliable data and lipid values are given as mean of direct and indirect determinations.

developed severe clinical manifestations. One of the most intriguing questions is whether probands of phenotype Apo E-D that have dys- but not hyperlipoproteinaemia are also at risk to develop premature atherosclerosis.

This work was supported by the Deutsche Forschungsgemeinschaft. The technical assistance of Miss H. Blüder is gratefully acknowledged.

G. UTERMANN
M. HEES
A. STEINMETZ

Institut für Humangenetik,
der Philipps-Universität,
Bahnhofstraße 7A,
3550 Marburg, FRG

Received 22 March; accepted 26 July 1977.

- 1 Gofman, J. W. *et al. Plasma* 2, 413 (1954).
- 2 Fredrickson, D. S. & Levy, R. I. in *The Metabolic Basis of Inherited Disease* (eds Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S.) 545 (McGraw-Hill, New York, 1972).
- 3 Havel, R. J. *Adv. Biol. Med. Phys.* 26, 57-70 (1972).
- 4 Morganroth, J., Levy, R. I. & Fredrickson, D. S. *Ann. int. Med.* 82, 158-174 (1975).
- 5 Fredrickson, D. S., Levy, R. I. & Lindgren, F. T. *J. clin. Invest.* 47, 2446-2457 (1968).
- 6 Bilheimer, D. W., Eisenberg, S. & Levy, R. I. *Circulation* 44, 11-56 (1971).
- 7 Hazzard, W. R. & Bierman, E. L. *Clin. Res.* 19, 476 (1971); *J. lab. clin. Med.* 86, 239-252 (1975).
- 8 Hazzard, W. R., O'Donnell, T. F. & Lee, Y. L. *Ann. int. Med.* 82, 141-149 (1975).
- 9 Utermann, G., Hees, M., Vogelberg, K. H. & Mühlfeßner, G. *5th int. Congr. hum. Genet.*, Mexico City 1976, abstract 124.
- 10 Utermann, G., Hees, M. & Vogelberg, K. H. *Proc. int. Symp. Electrophoresis Isotachophoresis*, Hamburg 1976 (eds Radola, B. J. & Graesslin, D.) 281-291 (de Gruyter, Berlin, 1977).
- 11 Utermann, G. *et al. Clin. Genet.* (in the press).
- 12 Utermann, G., Jaeschke, M. & Menzel, J. *FEBS Lett.* 56, 352-355 (1975).
- 13 Utermann, G. *Hoppe Seyler's Z. Physiol. Chem.* 356, 1113-1121 (1975).
- 14 Hazzard, W. R., Porte, D., Jr & Bierman, E. L. *Metabolism* 21, 1009-1019 (1972).
- 15 Mishkel, M. A. in *Protides of the Biological Fluids* (ed. Peeters, H.) 283-287 (Pergamon, New York, 1971).
- 16 Fredrickson, D. S., Morganroth, J. & Levy, R. I. *Ann. int. Med.* 82, 150-157 (1975).
- 17 Utermann, G., Beisiegel, U., Hees, M., Mühlfeßner, G., Pruin, N. & Steinmetz, A., in *Protides of the Biological Fluids* (ed. Peeters, H.) (Pergamon, New York, 1977).
- 18 Redgrave, T. G., Robert, D. C. K. & West, C. E. *Analyt. Biochem.* 65, 42-49 (1975).
- 19 Roeschlau, P. Z. *klin. Chem. klin. Biochem.* 12, 226 (1974).
- 20 Narayan, K. A., Narayan, S. & Kummerow, F. A. *Nature* 205, 246-248 (1965).

Human serum lipoproteins activate adipocyte plasma membrane adenylate cyclase

BROWN and Goldstein¹ and Stein *et al.*², have shown evidence for the role of a specific cell surface receptor in the binding and catabolism of serum low-density lipoproteins (LDL) by cultured human fibroblasts. This receptor does not seem to be unique to fibroblasts however, since it has been shown that circulating human lymphocytes³, human fat cells⁴ and cultured smooth muscle cells⁵ may also bind LDL; also, binding sites for LDL and very low density lipoproteins (VLDL) seem to be present in crude membrane preparations from various organs and tissues of the pig⁶. These findings suggest that lipoprotein receptors are ubiquitous in mammalian cells and raise the possibility that occupancy of such surface binding sites might modify some aspect of membrane function. Evidence for an alteration of membrane function by lipoproteins was recently reported by Shore and Shore⁷, who observed that human serum VLDL and LDL were capable of specifically activating the human erythrocyte membrane Mg²⁺-ATPase.

The plasma membrane of the adipocyte is intimately involved in lipid metabolism, particularly as a result of its role in the hormone-sensitive adenylate cyclase-triglyceride lipase system⁸. Since the properties and enzyme activities of this membrane may be modified by the components of its external environment, it seemed possible that circulating lipoproteins might affect its function. We describe here the activatory effect of human serum lipoproteins on the adenylate cyclase system of plasma membranes from human and rat adipocytes.

Adipocytes were isolated by collagenase digestion⁹ from the epididymal fat pads of male Wistar rats (150-180 g),

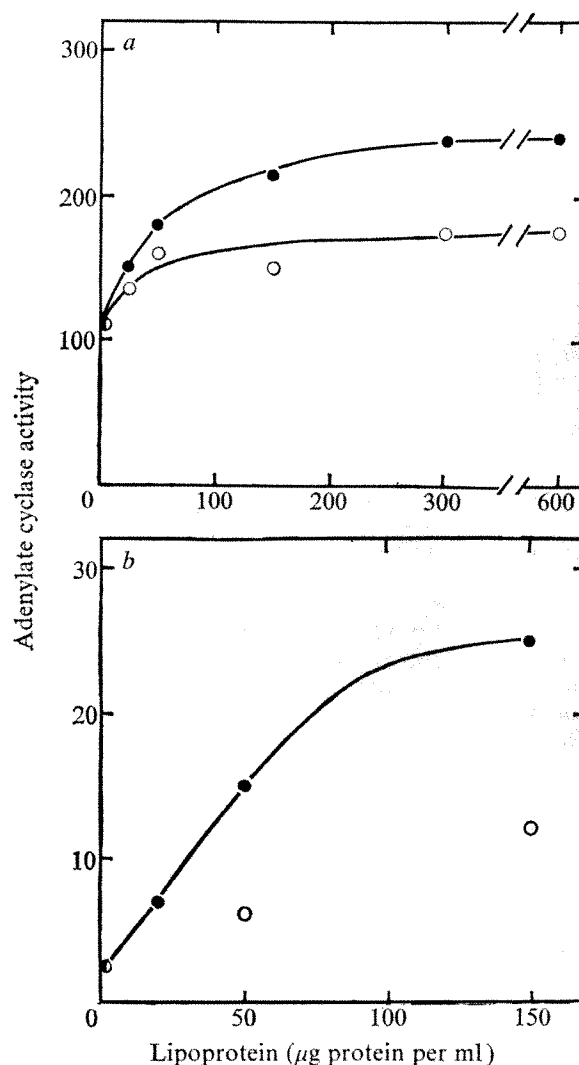


Fig. 1 Adenylate cyclase activity of plasma membranes from rat and human adipocytes as a function of serum lipoprotein concentration. The final incubation medium (50 μl) for the adenylate cyclase assay contained 0.2 mM [α -³²P]-ATP (0.5 μCi) (Radiochemical Centre, Amersham), 5 mM MgSO₄, 1 mM cyclic AMP, 1 mM EDTA, 1 mM dithiothreitol, 50 mM Tris-HCl (pH 7.8), an ATP-regenerating system consisting of 10 mM phosphocreatine (di-Tris salt, Sigma) and 0.5 IU creatine phosphokinase (Calbiochem). Plasma membranes from rat adipocytes (2 μg protein (a)) or from human adipocytes (1.5 μg protein (b)) were added to the reaction mixture (containing 0.2 mM ATP). Preincubation was carried out for 15 min at 30 °C in the presence of LDL (○) or VLDL (●); lipoprotein samples were added in a volume of 10 μl. At the end of the preincubation period, labelled ATP was added and the reaction allowed to proceed for 10 min at 30 °C. The accumulation of labelled cyclic AMP was linear as a function of time. The reaction was terminated according to White¹³ and the ³²P labelled-cyclic AMP was isolated by column chromatography on neutral alumina¹³. Each determination was performed in duplicate. Results are expressed as pmol cyclic AMP formed per min per mg protein. The amount of ATP present at the end of the incubation was determined by thin-layer chromatography on polyethyleneimine-cellulose developed in 1 M LiCl. In these conditions, residual ATP amounted respectively to 92% and 96% of its original concentration, in the absence and in the presence of 260 μg VLDL protein per ml. The product formed in the reaction mixture was identified as authentic ³²P-cyclic AMP by three procedures: (1) it migrated together with authentic cold cyclic AMP on cellulose plates developed in 1 M ammonium acetate and 95% ethanol (1/2.5, v/v); (2) the product of the reaction had the same specific activity when isolated either by the routine method described above or after two chromatographic steps through Dowex AG 50 W-X8 (H⁺ form) and neutral alumina¹⁴; and (3) 90% of the ³²P labelled product isolated in the presence or the absence of lipoproteins in the assay medium of adenylate cyclase was hydrolysed by 3', 5'-cyclic AMP phosphodiesterase from beef heart (Sigma, 20 μg; 20 min incubation at 30 °C).

Table 1 Activation by human serum VLDL of adenylate cyclase from rat adipocyte plasma membranes at several ATP and Mg^{2+} concentrations

ATP (μM)	Adenylate cyclase activity (pmol cyclic AMP per mg protein per min \pm s.e.)*					
	Mg^{2+} (mM)					
	1		5		50	
	0	VLDL (200 μg ml $^{-1}$)	0	VLDL (200 μg ml $^{-1}$)	0	VLDL (200 μg ml $^{-1}$)
10	2.8 \pm 0.6	6.6 \pm 0.4 (137)	16.6 \pm 1.2	27.5 \pm 0.9 (66)	13.1 \pm 0.2	20.1 \pm 0.6 (53)
50	6.8 \pm 0.7	13.6 \pm 1.5 (99)	40.2 \pm 1.6	68.5 \pm 1.5 (70)	54.5 \pm 0.1	79.5 \pm 0.1 (46)
200	6.6 \pm 2.2	7.6 \pm 0.6 (15)	61.3 \pm 8.8	96.7 \pm 6.2 (58)	127.8 \pm 2.1	181.5 \pm 0.2 (42)

Rat fat cell membranes (1.5 μg of protein) were incubated in the experimental conditions described in the legend to Fig. 1. The numbers in parentheses represent percentage cyclase activation relative to the basal level.

* Standard error of triplicate determinations.

while human adipocytes were prepared from subcutaneous fat obtained at surgery. Plasma membranes were separated as previously described¹⁰. Lipoproteins were isolated at 5 °C from the sera of normolipidaemic adult donors by sequential preparative ultracentrifugation (see Havel *et al.*¹¹); the detailed conditions have been previously outlined¹². The density intervals used for the various fractions were: VLDL, $d < 1.006$ g ml $^{-1}$; LDL, d 1.024–1.045 g ml $^{-1}$ and high-density lipoprotein (HDL), d 1.080–1.210 g ml $^{-1}$. All fractions were extensively dialysed at 5 °C against a solution containing 150 mM NaCl, 0.5 mM Na₂EDTA and 5 mM Tris-HCl at pH 7.5.

Human serum VLDL and LDL stimulated the basal activity of adenylate cyclase from human and rat adipocyte plasma membranes (homologous and heterologous systems respectively) in a dose-dependent manner (Fig. 1). Such activation was a saturating phenomenon; half-maximal activation occurred in the concentration range of 50–100 μg VLDL or LDL protein ml $^{-1}$ (Fig. 1). The stimulation of basal adenylate cyclase activity by VLDL was more potent than that elicited by LDL. The effect of HDL on enzyme activity was always inferior to or equal to that of LDL (data not shown).

The degree of activation of the adenylate cyclase system in a given membrane preparation varied with VLDL isolated from different sera. For example, the same human membrane preparation studied with three separate VLDL samples (protein concentration 150 μg ml $^{-1}$), elicited activations amounting to 250%, 1,400% and 1,970%, respectively of that of the basal adenylate cyclase activity determined in standard conditions (for details see Fig. 1 legend). Such observations may probably be attributed to variations in the relative proportions of the different particle species present in each VLDL fraction. A more constant but lower degree of stimulation by human VLDL was found in experiments with plasma membranes from rat

adipocytes (78 \pm 13%, mean value \pm s.e.m., $n = 6$ for 4 membrane preparations), that is, in the heterologous system.

The effect of substrate concentration on cyclase stimulation by VLDL was examined by variation of the ATP concentration in the range 10–200 μM at several Mg^{2+} concentrations in the assay medium. The results indicated a strong substrate ($MgATP$) dependency of the cyclase activation by VLDL which occurred at an Mg^{2+} concentration of 1 mM: at higher Mg^{2+} concentrations, the extent of enzyme activation by VLDL was essentially unchanged in the range 10–200 μM ATP (Table 1).

The possibility that lipoproteins could also modify the cyclic AMP-phosphodiesterase activity in the membrane preparations was investigated. The adenylate cyclase activity measured in the presence of 1 mM cyclic AMP was only 10–15% above that observed in its absence and the extent of cyclase activation by lipoproteins was unaffected by the presence of cyclic AMP. Also, additional experiments indicated that VLDL were without effect on the membrane phosphodiesterases of low and high K_m , whose activities were measured by the method of Thompson and Appleman¹⁵ (data not shown). We conclude therefore that the increased synthesis of ^{32}P -labelled cyclic AMP which we have observed results specifically from an effect of serum lipoproteins on the adenylate cyclase system.

Activation by serum lipoproteins of adenylate cyclase in both homologous and heterologous experimental systems also occurred when the enzyme was maximally stimulated by 0.1 mM adrenaline (bitartrate), by 10 mM fluoride ion or by 0.1 mM guanylyl (β,γ -methylene)-diphosphonate (Gpp(CH₂)p) (Table 2). These findings show that serum lipoproteins do not seem to modify the responsiveness of the cyclase system to hormones or other effectors; similar effects have been reported in other systems following the addition of polycationic peptides¹⁶ or after limited proteolysis¹⁷. Our present observations are, however, consistent with the sug-

Table 2 Effect of human serum VLDL and LDL on adenylate cyclase activity in human and rat fat cell membranes stimulated by adrenaline, fluoride ion and Gpp(CH₂)p

Plasma membranes	Addition	Adenylate cyclase activity (pmol cyclic AMP per mg protein per min \pm s.e.)*			
		None (basal)	Adrenalin (0.1 mM)	NaF (10 mM)	Gpp(CH ₂)p (0.1 mM)
	0	2.5 \pm 0.5	3.5 \pm 0.6	30.3 \pm 7.2	10.1 \pm 1.8
Human	VLDL	25.8 \pm 1.5	28.6 \pm 3.2	220.0 \pm 22.6	81.0 \pm 0.9
	LDL	11.8 \pm 1.5	16.0 \pm 0.8	158.0 \pm 34.0	76.0 \pm 2.6
Rat	0	92.0 \pm 2.3	391.0 \pm 31.0	434.0 \pm 14.3	229.0 \pm 10.4
	VLDL	175.0 \pm 1.6	593.0 \pm 18.2	874.0 \pm 52.1	350.5 \pm 8.5
	LDL	112.3 \pm 1.5	508.0 \pm 21.2	n.d.	n.d.

The experimental conditions were as described in the legend to Fig. 1, except that incubation was performed at 37 °C; the ATP and Mg^{2+} concentrations were 0.2 mM and 5 mM respectively; the amount of membrane protein incubated was 2.2 μg (rat) and 1.5 μg (human). The concentration of lipoproteins was 200 μg protein ml $^{-1}$.

* Standard error of triplicate determinations.

n.d., Not determined.

gestion that VLDL transform the catalytic site of the cyclase into an activated state, in which it retains its susceptibility to stimulation by various effectors.

The mechanism involved in the observed activation by serum lipoproteins of the adenylate cyclase system in adipocyte plasma membranes remains indeterminate. The effect may, however, be dependent on some aspect of the native lipoprotein molecule, since isolated phospholipids (egg yolk lecithin and lysolecithin, added at concentrations equivalent to their content in native VLDL) lead to inhibition of the adenylate cyclase activity in fat cell membranes (preliminary observations). The observed activation of the adenylate cyclase system by VLDL might occur via an intermediate coupling step, involving lipoprotein binding sites on the adipocyte plasma membrane. Thus it seems likely that the apoprotein would be directly concerned, as has been demonstrated in the activation of the erythrocyte membrane Mg^{2+} -ATPase by VLDL (ref. 7). Alternatively, the activation of plasma membrane adenylate cyclase might occur by virtue of a physicochemical interaction between the serum lipoprotein particles themselves and plasma membranes. Thus, evidence has been provided for the exchange and transfer of cholesterol and of phospholipids between serum lipoproteins and a variety of cell membranes (for a review see ref. 18). Such processes, which may occur *in vivo*, may maintain or modify membrane fluidity and are thus implicated in the modulation of the immediate environment of the cyclase system; several lines of evidence indicate that the catalytic activity¹⁸⁻²¹ and hormone responsiveness^{20, 22-25} of the adenylate cyclase system are strongly dependent on the nature of its surrounding lipids.

Papahadjopoulos²⁶ has suggested that serum β -lipoproteins may regulate the activity of several key membrane-bound enzymes, such as the $(Na^+ + K^+)$ ATPase and adenylate cyclase. This hypothesis is consistent with the present observations which indicate that serum lipoproteins may stabilise or modify the adenylate cyclase system of fat cell membranes.

Dominique Lagrange and Françoise Lecot gave excellent technical assistance. Nelly Scharapan kindly prepared the typescript. We gratefully acknowledge Dr M. H. Laudat for her interest in this work. We thank Professor D. Mellièrè for provision of human fat samples. This study was supported by a grant from the Délégation Générale à la Recherche Scientifique et Technique. M.J.C. was the recipient of a French Exchange Fellowship from the Medical Research Council.

JACQUES PAIRAULT
JACQUELINE LEVILLIERS
M. JOHN CHAPMAN

Unité de Recherches sur le
Métabolisme des Lipides,
INSERM U 35
Hôpital Henri Mondor,
94010 Creteil, France

Received 6 April; accepted 11 August 1977.

1. Brown, M. S. & Goldstein, J. L. *Proc. natn. Acad. Sci. U.S.A.* **71**, 788-792; Goldstein, J. L. & Brown, M. S. *J. biol. Chem.* **16**, 5153-5162 (1974).
2. Stein, O., Weinstein, D. B., Stein, Y. & Steinberg, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 14-18 (1976).
3. Ho, Y. K., Brown, M. S., Bilheimer, D. W. & Goldstein, J. L. *J. clin. Invest.* **58**, 1465-1474 (1976).
4. Angel, A. & D'Costa, M. A. *19th International Conference on the Biochemistry of Lipids*, 6-8 Sept 1976, Paris. Abstract book p. 70 (ed. Faculté de Médecine St Antoine, Paris).
5. Steinberg, D., Carew, T. E., Weinstein, D. B. & Koschinsky, T. in *Lipoprotein Metabolism* (ed. Gerten, H.) 90-98 (Springer-Verlag, Heidelberg and New York, 1976).
6. Bachorik, P. S., Livingston, J. N., Cooke, J. & Kwiterovich, P. O. *Biochem. biophys. Res. Commun.* **69**, 927-935 (1976).
7. Shore, V. & Shore, B. *Biochem. biophys. Res. Commun.* **65**, 1250-1256 (1975).
8. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (eds) in *Cyclic AMP* 285-316 (Academic, New York and London, 1971).
9. Rodbell, M. *J. biol. Chem.* **239**, 375-380 (1964).

10. Laudat, M. H., Pairault, J., Bayer, P., Martin, M. & Laudat, Ph. *Biochim. biophys. Acta* **255**, 1005-1008 (1972).
11. Havel, R. J., Eder, H. A. & Bragdon, J. H. *J. Clin. Invest.* **34**, 1345-1353 (1955).
12. Chapman, M. J. & Goldstein, S. *Atherosclerosis* **25**, 267-291 (1976).
13. White, A. A. in *Methods in Enzymology*, **38** part C (eds Hardman, J. G. & O'Malley, B. W.), 41-46 (Academic, New York, 1974).
14. Salomon, Y., Londos, C. & Rodbell, M. *Anal. Biochem.* **58**, 541-548 (1974).
15. Thompson, W. J. & Appleman, M. M. *Biochemistry* **10**, 311-316 (1971).
16. Wolf, J. & Cook, G. H. *J. biol. Chem.* **250**, 6897-6903 (1975).
17. Hanoune, J., Stengel, D., Lacombe, M. L., Feldmann, G. & Coudrier, E. *J. biol. Chem.* **252**, 2039-2045 (1977).
18. Bruckdorfer, K. R. & Graham, J. M. in *Biological Membranes* (eds Chapman, D. & Wallach, D. F. H.) **3**, 103-152 (Academic, London, New York and San Francisco, 1976).
19. Rethy, A., Tomasi, V. & Trevisani, A. *Archs Biochem. biophys.* **147**, 36-40 (1971).
20. Houslay, M. D., Hesketh, T. R., Smith, G. A., Warren, G. B. & Metcalfe, J. C. *Biochim. biophys. Acta* **436**, 495-504 (1976).
21. Engelhard, V. H., Esko, J. D., Storm, D. R. & Glaser, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4482-4486 (1976).
22. Levey, G. S. *Biochem. biophys. Res. Commun.* **43**, 108-113 (1971).
23. Pohl, S. L., Krans, H. M. J., Kozireff, V., Birnbaumer, L. & Rodbell, M. *J. biol. Chem.* **246**, 4447-4454 (1971).
24. Levey, G. S. *J. biol. Chem.* **246**, 7405-7407 (1971).
25. Puchwein, G., Pfeuffer, T. & Helmreich, E. J. M. *J. biol. Chem.* **249**, 3232-3240 (1974).
26. Papahadjopoulos, D. *J. theor. Biol.* **43**, 329-337 (1974).

Interaction of a fluorescent probe with acetylcholine-activated synaptic membrane

GRÜNHAGEN and Changeux have shown^{1,2} that *Torpedo* membrane fragments stained with the antimalarial, quinacrine, show a rapid increase in fluorescence after mixing with cholinergic agonists (see ref. 3). These authors suggest that the fluorescence intensity of quinacrine associated with cholinergic receptors increases during the transition of the receptor from its resting to its active state. We report here an electrophysiological analysis of the kinetic behaviour of the postsynaptic membrane of intact vertebrate muscle fibres treated with quinacrine. The kinetic data from the fluorescence and electrophysiological experiments show remarkable parallels, and we suggest that both experimental procedures reveal the binding of quinacrine to the activated receptor.

The experiments were carried out at room temperature (20-23 °C) on frog endplates using the voltage jump relaxation technique⁴⁻⁷. An endplate was voltage-clamped using a conventional two-electrode arrangement. The endplate current induced by iontophoretic or bath application of acetylcholine (ACh) or carbachol was measured either using extracellular focal recording⁴, or as the total current after subtracting current flowing in the absence of agonist⁵. The first set of experiments was performed using focal current recording and iontophoretic ACh application. The focal recording method yields very small currents recorded during voltage steps in the absence of agonist (upper traces in Fig. 1a, b, c). During ACh action but before treatment with quinacrine, control relaxations were obtained by stepping the membrane potential to a new level and observing the resulting changes in agonist-induced current (Fig. 1a). Following a step hyperpolarisation (marked by a downward arrow) the agonist-induced current jumps very rapidly from its previous steady level to the new level expected from the change in driving force on those channels that are already open just before the jump. The current then increases exponentially to a new steady level, as the ACh-evoked conductance increases from the equilibrium level appropriate to the original holding potential to the level appropriate to the potential during the jump. This conductance increase reflects opening of additional endplate channels. Over the range of agonist concentrations used in the present study, the normal open-close kinetics of the channel behave as if a single molecular inter-conversion takes place



The forward rate constant is increased by increasing the agonist concentration^{6,8} and the back rate constant is decreased by hyperpolarising the membrane⁹.

In the presence of 0.5 μ M quinacrine, the time course of the fast

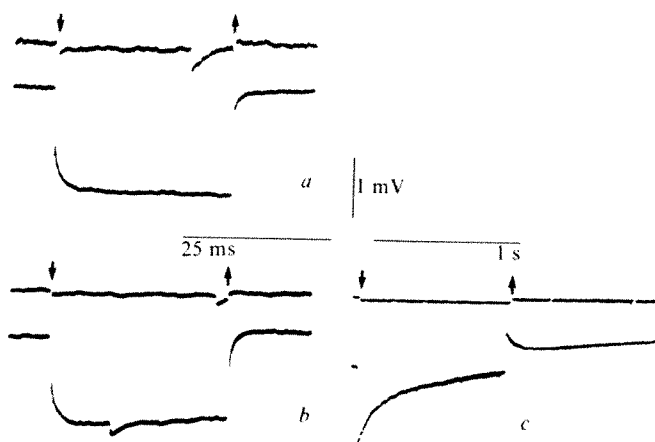


Fig. 1 Effect of quinacrine ($0.5 \mu\text{M}$) on relaxations induced by rectangular voltage jumps (from -60 mV to -100 mV at the downward arrow, and back to -60 mV at the upward arrow) during the action of iontophoretically applied ACh at a voltage-clamped sartorius endplate. Membrane current was recorded as the potential difference between a Ringer-filled fire-polished micropipette placed on the nerve terminal and a similar reference micropipette nearby. Downward deflections represent inward membrane currents. In each pair of traces, the upper trace shows the negligible leakage current during voltage jumps in the absence of ACh. The lower traces show responses to jumps during ACh iontophoretic applications (the absolute vertical shift between the upper and lower traces does not indicate the absolute value of the ACh-induced current at -60 mV). *a*, Control in the absence of quinacrine; *b*, and *c*, in the presence of $0.5 \mu\text{M}$ quinacrine. The same agonist dose was applied in *a*, *b* and *c*. A short jump was applied in *a* and *b* (25-ms time calibration), and a long jump in *c* (1-s time calibration). The extra ACh current which normally develops during the step was greatly decreased in the presence of quinacrine. In *c*, an additional slow inactivation process reflecting desensitisation shows up as an inclined base line. Miniature endplate currents are visible in the left-hand traces.

increase in conductance following a step from -60 mV to -100 mV was little changed (Fig. 1*a**b*). The initial relaxation, however, was now followed by a slow decrease in current (Fig. 1*b*), and if the hyperpolarisation was maintained, the extra agonist-induced current fell back to a low level (Fig. 1*c*). Inactivation of agonist-induced current following a step hyperpolarisation was not seen in the absence of quinacrine in the conditions of the present experiments. On stepping back from -100 mV to -60 mV (upward arrows) there occurred, successively, a very fast ohmic decrease in current, a fast relaxational decrease in current and finally a slow increase in current (Fig. 1*b* and *c*). When applying ACh iontophoretically, the slow relaxation was not always exponential, probably because of local variations in agonist concentration or concurrent desensitisation.

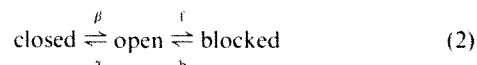
The slow relaxation was then studied in more detail using bath agonist application, total current recording and a fixed quinacrine concentration of $1 \mu\text{M}$. In these conditions (Fig. 2), the slow relaxation, measured after subtracting currents flowing during the step in the absence of agonist, was exponential. Its time constant became smaller as the concentration of agonist (ACh or carbachol) was increased (Figs 2 and 3). The times for half decay of the slow relaxation for -60 mV to -140 mV jumps are shown as a function of agonist concentration in Fig. 3. Individual half-decay times ranged from 2 to 0.04 s . Higher concentrations of carbachol than of ACh were needed to give equal half-decay times.

Our results show that even at low concentrations, quinacrine profoundly modifies the normal kinetic behaviour of the cholinergic membrane, and that in steady-state conditions, it gives rise to a blocking effect more pronounced at more hyperpolarised potentials. This effect of quinacrine at the endplate strikingly resembles the action of procaine on *Aplysia* neurones¹⁰, and may thus reflect the local anaesthetic action of quinacrine postulated by Grünhagen and Changeux¹¹.

The kinetic data reported here, obtained electrophysiologically, and those revealed by fluorescence^{2,3}, display resemblances when

using the same two agonists, in spite of differences in the techniques and preparations used. Since in the present experiments the slow process corresponds to a decrease in conductance, it is possible that the fluorescence increase seen when adding the agonist to microsac suspensions and which has a similar slow time course, does not monitor directly the activation of the receptor. Instead, we propose that quinacrine, like other local anaesthetics^{12,13}, blocks activated receptor-channel complexes. One interpretation of the voltage dependence of the quinacrine effect would thus be voltage sensitivity of the partitioning of cationic quinacrine between the external membrane surface and a blocking site within the channel¹⁴.

In the presence of quinacrine, the scheme describing endplate channel kinetics can be modified to



This scheme predicts two relaxation processes, as observed. It may be shown that if the two relaxation times are well separated, scheme 2 leads to the following expressions for the fast (τ_f) and slow (τ_s) relaxation time constants

$$1/\tau_f = \alpha + \beta + f + b$$

$$1/\tau_s = \frac{\beta f + \alpha b + \beta b}{\alpha + \beta + f + b}$$

In our experiments, the time constant of the control relaxation, $1/(\beta + \alpha)$, was very similar to that of the fast relaxation in the presence of quinacrine. This implies $(\beta + \alpha) \gg (f + b)$. With this condition the equation for τ_s simplifies to $1/\tau_s = f\beta/(\beta + \alpha) + b$.

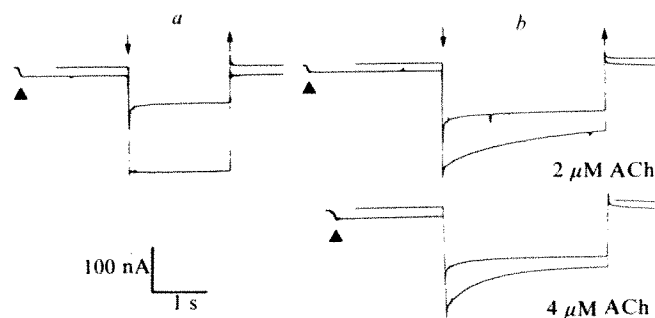


Fig. 2 Slow quinacrine-induced relaxations during bath application of ACh to a voltage-clamped frog muscle endplate. ACh responses to fast agonist superfusion had times to peak less than 10 s . In each pair of traces, the upper trace shows the total clamp current recorded in the absence of ACh before, during and after rectangular hyperpolarising voltage jumps (from -60 mV to -140 mV , downward arrow, and back to -60 mV , upward arrow). Apart from the initial capacity transients, the passive currents are almost constant during the jumps. The lower traces in each pair show inward currents recorded during ACh action at 1-s time calibration, except for the beginning of each trace which shows the onset of the response to ACh perfusion (\blacktriangle) at a tenfold slower time base. The difference between the upper and lower traces in each block represents the ACh-induced endplate current. In the absence of quinacrine (*a*) the additional ACh-induced ($2 \mu\text{M}$) current flowing during the step is well maintained. In the presence of $1 \mu\text{M}$ quinacrine (*b*), the ACh current is not well maintained and shows a progressive decrease. This slow relaxation is clearly faster for $4 \mu\text{M}$ ACh than for $2 \mu\text{M}$. Note that the current at the holding potential evoked by $2 \mu\text{M}$ ACh is only slightly depressed by quinacrine. The fast relaxations in the absence and the presence of quinacrine cannot be resolved with total current recordings. In all bath application experiments using ACh, endplate cholinesterase was inactivated by a 90-min treatment with methyl, *O*-ethyl, 5-ethyldipropylamine phosphorothiolate at $0.5 \mu\text{M}$, followed by extensive washing. After the treatment, $25\text{--}30 \mu\text{M}$ carbachol was needed to match the response to $1 \mu\text{M}$ ACh; this was used as an indication of cholinesterase block. Cholinesterase was blocked so that the ACh concentration in the synaptic cleft would equal the bath concentration.

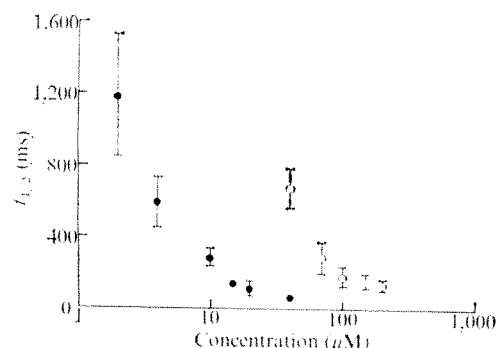


Fig. 3 Mean half-decay times (evaluated from semilog plots of relaxing currents) of slow quinacrine-induced relaxations at -140 mV membrane potential. The quinacrine concentration was $1 \mu\text{M}$, and the agonists were applied in the bath. The bars show ± 2 s.e.m., where this exceeds the symbol size. Experiments were from 13 *Rana pipiens* fibres. The agonist concentration (abscissa) is on a log scale. The data were plotted in this manner to facilitate comparison with Fig. 2 of ref. 2. ●, Acetylcholine; ○, carbachol.

Noise and relaxation experiments^{6,8} in the absence of quinacrine, which provide a direct measure of $(\beta + \alpha)$, show that β increases monotonically as the agonist concentration is increased, in a range comparable with that used here. Our model therefore predicts the monotonic decrease in τ , as the agonist concentration is increased (Fig. 3). The observation that for high agonist concentrations τ seems to reach a limiting value which is larger for carbachol than for ACh (Fig. 3 and Fig. 2 of ref. 3) suggests that carbachol cannot open all the endplate channels, even when acting at high concentration. In this model, the electrophysiological and fluorescence data are to be interpreted in slightly different terms, since the first would reflect the number of conducting channels remaining after quinacrine treatment, whereas intensity of fluorescence would directly monitor the amount of quinacrine bound to the receptor-channel complex.

This work was supported by grants from the CNRS, the DGRST and the ISERM. We thank Rhône-Poulenc for quinacrine.

P. R. ADAMS*
A. FELTZ

Laboratoire de Neurobiologie,
Ecole Normale Supérieure,
46, rue d'Ulm,
75230 PARIS CEDEX 05, France

Received 20 May; accepted 11 August 1977

*Present address: Department of Physiology and biophysics, U.T.M.B., Galveston, Texas 77550.

1. Grünhagen, H. H. & Changeux, J. P. *J. molec. Biol.* **106**, 517-535 (1976).
2. Grünhagen, H. H., Iwatsubo, M. & Changeux, J. P. *C. r. hebdom. Séanc. Acad. Sci., Paris* **D283**, 1105-1108 (1976).
3. Grünhagen, H. H. in *Electrical Phenomena at the Biological Membrane* (ed. Roux, L.), 171-183 (Elsevier, Amsterdam, 1977).
4. Adams, P. R. *Br. J. Pharmacol.* **53**, 308-310 (1975).
5. Neher, E. & Sakmann, B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2140-2144 (1975).
6. Sheridan, R. E. & Lester, H. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3496-3500 (1975).
7. Marty, A., Neild, J. & Ascher, P. *Nature* **261**, 502-504 (1976).
8. Sakmann, B. & Adams, P. R. *Pflügers Arch.* **365**, 145 (1976).
9. Magleby, K. & Stevens, C. F. *J. Physiol., Lond.* **223**, 173-197 (1972).
10. Ascher, P., Marty, A. & Neild, J. *J. Physiol., Lond.* **263**, 121-122P (1976).
11. Grünhagen, H. H. & Changeux, J. P. *J. molec. Biol.* **106**, 497-516 (1976).
12. Adams, P. R. *J. Physiol., Lond.* **246**, 61-63P (1975).
13. Ruff, R. L. *J. Physiol., Lond.* **264**, 89-124 (1977).
14. Woodhull, A. M. *J. gen. Physiol.* **61**, 687-708 (1973).

Role of intracellular calcium in the transient outward current of calf Purkinje fibres

CALCIUM-activated currents are known in red blood cells¹, invertebrate neurones², vertebrate spinal motoneurones³ and skate electroreceptors⁴, and it has been suggested that intracellular calcium increases outward currents in cardiac muscle⁵⁻⁷. The possibility of calcium-activated currents in heart and other muscle^{8,9} is interesting because these cells have intracellular stores that release calcium. Some of the apparent complexity of membrane currents in muscle might be explained as a manifestation of excitation-contraction coupling. If internal calcium (Ca_i) en-

hanced a surface membrane conductance, one might expect a phasic current which reflected the increase and decrease in Ca_i during contraction. No such current has been identified in heart. We report evidence here that intracellular calcium controls the transient outward current which underlies the early repolarisation in cardiac Purkinje fibres^{10,11}. Thus the transient outward current is inhibited by blockers of calcium current, varies directly with the external calcium concentration and correlates with twitch amplitude in conditions which vary the amount of calcium released.

Short Purkinje fibre preparations from calf hearts were voltage-clamped by a conventional two-microelectrode method. In some experiments, contractile force was measured with a piezoelectric force transducer (Endevco 87102). Interference from the excitatory sodium current was prevented by blocking sodium channels with tetrodotoxin ($20 \mu\text{M}$) or inactivating them with a depolarised holding potential (~ -40 mV). The transient outward current is referred to as I_{qr} because the kinetic variables q and r have been used previously to describe its activation and inactivation^{11,12}.

In Fig. 1a, trace 1 shows a record of membrane current during a clamp depolarisation in the range of the action potential plateau. The outward transient current (I_{qr}) is dominant for the first 150 ms after the step depolarisation and obscures the slow inward calcium current (I_{Ca}). I_{Ca} turns on within a few ms after the depolarisation and then inactivates slowly¹³; the inactivation is largely responsible for the slow current change during the last 300 ms of the pulse. Efforts at quantitative analysis of I_{Ca} in Purkinje fibres have been hampered by such overlap with I_{qr} (refs 14, 15). We tried to determine I_{Ca} without interference from I_{qr} by a pharmacological dissection involving D600, a blocker of calcium currents¹⁶. But D600 inhibited the outward transient as well as I_{Ca} . In preparations showing large I_{qr} (Fig. 1a) the early current in the absence of D600 (1) was more outward than the current in its presence (2). Thus, the 'D600-sensitive current' (1-2, Fig. 1b) displayed a region of net outward current. We attribute the outward region to I_{qr} (shaded area) in combination with I_{Ca} . The inward spike in Fig. 1b is explained as a rapid activation of I_{Ca} preceding the activation of I_{qr} . Similar net current waveforms have been observed in crustacean muscle and have been analysed as a transient outward current superimposed on an inward calcium current^{9,17}.

The consistent reduction of I_{qr} obtained with D600 invalidates the use of this drug to determine I_{Ca} . But the results also suggested that I_{qr} is dependent on intracellular calcium. On this view, the reduction of I_{qr} would be accounted for as a natural consequence of the inhibition of calcium entry. We explored this hypothesis using procedures believed to modify calcium influx. Figure 1c shows that manganese ion, another widely-used blocker of calcium currents^{13,14}, resembles D600 in the ability to reduce I_{qr} . Figure 1d shows the effects of increasing the extracellular calcium concentration from 1.8 to 5.4 mM. Increased slow inward current is evident from the augmented early spike (arrows) and the downward displacement of current during the last 400 ms of the clamp pulse. The increase in external calcium (Ca_o) also produced a clear enhancement of I_{qr} , which was fully reversible (record not shown). An increase of Ca_o from 5.4 to 9.0 mM caused a further increase in I_{qr} .

Another approach to the possible involvement of intracellular calcium relied on contractile force to report changes in myoplasmic calcium activity. We took the amplitude of the twitch as a rough index of the calcium transient and compared it with the surge of I_{qr} during depolarising clamp pulses. The size of the calcium transient was varied by manipulating the previous history of membrane potential¹⁸. Figure 2a shows a typical voltage clamp protocol consisting of two identical depolarising pulses separated by a variable interval. The first pulse followed a 5-s quiescent period and produced a large transient outward current and a sizeable twitch contraction. The second pulse was applied after a 1.5-s repolarisation and evoked a smaller I_{qr} and smaller twitch. Earlier experiments of this type have shown that the level and duration of the repolarisation strongly influence the restitution of the second contraction¹⁸ and the recovery of the outward transient current¹¹. Here the question is whether these recovery processes share the same time and voltage dependence. Because this

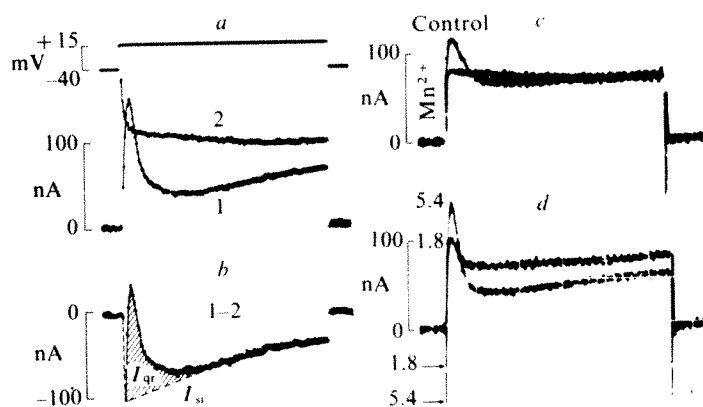


Fig. 1 Effects of D600, manganese and calcium on the transient outward current (I_{qr}). *a*, Membrane current in the absence of drug (1) and after exposure to D600 (5×10^{-6} g ml $^{-1}$) for 30 min (2). The current records accompanied a rectangular membrane depolarisation from -40 to $+15$ mV for 500 ms (upper trace). Signals were recorded by a digital computer, sampling once every 3 ms. The smooth line was drawn by eye for clarity; preparation S22-1. *b*, 'D600-sensitive current', obtained by subtracting trace (2) from trace (1). Shaded area is estimate of I_{qr} , dashed line estimate of I_{qr2} . *c*, Membrane current in a control run and after exposure to 3 mM $MnCl_2$ for 9 min. The currents were evoked by a 500-ms clamp pulse from -38 to $+13$ mV. *d*, Membrane current in 1.8 mM Ca and 10 min after increasing the external calcium concentration to 5.4 mM. The arrows indicate the peak inward spike of current, an approximate measure of peak I_{qr} . Currents were in response to a 520-ms clamp pulse from -39 to $+13$ mV. *c* and *d*, Preparation R33-2. The composition of the Tyrode solution used in these experiments was 150 mM NaCl, 4 mM KCl, 10 mM Tris-HCl buffer, pH 7.4, 0.5 mM $MgCl_2$, 5.4 mM $CaCl_2$ and 5 mM glucose.

comparison depends on a fairly accurate determination of I_{qr} , we reduced interference from I_{si} by choosing preparations with relatively large I_{qr} , by using strong depolarisations to increase the driving force for outward current and by estimating I_{qr} with a graphical procedure (Fig. 2a) which made some allowance for I_{si} .

Figure 2b shows the influence of potential level on the recovery of the twitch and of I_{qr} . The results were obtained with a 1.5-s repolarisation interval and were normalised relative to the responses evoked by the first pulse. The contraction and the transient outward current show nearly identical dependence on membrane potential. Similar correspondence was observed in five out of six preparations. Figure 2c shows the recovery of the twitch and I_{qr} as the repolarisation interval increased progressively. Both signals show a similar time course. Temporal correlation was observed in six out of seven experiments of this kind, and was found at -70 mV as well as near -40 mV. The occasional lack of correlation might be explained by exceptionally severe contamination by I_{si} .

Although parallelism between I_{qr} and contractile force is clear and rather consistent, the agreement could be considered fortuitous. A purely coincidental explanation is not supported, however, by experiments in which the recovery time course was manipulated drastically. Cardiac glycosides and aglycones were useful for this because they modify the time course of contractile restitution as well as increasing the strength of contraction¹⁹. Figure 3 shows the influence of strophanthidin on the recovery of the twitch and of I_{qr} . In the absence of the drug, both transients showed a monotonic recovery with increasing repolarisation interval as in Fig. 2c. After exposure to 1 μ M strophanthidin, contractile restitution and recovery of I_{qr} both became oscillatory, with the same period and phase. The discrepancy in the height of the oscillatory peaks at 0.5 s could be explained if I_{qr} were more seriously underestimated at this time. Contamination from I_{si} may have been more severe at the peak because other experiments have shown that strophanthidin evokes a clear oscillation in the repriming of I_{si} (ref. 20). The inset in Fig. 3 illustrates another experiment using strophanthidin in which the time courses of I_{qr}

and I_{si} were particularly distinct. Clearly the amplitudes of I_{qr} , I_{si} and twitch force were each enhanced by a second pulse relative to their size during the first pulse.

These kinetic correlations between I_{qr} and twitch force, taken together with the actions of D600, manganese and extracellular calcium, strongly suggest that the transient outward current is controlled by intracellular calcium activity. The myoplasmic calcium could be supplied by release from intracellular stores or entry across the surface membrane. Contractile force provides a convenient and sensitive bioassay for the size of the intracellular calcium transient. This approach has drawbacks, however, since the peak of the twitch must lag behind the peak of calcium activity near the surface membrane because of diffusional, chemical and mechanical delays. It is possible that the waveform of I_{qr} is a more faithful representation of the time course of calcium activity than the externally measured force. Direct measurements of the calcium transient may soon be possible using optical indicators^{21,22}. Such experiments would provide another test for the present hypothesis and might determine whether intracellular calcium governs the time course of I_{qr} or if it merely acts as a trigger for a phasic conductance change.

Our results suggest that some of the time and voltage dependence of the transient outward current arises from the same mechanisms which underly activation and inactivation of contraction. The ionic nature of the outward current remains unsettled. Chloride was originally thought to carry I_{qr} because the outward transient was reduced by replacing chloride with larger anions^{10,11}. But Kenyon and Gibbons²³ have shown that the

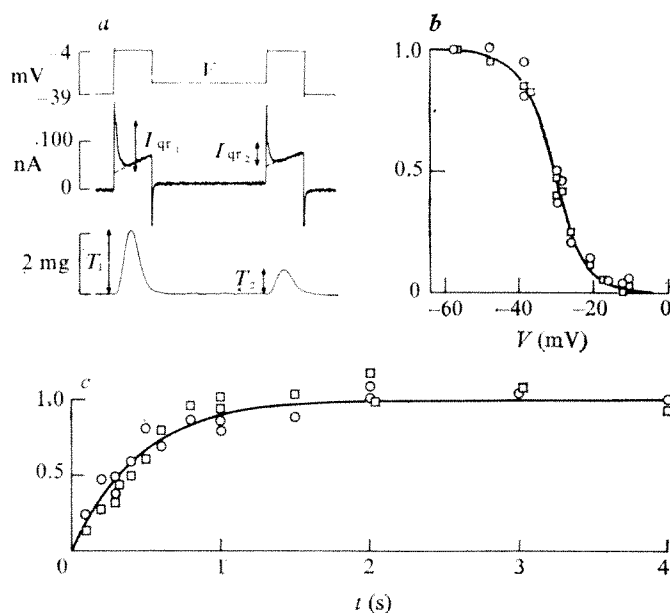


Fig. 2 *a*, Experimental records illustrating the protocol for obtaining the curves in (b) and (c). The traces (records from a Brush 440 pen recorder) show, from top to bottom, membrane voltage, membrane current and contractile force. The magnitude of I_{qr} was measured as the difference between the peak of the outward transient and a linear extrapolation of the late current to the time of peak I_{qr} (dashed line). This method will underestimate I_{qr} because it underestimates I_{si} . To correct for small progressive changes during the run, currents and tension during the second pulse were normalised by the response to the first pulse. *b*, Relationship between repriming potential (V) and the inactivation of I_{qr} (\square) and tension (\circ). The normalised I_{qr} and tension at a given repriming potential were divided by the normalised values for $V = -57$ mV to yield the inactivation curve. The continuous line was drawn according to $r = [1 + \exp((V + 31)/4)]^{-1}$, where r is a Hodgkin-Huxley type variable with values between 0 and 1. *c*, Comparison of the recovery from inactivation of I_{qr} and tension. Two depolarising voltage-clamp pulses were separated by repolarisation to the holding potential for a variable interval (t). Normalised I_{qr} and tension were plotted as a function of t . Symbols are as in (b). The continuous line is an exponential with a time constant of 450 ms. Voltage-clamp pulses in (a), (b) and (c) depolarised the membrane to -4 mV for 500 ms from a holding potential of -39 mV; preparation R25-2.

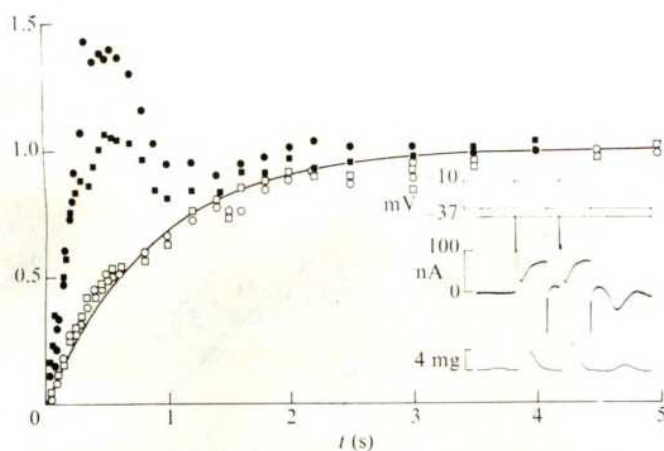


Fig. 3 Alteration of the repriming time courses of I_{qr} and tension by strophanthidin. In the absence of strophanthidin the repriming time courses of I_{qr} (\square) and tension (\circ) are very similar. The continuous line is an exponential with a time constant of 880 ms. The closed symbols were obtained 15 min after addition of $1 \mu\text{M}$ strophanthidin. Both repriming curves then show distinct oscillations. Repriming curves obtained with 500-ms clamp pulses from -40 mV to -10 mV . Preparation R27-2. The inset shows a chart recording from a less complete experiment in the presence of $1 \mu\text{M}$ strophanthidin; preparation R26-7.

substitute anions act by reducing extracellular calcium activity. Their results are consistent with the sensitivity of I_{qr} to deliberate variations in Ca_0 (Fig. 1d). Reports of calcium-activated potassium currents in other tissues raise the possibility that potassium ions help carry I_{qr} . Whatever the ionic basis of I_{qr} , its apparent dependence on intracellular calcium raises fundamental difficulties for analysis of the slow inward current in Purkinje fibres. A drug which interacts specifically with Ca_i channels will still have indirect effects on I_{qr} . Thus proper pharmacological dissection of Purkinje fibre plateau currents will require a method for selectively abolishing I_{qr} .

We thank Dr Peter McNaughton for suggesting that the transient outward current might be activated by intracellular calcium, and Drs K. Beam, T. Colatsky, W. R. Gibbons, J. L. Kenyon and R. Weingart and Mr W. Gilly for helpful discussions. This work was supported by a grant from the USPHS.

S. A. SIEGELBAUM
R. W. TSJEN
R. S. KASS

Departments of Pharmacology and Physiology,
Yale University School of Medicine,
333 Cedar Street,
New Haven, Connecticut 06510

Received 14 June; accepted 16 August 1977

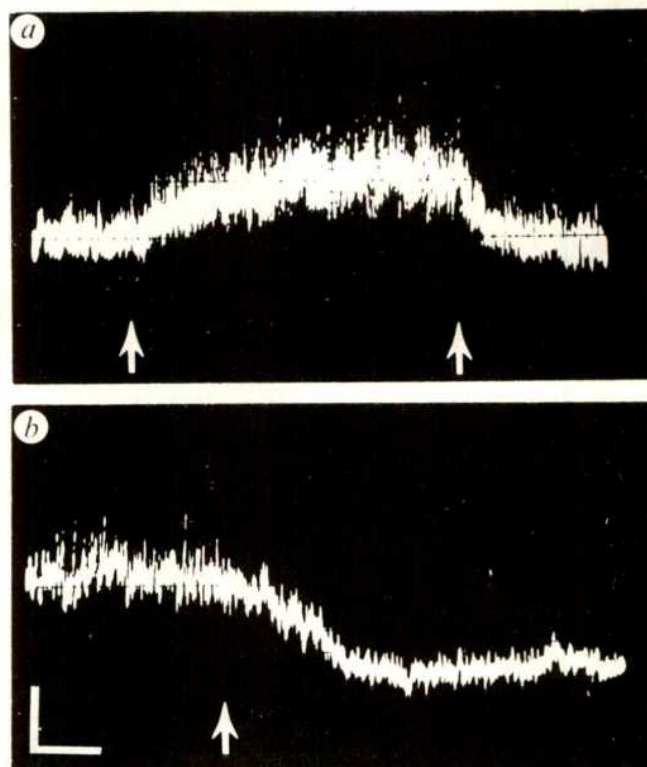
1. Lev, V. L. & Beauge, L. in *Transport across Biological Membranes* (eds Giebisch, G., Tosteson, D. C. & Ussing, H. H.) 2 (Springer, Berlin, 1977).
2. Meech, R. W. & Standen, N. B. *J. Physiol., Lond.* **249**, 211-239 (1975).
3. Krnjević, K. & Lisiewicz, A. *J. Physiol., Lond.* **225**, 363-390 (1972).
4. Clusin, W. T. & Bennett, M. V. L. *J. gen. Physiol.* **69**, 145-182 (1977).
5. Isenberg, G. *Nature* **253**, 273-274 (1975).
6. Bassingthwaite, J. B., Fry, C. H. & McGuigan, J. A. S. *J. Physiol., Lond.* **262**, 15-37 (1976).
7. Colatsky, T. J. & Hogan, P. F. *Fed. Proc.* **34**, 375 (1975).
8. Vassort, G. *J. Physiol.* **252**, 713-734 (1975).
9. Mounier, Y. & Vassort, G. *J. Physiol., Lond.* **251**, 609-625 (1975).
10. Dudel, J., Peper, K., Rudel, R. & Trautwein, W. *Pflügers Arch. ges. Physiol.* **295**, 197-212 (1976).
11. Fozzard, H. A. & Hiraoka, M. *J. Physiol., Lond.* **234**, 569-586 (1973).
12. McAllister, R. E., Noble, D. & Tsien, R. W. *J. Physiol.* **251**, 1-59 (1975).
13. Reuter, H. *Prog. Biophys. molec. Biol.* **26**, 1-43 (1973).
14. Vitek, M. & Trautwein, W. *Pflügers Arch. ges. Physiol.* **323**, 204-218 (1971).
15. Gibbons, W. R. & Fozzard, H. A. *J. gen. Physiol.* **65**, 367-384 (1975).
16. Kohlhardt, M., Bauer, B., Krause, H. & Fleckenstein, A. *Pflügers Arch. ges. Physiol.* **335**, 309-322 (1972).
17. Henck, M. & Zachar, J. *J. Physiol., Lond.* **268**, 51-71 (1977).
18. Gibbons, W. R. & Fozzard, H. A. *Circ. Res.* **28**, 446-460 (1971).
19. Ferrer, G. R. *Circ. Res.* (in press).
20. Kass, R. S., Tsien, R. W. & Weingart, R. *Biophys. J.* **17**, 4a (1977).
21. Blinks, J. R., Prendergast, F. G. & Allen, D. G. *Pharmacol. Rev.* **28**, 1-93 (1976).
22. Brown, J. E. *et al.* *Biophys. J.* **15**, 1155-1160 (1975).
23. Kenyon, J. L. & Gibbons, W. R. *J. gen. Physiol.* (in press).

Voltage noise from hair cells during mechanical stimulation

THE generator potential of mechanoreceptor hair cells is thought to arise from a change in conductance of the receptor membrane¹⁻⁴. A mechanical force in the environment acts on hairs to cause the generator potential. The conductance change associated with the generator potential may be composed of many elementary conductances whose frequency or number vary with mechanical stimulation. From this hypothesis, one would expect to observe random fluctuations, or noise, associated with the generator potential. We report here the presence of such fluctuations in an invertebrate statocyst; the analysis of the fluctuations gives an estimate of the size of the underlying events associated with the generator potential during mechanical stimulation.

Hair cells of the invertebrate statocyst are presumably stimulated by statoconia. Increased interaction between statoconia and the cilia depolarises the hair cells; decreased interaction hyperpolarises them. The statoconia, however, are in constant motion. Random movement of these particles should cause a baseline noise¹⁻³. Figure 1 shows intracellular recordings from a hyperpolarised hair cell body of *Hermisenda crassicornis*. The cells were hyperpolarised by injecting current through the recording electrode to suppress spontaneous firing which normally occurs near rest at -55 mV . The preparation and the rotational method of physiological stimulation have been described elsewhere³. Hair cell somata were passively invaded by action potentials arising in the axon $80 \mu\text{m}$ from the soma, and received synaptic input at axonal branches $100 \mu\text{m}$ from the soma. The generator potential and voltage noise can be studied in the absence of impulses and synaptic interactions by cutting the distal axon (cut N⁵). Fig. 1a shows a depolarising response from a hair cell (cut N) in front of the force vector. In the absence of rotation, the variance in the voltage noise was $9.3 \times 10^{-8} \text{ V}^2$. A rotational velocity of 60 r.p.m.

Fig. 1 Intracellular recordings (0-313 Hz) from statocyst hair cells. The vertical bar represents 5 mV, the horizontal bar 5 s. *a*, A hair cell in front of the force vector with cut axon (cut N) to remove synaptic input. Rotation begins and ends at the arrows, and produces a maximum steady value of 0.8 g midway during the response. The holding potential is -100 mV . *b*, A hair cell with intact axon behind the force vector. Rotation begins at the arrow and produces a maximum steady value of 1.4 g. The holding potential is -80 mV .



for a cell located 19 cm from the centre of rotation, produced an average depolarisation of 5.5 mV and an increase in the variance to $29.3 \times 10^{-8} \text{ V}^2$. Figure 1b shows the opposite effect from a cell behind the force vector, the axon of which was left intact. The noise variance decreased from 12.0×10^{-8} to $2.1 \times 10^{-8} \text{ V}^2$ as a result of a rotational velocity of 75 r.p.m. The mean hyperpolarisation was 9 mV.

The variance of the microelectrode and amplifier noise, measured in the external bath, was less than 10^{-8} V^2 . The noise increased as the microtip entered the statocyst. Hyperpolarising the membrane with current further increased the noise (Fig. 2). Microelectrode noise measured in the external bath increased negligibly over the current range used. The voltage was held between -80 and -100 mV in the rotation experiments. In this range, the 'background noise', that is, membrane noise plus microelectrode and amplifier noise, varied between 2×10^{-8} and $10 \times 10^{-8} \text{ V}^2$. Changes were then studied as a function of the physiological stimulus.

Autocorrelation functions of the noise are shown in Fig. 3. At -80 mV and zero rotational velocity, the correlation function is approximated by a single exponential with time constant 74 ms. The 7-mV hyperpolarisation caused by rotation reduced the noise and increased the time constant to 168 ms. An increase in the speed of rotation, increased the hyperpolarisation to -9 mV and reduced the noise, with little further effect on the time constant. The noise level at -9 mV approached that caused by microelectrode and amplifier noise. A cell with similar background noise is shown for the depolarising response. Depolarisation to 16 mV increased the noise variance with no appreciable change in time constant. The smallest time constant observed for a depolarising response was 62 ms. The largest time constant observed for a hyperpolarising response was 178 ms.

The results of many experiments are summarised in Fig. 4 in which the change in noise variance is compared with the mean change in membrane potential, without regard to sign. Hyperpolarising and depolarising responses are presented for both intact hair cells and from hair cells with cut axons (cut N). The change in variance was roughly proportional to the change in the mean. A solid line, approximately determined by the data points, has a

Fig. 2 Intracellular recordings from statocyst hair cells, hyperpolarised from their resting value by current injection. The average resting potential is about -55 mV. One very noisy cell (upper dashed line) and one very quiet cell (lower dashed line) are shown. The solid line is drawn through pooled data from many cells.

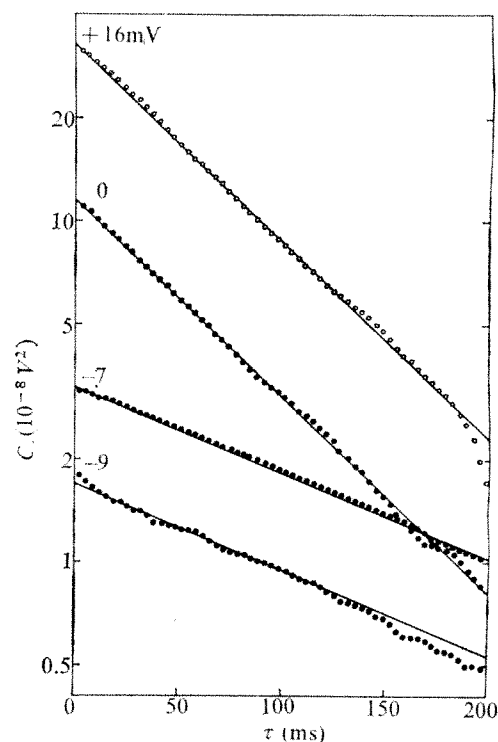
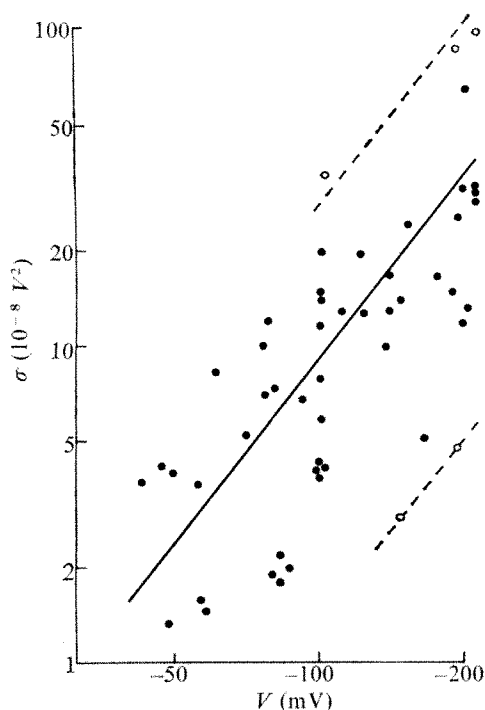


Fig. 3 Autocorrelation functions of the noise in various conditions of rotation. The sample length was 27.31 s during steady rotation. The data were recorded on FM tape at 3.75 i.p.s. (0–625 Hz) after passing the signal through an RC high pass filter with a 2.2 s time constant. Processing was carried out off-line with a d.c.-coupled HP 3721A correlator after passing the signal through a RC high pass filter with an 8 s time constant. ●, Noise from a hair cell behind the force vector, at zero rotation and two levels of rotation which produce steady hyperpolarisations of 7 mV at 1.2 g and 9 mV at 1.4 g. ○, Depolarising response of 16 mV at 1.2 g from a cell with approximately the same zero rotation background noise. The lines represent exponentials with time constants 74 ms (upper) and 168 ms (lower). C is the value of the autocorrelation for relative time displacement τ .

slope of $12 \mu\text{V}$. This slope may be taken as the order of magnitude of the elementary voltage event associated with mechanotransduction. The dashed line in Fig. 4 represents the expected distortion of the variance-to-mean relationship produced by the summation of elementary increases in conductance. The soma membrane resistance with rest potential E is taken in parallel with the receptor membrane resistance with reversal potential e . The correction factor to $\Delta\sigma^2$ is f^4 and to ΔV is f , where $f = V_0/(V_0 - \Delta V)$ and $V_0 = e - E$ is the maximum possible ΔV . This analysis is similar to that applied to acetylcholine-induced noise at the neuromuscular junction⁶ and to changes in noise induced by light in bipolar cells and photoreceptors⁷.

Two experiments in Fig. 4 are from an abnormal *Hermisenda* statocyst which has only one or two statoconia instead of several hundred. Such a statocyst, which may be a mutant form, offers the possibility of studying the elementary statoconium-hair cell interaction more directly.

Are the changes in noise produced by rotation caused by the mechanotransduction or by changes in membrane potential? A change in membrane potential by current injection has the opposite effect to a change caused by physiological stimulation. For example, a hyperpolarisation caused by current increased the noise (Fig. 2), but a hyperpolarisation caused by rotation decreased the noise (Figs 1 and 4). The converse was true for the depolarising direction. We conclude that the changes in hair cell noise analysed here are caused by mechanotransduction.

Since the change in variance is roughly proportional to the mean, the potential changes due to mechanotransduction can be considered as the sum of random events according to a simple shot model. The model is discussed fully by Katz and Miledi⁶. The basic relationship for a random sum of exponential events, each of height a is: $a = 2\Delta\sigma^2/\Delta V = 24 \mu\text{V}$. The spread of data in Fig. 4 is

due to a variation in the time constant with different levels of rotation (Fig. 3) and to the subtraction procedure.

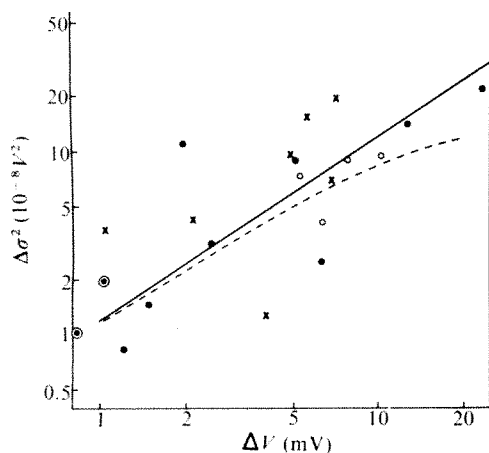
The largest time constant previously observed by the displacement of voltage with current pulses was 45 ms¹. The relaxation times shown in Fig. 3 may be caused by transduction or by an induced change in input resistance. The input resistance could be expected to decrease for the depolarising response and increase for the hyperpolarising response, although we have no direct evidence that this occurs. In either case, a random event with a single relaxation time for a particular stimulus describes the effect shown in Fig. 4. The input resistance of the hair cells at rest is 80–100 MΩ. If the amplitude of an individual event is taken as 24 μV, one calculates an elementary current of approximately 0.3 pA for each event. It was shown recently that hair cell generator potentials and voltage noise are dependent equally on sensory stimuli, membrane potential, external sodium concentration, and treatment with the anaesthetic, chloral hydrate (D.L.A., Y. Grossman and E. Heldmann, unpublished).

These observations support the hypothesis that the voltage noise reported here involves the same mechanism that underlies the generator potential. The generator potential arises from a conductance increase with a reversal potential of about 30 mV positive with respect to rest¹. From an average holding potential of -90 mV, we assume a driving force of 60 mV. The elementary conductance change associated with each event is therefore about 5 pS. This is the order of conductance found for individual ionic channels⁸. Although there is considerable uncertainty in the reversal potential, even if 30 mV positive with respect to zero is assumed, the estimate for the elementary conductance change is merely halved.

Our observations show that the changes in hair cell membrane potential do not account for the changes in noise produced by a physiological stimulus. The changes in noise produced by rotation are primarily caused by the transduction process itself, indicating a common origin for voltage noise caused by rotation and by the hair cell generator potential. We conclude that the generator potential is the result of a random sum of elementary events which have an average effect (the generator potential) and a fluctuating component (the voltage noise). Local potential phenomenon previously reported by Alkon³ do not occur at the holding potentials used here, nor do they occur in hair cells with cut axons. Thus, such effects could not contribute to the noise studied here.

The number of hairs interacting with one statoconium is

Fig. 4 Change in noise variance ($\Delta\sigma^2$) against the change in mean membrane potential (ΔV) at different levels of rotation. \times , Hair cells with cut axons (cut N) showing a depolarising response. Hair cells with intact axons showing a depolarising (○) or hyperpolarising (●) response. (●) From an abnormal statocyst with one or two statoconia. Eleven different cells are represented in this figure. The solid line represents $\Delta\sigma^2/\Delta V = 12 \mu V$. The dashed curve represents the expected nonlinear effect of increased conductance on the depolarising response assuming a maximum ΔV of 90 mV. The variance was taken as the first point of autocorrelation functions calculated from 13.65 or 27.31 s of data.



estimated to be between 1 and 20, based on scanning electron micrographs (D.L.A., Y. Grossman and E. Heldmann, unpublished). Discrete depolarising waves observed in the voltage noise near rest³ probably reflect the interaction of a statoconium with one or more cilia. These depolarising waves range from 1–10 mV. If the lower limit of 1 mV is taken to approximate the interaction of a single statoconium with a single hair, 40 elementary events, each causing a 5-pS increase of conductance, would be necessary. Noise analysis offers the possibility of relating molecular membrane phenomena, which are not directly observable, to the mechanism of the generator potential of a sensory receptor.

LOUIS J. DEFELICE

Department of Anatomy,
Emory University,
Atlanta, Georgia 30322

DANIEL L. ALKON

Laboratory of Biophysics,
NINCDS, NIH,
Marine Biological Laboratory,
Woods Hole, Massachusetts 02543

Received 28 February; accepted 1 August 1977.

- ¹ Alkon, D. L. & Bak, A. *J. gen. Physiol.* **61**, 619–637 (1973).
- ² Wiederhold, M. L. *Brain Res.* **78**, 490–494 (1974).
- ³ Alkon, D. L. *J. gen. Physiol.* **66**, 507–530 (1975).
- ⁴ Hudspeth, A. J. & Corey, D. P. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2407–2411 (1977).
- ⁵ Detwiler, P. & Alkon, D. L. *J. gen. Physiol.* **62**, 618–643 (1973).
- ⁶ Katz, B. & Miledi, R. *J. Physiol. Lond.* **224**, 665–699 (1972).
- ⁷ Simon, E., Lamb, T. D. & Hodgkin, A. L. *Nature* **256**, 661–662 (1975).
- ⁸ DeFelice, L. J. *Int. Rev. Neurobiol.* **20** (in the press).

Stimulation of the sympathetic nervous system during sucrose feeding

THE activity of the sympathetic nervous system in the rat is markedly reduced by 2 d of fasting, and this suppression is reversed by feeding¹. Studies of urinary catecholamine excretion in fasting rats have corroborated these findings and in addition have shown that urinary adrenaline and noradrenaline (NA) excretion actually exceed baseline levels on the second and third days of refeeding². Since dietary intake in these previously fasted animals was greater than baseline during the refeeding period, the possibility arose that overfeeding might be associated with increased sympathetic activity and led to the present investigation. Work by others has provided evidence that supplementation of a standard rat chow diet with sucrose-containing drinking solutions increases total caloric intake of the rat by approximately 20%³. Thus the experimental design for voluntary overfeeding in the rat is based on the addition of sucrose-containing drinking solutions to the animals' *ad libitum* access to rat chow and water during the experimental period. In this report we describe increased sympathetic activity in the hearts of overfed animals after 3 d of sucrose supplementation. Such an increase in sympathetic activity may contribute to the enhanced thermogenesis associated with overfeeding.

The use of NA turnover techniques in assessing the functional state of the unperturbed sympathetic nervous system permits the estimation of organ-specific sympathetic activity in each sympathetically innervated organ. NA, the sympathetic neurotransmitter, is synthesised and stored within the sympathetic nerve endings and is released in response to neural impulses. An active transport mechanism in the neuronal membrane recaptures a major fraction of the released NA. As the activities of both NA biosynthesis and NA re-uptake are coupled to the level of impulse traffic, the endogenous level of NA within the nerve terminals in a particular organ is relatively constant despite wide fluctuations in sympathetic activity. The kinetic measurements of NA turnover used here use these neuronal processes of NA biosynthesis and active transport to quantitate changes in the level of sympathetic activity in experimental conditions. The ³H-NA turnover

technique involves the intravenous (i.v.) administration of tracer doses of ^3H -NA. After uptake of the label into the sympathetic nerve endings and equilibration with intraneuronal stores, the rate of disappearance of the tracer provides an indication of sympathetic activity within a particular organ. Previous work with this technique has established its usefulness as a measure of sympathetic activity in various physiological and pathophysiological conditions⁴⁻⁶. A second method of assessing NA turnover involves inhibition of NA biosynthesis with α -methyl-*P*-tyrosine (α MPT), an inhibitor of tyrosine hydroxylase, the rate-limiting step in NA biosynthesis. In the presence of such inhibition endogenous NA levels cannot be maintained and the rate of fall of endogenous NA reflects NA turnover. Intraneuronal metabolism of NA contributes to a limited extent to these measurements of NA turnover, but is not altered by the experimental conditions described here.

Female Sprague-Dawley rats (150–200 g) were used in these experiments. These animals were allowed free access to Purina rat chow and water except as noted in the experimental protocols. The effect of 3 d of sucrose feeding on NA turnover as measured by the rate of disappearance of ^3H -NA is shown in Fig. 1. In this experiment control (normal diet) and sucrose-fed animals received an i.v.

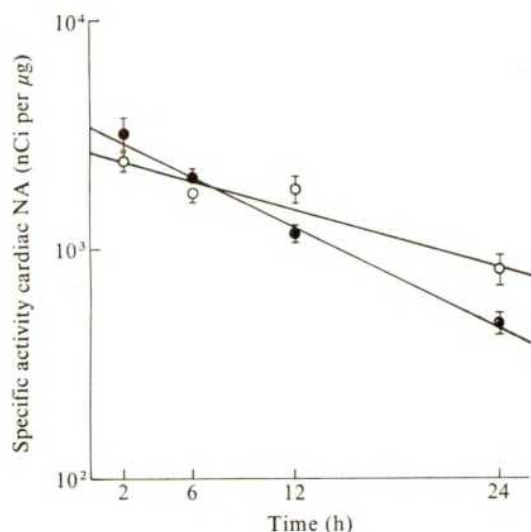


Fig. 1 ^3H -NA turnover in rat heart in control and sucrose-fed animals. Female Sprague-Dawley rats (150–200 g) were divided into two groups 3 d before the start of the NA turnover experiment. Control animals (○) remained on their *ad libitum* diet of rat chow and water; sucrose-fed animals (●) were provided access to an 8% sucrose solution in addition to their rat chow and water. At the beginning of the turnover, each animal received an i.v. injection of ^3H -NA (250 μCi per kg body weight; specific activity 24.4 Ci mmol⁻¹; New England Nuclear) by tail vein. At 2, 6, 12 and 24 h after injection, five to seven rats from each group were killed, their hearts removed and quickly frozen on dry ice. The organs were stored at -20°C until analysed within 2–3 d. After the organs were homogenised in ice-cold 0.4 M perchloric acid, NA was isolated chromatographically with alumina and the eluates analysed for ^3H -NA and NA content, as previously described⁶. Values were corrected for recovery (80–90%) as determined in each experiment. Specific activity of cardiac NA was determined for each time point, expressed as mean \pm s.e.m. The line representing the decline in specific activity with time was calculated by the method of least squares. For both control and sucrose-fed groups, the significance of each slope is $P < 0.001$. For the control group the slope or fractional turnover rate is $4.80 \pm 0.16\%$ per h with a NA turnover rate of 21.4 ± 1.9 ng NA per heart per h (95% confidence interval). For the sucrose-fed group the fractional turnover rate is $8.49 \pm 0.13\%$ per h and the NA turnover rate 30.6 ± 2.3 ng NA per heart per h (95% confidence interval). The slopes are significantly different at the $P < 0.001$ level using the Student's *t* test. Sucrose intake averaged 5.5 g per rat per d for the experiment. For control, $t_1 = 14.5 \pm 0.5$ h; for sucrose-fed rats, $t_1 = 8.2 \pm 0.1$ h.

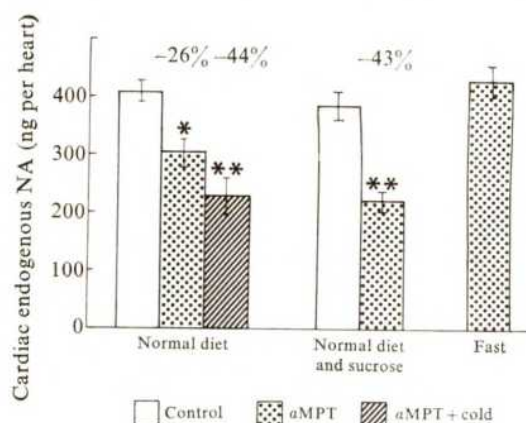


Fig. 2 Effect of inhibition of NA biosynthesis on endogenous NA levels in rat heart from control (normal diet), fasted and sucrose-fed animals. Animals were prepared as described in Fig. 1, except that in this experiment, a single group of animals was fasted for 48 h before the start of the experiment. Animals were injected i.p. with the methyl ester of α MPT (Sigma) dissolved in saline, 250 mg per kg at zero time and 125 mg per kg at 3.5 h; controls received the diluent alone in two injections. Animals were killed at 7 h, and the endogenous NA levels in heart determined. Open bars represent the saline-treated animals, the shaded bars the α MPT-treated animals, and the striped bar a single group of α MPT-treated animals that was placed in cages in a cold (4°C) room during the course of the experiment. Each group contained eight or nine animals. * Represents $P < 0.005$ and ** represents $P < 0.001$, when compared with the corresponding diluent control. The endogenous NA content in α MPT-treated animals is significantly less ($P < 0.02$) in those fed sucrose compared with those maintained on the control diet. Sucrose intake averaged 6.2 g per rat per d during the experiment.

injection of ^3H -NA at t_0 . At 2, 6, 12 and 24 h after injection, 5–7 rats from each group were killed and their hearts analysed for ^3H -NA and endogenous NA. As shown in the semi-logarithmic plot of cardiac NA specific activity against time (Fig. 1), sucrose feeding was associated with a highly significant ($P < 0.001$) decrease in the calculated half-time of disappearance (t_1), from 14.5 ± 0.5 h in the control group to 8.2 ± 0.1 h in the sucrose-fed group. (Similar differences were observed when cardiac ^3H -NA content was plotted against time.) The fractional NA turnover rate was increased significantly ($P < 0.001$) during sucrose feeding (from $4.80 \pm 0.16\%$ per h in the control group to $8.49 \pm 0.13\%$ per h in the sucrose-fed group). Endogenous levels of cardiac NA decreased significantly ($P < 0.02$) in the sucrose-fed rats compared with the control animals (360 ± 21.7 and 446 ± 25.4 ng NA per heart, respectively). NA turnover rate, calculated as the product of the fractional turnover rate and the endogenous level, was increased in the hearts of sucrose-fed animals (21.4 ± 1.9 and 30.6 ± 2.3 ng NA per heart per h for the 95% confidence intervals in control and sucrose-fed animals, respectively). Sucrose feeding for 3 d, therefore, is associated with increased ^3H -NA turnover.

The effect of inhibition of NA biosynthesis on endogenous levels of NA in hearts of control (normal diet), fasted and sucrose-fed animals is shown in Fig. 2. With both control and sucrose-fed groups significantly ($P < 0.005$ and $P < 0.001$, respectively) decreases in the NA content of rat heart followed 7 h of inhibition of NA biosynthesis; cardiac NA levels were significantly ($P < 0.02$) less in the α MPT-treated control animals, and were similar to the NA levels observed in control animals exposed to cold (4°C). Fasted animals exhibited no change in cardiac NA content after α MPT treatment as previously reported. Thus, two independent methods for assessing NA turnover demonstrate increased NA turnover during sucrose feeding.

Ganglionic blockade interrupts central sympathetic

outflow and as a result decreases peripheral sympathetic activity. The effect of such blockade on endogenous NA and on the residual ^3H -NA remaining in the hearts of control and sucrose-fed animals 10 h after administration of label is shown in Fig. 3. In this experiment chlorisondamine (5 mg per kg, body weight intraperitoneally), a long-acting ganglionic blocking agent, was administered at 5 min, and again at 5 h, after injection of tracer NA into control and sucrose-fed animals. The animals were killed 10 h after the start of the experiment. In the control animals the residual ^3H -NA was 41% ($P < 0.025$) and the endogenous NA 14% (not significant) greater in the group treated with ganglionic blockade compared with the group given saline. In the sucrose-fed animals ganglionic blockade increased residual ^3H -NA 109% ($P < 0.001$) and endogenous NA 23% (not significant). The hearts of sucrose-fed animals without ganglionic blockade contained less ^3H -NA and endogenous NA than did those of the control animals ($P < 0.02$ and $P < 0.05$, respectively) consistent with increased NA turnover while no differences in either residual ^3H -NA or endogenous NA were observed in the hearts of sucrose-fed and control animals with ganglionic blockade. This abolition of differences in cardiac ^3H -NA content between control and experimental groups with ganglionic blockade indicates that increased central sympathetic outflow underlies the accelerated NA turnover observed in the hearts of sucrose-fed animals.

This report, in addition to our previous finding that sympathetic activity is suppressed during fasting, suggests that central sympathetic outflow is influenced by the nutritional state of the animal. The nutritional signal to the central nervous system that leads to the observed alterations in sympathetic activity is unknown. Nutritional modification of central sympathetic outflow may have several important implications. As catecholamines are considered to be the principal mediators of cold-induced thermogenesis^{7,8} and as the mechanisms of thermogenesis are presumed to be similar during cold exposure and with

overfeeding⁹, dietary-induced changes in sympathetic activity may have a central role in nutritionally-determined thermogenesis¹⁰. The physiological importance for cardiovascular disease of the increased sympathetic activity in heart associated with over-feeding is unknown, but dietary alterations in sympathetic activity may be a possible link between the increase in calorie and/or sucrose consumption and the rise in cardiovascular disease observed in Western countries¹¹.

JAMES B. YOUNG
LEWIS LANDSBERG

Thorndike Laboratory and Department of Medicine,
Harvard Medical School and Beth Israel Hospital,
Boston, Massachusetts 02215

Received 23 May; accepted 8 August 1977.

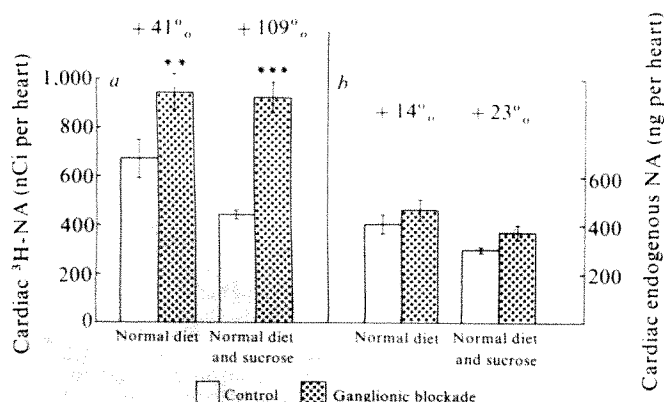
1. Young, J. B. & Landsberg, L. *Science* **196**, 1473-1475 (1977).
2. Young, J. B. & Landsberg, L. *Clin. Res.* **25**, 404A (1977).
3. Kanarek, R. B. & Hirsch, E. *Fedn Proc.* **36**, 154-158 (1977).
4. Neff, N. H., Tozer, T. N., Hammer, W., Costa, E. & Brodie, B. B. *J. Pharmac. exp. Ther.* **160**, 48-52 (1968).
5. Landsberg, L. & Axelrod, J. *Circulat. Res.* **22**, 559-571 (1968).
6. Taubin, H., Djahanguiri, B. & Landsberg, L. *Gut* **13**, 790-795 (1972).
7. Gale, C. C. *A. Rev. Physiol.* **35**, 381-430 (1973).
8. Jansky, L. *Biol. Rev.* **48**, 85-132 (1973).
9. Sims, E. A. H. *Clin. endocrinol. Metabol.* **5**, 377-395 (1976).
10. Stirling, J. L. & Stock, M. J. *Nature* **220**, 801-802 (1968).
11. Yudkin, J. *Nature* **239**, 197-199 (1972).

Occurrence of a new class of tetrahydroisoquinoline alkaloids in L-dopa-treated parkinsonian patients

THE chemotherapeutic administration of massive doses of L-3,4-dihydroxyphenylalanine (L-dopa) with or without peripheral decarboxylase inhibitors to parkinsonian patients can lead to aberrant metabolism which may influence the efficacy of the regime. It is generally agreed that the transformations can include spontaneous chemical reactions facilitated by the inherent reactivity of the catechol nucleus. For example, Sandler *et al.*¹ reported the excretion of tetrahydroisoquinoline alkaloids (TIQs), tetrahydropapaveroline (norlaudanoline) and salsolinol by parkinsonian patients who had consumed large doses of L-dopa and ethanol. The same two TIQs have been detected in brain and urine of mammals administered alcohol and a second compound—for example, L-dopa or pyrogallol; and it has been proposed that these Pictet-Spengler condensation products (of dopamine and dopaldehyde or acetaldehyde, respectively) may be responsible for addiction in alcoholism²⁻³. We report here the occurrence of a new class of TIQs, norlaudanolinecarboxylic acids (NLCAs), in the urine of parkinsonian patients on L-dopa with or without carbidopa. Comparative studies with rats on a similar regimen reveal the presence of NLCAs in brain as well as in urine.

NLCAs arise by Pictet-Spengler condensation of phenylpyruvic acids and dopamine (Fig. 1). The parent compound, NLCA, derived from the transamination and decarboxylation products of L-dopa, has been previously characterised and implicated in *Papaver* alkaloid biosynthesis in higher plants^{6,7}. Norcoclaurinecarboxylic acid (NCCA), 3'-O-methylnorlaudanolinecarboxylic acid (MNLCA), and 3',4'-desoxynorlaudanolinecarboxylic acid were synthesised from dopamine and *p*-hydroxyphenylpyruvate, 3-methoxy-4-hydroxyphenylpyruvate and phenylpyruvate respectively, as described for NLCA (ref. 6). Their ultraviolet, nuclear magnetic resonance, infrared and mass spectra were consistent with assigned structures. NLCAs were detected in urine by mass fragmentography on an LKB-9000 gas-liquid chromatograph mass spectrometer. The presence of a carboxyl group dictated a two-step derivatisation of the alkaloids, entailing esterification with

Fig. 3 Effect of ganglionic blockade on *a*, cardiac ^3H -NA and *b*, cardiac endogenous NA in control (normal diet) and sucrose-fed rats. Animals were prepared for this experiment by 3 d of sucrose feeding as in Fig. 1: 5 min and 5 h after the i.v. injection of the ^3H -NA (200 μCi per kg) chlorisondamine (5 mg per kg; Ecolid, Ciba), a ganglionic blocking agent, was administered intraperitoneally. Control animals received similar i.p. injections of diluent. Animals were killed 10 h after injection of the ^3H -NA. Open bars represent saline-treated animals; shaded bars the chlorisondamine groups. Each group contained eight or nine rats. The percentage shown over the bar is the increase over the group without ganglionic blockade. ** Represents $P < 0.025$ and *** $P < 0.001$ compared with control without ganglionic blockade. ^3H -NA and endogenous NA content are significantly lower ($P < 0.02$ and $P < 0.05$, respectively) in the hearts of diluent-treated, sucrose-fed animals compared with diluent-treated, control animals, while no significant differences obtain in control and sucrose-fed animals in the presence of ganglionic blockade. Sucrose intake averaged 8.8 g per rat per d.



pentafluoropropanol followed by acylation of the phenolic and amino groups with heptafluorobutyric anhydride by a modification of a procedure for simple catechol carboxylic acids⁸.

In early experiments single ion monitoring was used and the instrument was focused on the debenzylated fragment (m/e 928), which represented the base peak in mass spectra of all NLCA derivatives. Subsequently the mass spectrometer was interfaced with a Logos Spectrotek computer system which allowed repetitive scanning of the gas-liquid chromatography effluent and data storage. Typical fragment ion current chromatograms generated by the computer from runs with standards and a urine sample are shown in Fig. 2.

The alkaloids were detected by monitoring both the base peak at m/e 928 and benzyl fragment ions (m/e 303 for NCCA, m/e 333 for MNLCA, m/e 91 for 3', 4'-desoxy NLCA). The benzyl fragment ion of NLCA had too low an intensity for routine detection.

With these semi-quantitative techniques the urine of normal subjects (controls) and parkinsonian patients on L-dopa, L-dopa-carbidopa (10:1, Sinemet) or no chemotherapy, were analysed (Table 1). Low but reproducible levels of MNLCA were detectable in urine of L-dopa- and Sinemet-treated parkinsonian patients. NLCA and MNLCA values were obtained for controls and non-treated patients but these were too close to minimal levels of detection to be conclusive. This was also the case for NLCA in treated parkinsonian subjects.

Comparative studies have been carried out with 250 g male Sprague-Dawley rats maintained on 2.5 mg L-dopa or 1.1 mg L-dopa-carbidopa (10:1, Sinemet) per ml drinking water (*ad libitum*) for 1 week. The animals consumed 5–30 mg L-dopa per day and excreted NLCA and MNLCA at a rate of 0.05–48 μ g per 24 h. Brain levels of MNLCA for L-dopa-treated rats averaged 202 ng per g tissue, a value confirmed independently by ion pair reversed phase high-pressure liquid chromatography.

After incubations in physiological conditions (0.2 M phosphate buffer, pH 6.0, at 37 °C for 30 min), 3,4-dihydroxyphenylpyruvate (14 mM) and dopamine (18 mM) reacted to produce 2–3% NLCA in the presence of cell-free homogenates of rat brain tissue. The fact that comparable yields were realised in the absence of brain tissue extracts or with boiled homogenates proved that the condensation was not enzyme dependent. Furthermore, enzymes such as monoamine oxidase, which were demonstrated to be active in these homogenates, did not interfere with the Pictet-Spengler condensation.

In vivo synthesis of MNLCA was demonstrated with rats fed Sinemet for 1 week as described above. The animals were injected intraperitoneally with 0.5 mCi 2,3-³H-L-dopa and killed 1.5–2 h later. Carrier MNLCA (10 mg) was added to *n*-butyl alcohol-ethyl acetate (3:2) extracts of brain

Fig. 1 Pictet-Spengler condensation of dopamine and phenylpyruvates.

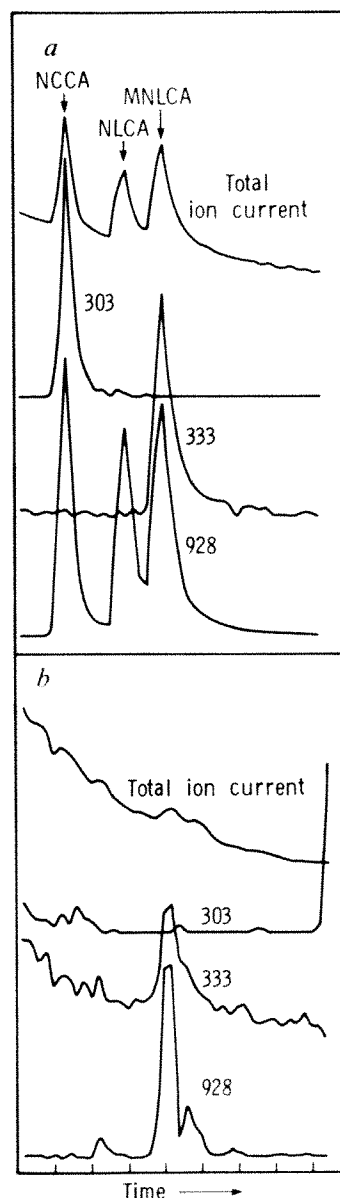
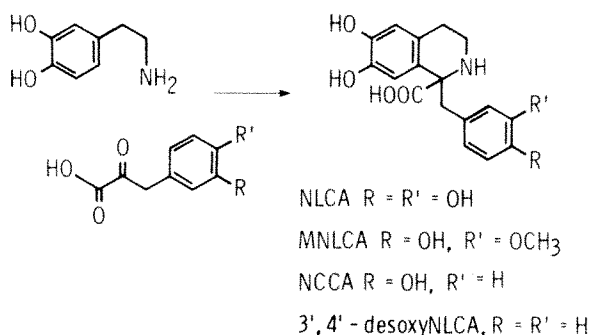


Fig. 2 Fragment ion current chromatograms of derivatised NLCA standards and urine samples by computerised gas-liquid chromatography and mass spectroscopy. Solid NaCl was added to 5 ml urine until saturation, and the solution was extracted with 4 \times 5 ml *n*-butyl alcohol-ethyl acetate (3:2). The combined organic phase was evaporated to dryness and taken up in a buffer solution of 0.2 M sodium acetate, pH 8.6, 0.1% EDTA and 1% sodium metabisulphite. Acid-washed alumina (500 mg) was added to this solution and the pH adjusted to 8.6 \pm 0.1. This mixture was added to a column of acid-washed alumina (750 mg) pre-equilibrated with buffer and the column was washed with 4 volumes of 1 M HCl. The eluate was saturated with solid NaCl and extracted with 4 \times 0.75 ml *n*-butyl alcohol-ethyl acetate (3:2). The combined organic phase was taken to dryness. Recovery of NLCAs in this process ranged from 50–80%. Pre-incubation of urine with glucosylase did not increase yields appreciably. Derivatisation was accomplished by adding to the dry sample 100 μ l 1-H,1-H-pentafluoropropanol-1 (PCR) and 200 μ l heptafluorobutyric anhydride (Aldrich) and heating at 90 °C for 1–3 h. The sample was then taken to dryness with a stream of dry nitrogen gas; 100 μ l heptafluorobutyric anhydride was added and the sample was heated at 90 °C for 15 min. After removal of excess reagent with nitrogen, the sample was taken up in 200 μ l of either anhydrous ethyl acetate or acetonitrile containing 10% (v/v) heptafluorobutyric anhydride. The sample (1–5 μ l) was injected on to a 6-feet silanised glass column (4 mm internal diameter) packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) in the following conditions: carrier gas, nitrogen; flow rate 30 ml min⁻¹; ion source temperature 290 °C; flash heater 230 °C; column temperature 160 °C; accelerating voltage 2.5 kV; ionising energy 30 or 70 eV; trap current 60 or 120 μ A. Upscans were 4 s and rescans took 1.8 s. The urine sample shown here was obtained from a parkinsonian patient on 1.5 g L-dopa per day. a, Standards; b, urine analysis.

Table 1 Urinary levels of NLCA in controls and parkinsonian patients

Drug regimen	NLCA (μg per 24 h)	MNLCA (μg per 24 h)	Dopamine (mg per 24 h)
Controls (3)	0	8.4 [0-39]	1.19 \pm 0.27
Sinemet (6)	1.1 [0-7.1]	40.0 [10.9-149.5]	15.2 \pm 3.3
L-Dopa (3)	2.2 [0.6-3.8]	127.7 [55.5-235.9]	204 \pm 66
None (2)	0	3.3 [1.3-5.3]	0.66 \pm 0.18

Urine samples (24 h) were collected from normal subjects (controls) and parkinsonian patients on Sinemet (0.3-0.8 g L-dopa and 30-80 mg carbidopa per day), L-dopa (1.5-4.0 g per day), or no chemotherapy. Urine was stored immediately at -20°C after addition of either 5 ml 6 M HCl or 10 g sodium metabisulphite. TIQ levels did not change with time in these conditions. The values in parentheses indicate the number of subjects used. TIQ values were obtained by mass fragmentography and for MNLCA represent the average of at least two determinations for each individual, with the range of values given in brackets. Dopamine values (mean \pm s.e.m.) were obtained by paired ion high pressure liquid chromatography on a reversed phase column (unpublished results). Levels of 3,4-dihydroxyphenylpyruvate and 3-methoxy-4-hydroxyphenylpyruvate were below the limits of detection of the latter method ($< 0.1 \mu\text{g}$ per ml urine).

and intestinal tissues; it was then recrystallised to constant specific activity⁸. Final specific activities of MNLCA from gut of four different rats were 536-3,342 d.p.m. per mg ($6.5 \times 10^{-4}\%$ incorporation), whereas MNLCA from brain tissue of two rats gave constant specific activities of 231 and 599 d.p.m. per mg ($2.1 \times 10^{-4}\%$ incorporation). Labelled MNLCA was not found in rat liver or kidney in these experiments.

To determine whether endogenous levels of L-dopa metabolites had diluted the tracer, thereby depressing incorporation rates, an experiment was carried out in which an untreated rat was injected intraperitoneally first with carbidopa (1 mg per kg) and 0.5 h later, with 0.5 mCi $2,3\text{-}^3\text{H}$ -L-dopa. The percentage incorporation (1.64×10^{-4}) of L-dopa into MNLCA in the gut of this rat did not differ appreciably from the *in vivo* tracer data obtained with Sinemet-treated rats. Rates of *in vivo* conversion of L-dopa to MNLCA were more consistent with a spontaneous chemical reaction than with an enzyme-catalysed reaction. MNLCA formation in gut, in contrast to liver and kidney, reflects the intensive metabolism of L-dopa in the intestine of the rat⁹. Despite the low rates of incorporation, brain levels of MNLCA suggest accumulation can occur, possibly analogous to non-enzymatic melanin formation and deposition in brain.

To determine whether NLCA can interfere with normal catechol metabolism, they were included in enzyme incubations. Neither 0.1 mM NLCA nor MNLCA had an effect on V_{max} or the apparent K_m of tyrosine aminotransferase (29 nmol per min per mg protein and 2.1 mM, respectively) or L-aromatic amino acid decarboxylase (43 nmol per min per mg protein and 0.60 mM, respectively) in crude rat liver cytosolic fractions. Low concentrations of NLCA also did not seem to affect hepatic catecholamine catabolic enzymes *in vivo*. If 0.2 mg NLCA, MNLCA or 3',4'-desoxyNLCA and $7\text{-}^3\text{H}$ -L-noradrenaline (2 μCi , 0.2 nmol) were injected simultaneously into the tail vein of male Swiss albino mice (17-20 g) the percentage ^3H label in hepatic normetanephrine and vanillyl glycol was not appreciably different from control experiments in which noradrenaline was injected alone. Specific radioactivities were determined after fractionation by high-pressure liquid chromatography.

The question has been raised as to whether the efficacy of L-dopa chemotherapy in parkinsonism can be attributable to dopamine replacement alone. TIQs have been proposed as possible therapeutic agents¹⁰, whereas some investigators^{11,12} have postulated that they may cause side-effects such as the 'on-off' phenomena observed

clinically in L-dopa chemotherapy. These hypotheses imply interaction of TIQs with catecholaminergic receptors, and evidence exists for transmitter-like behaviour of simple aldehyde-derived TIQs⁴. Thus, the occurrence of NLCA in human urine and rat urine and brain demonstrated here, provides a basis for further investigation of theories concerning the role of TIQs in parkinsonian chemotherapy.

This study was supported by the NIH. We thank W. Frasure and T. Chyn for assistance.

C. J. COSCIA
W. BURKE
G. JAMROZ
J. M. LASALA
J. MCFARLANE
J. MITCHELL
M. M. O'TOOLE
M. L. WILSON

E. A. Doisy Department of Biochemistry,
St Louis University School of Medicine,
St Louis, Missouri 63104

Received 6 June; accepted 1 August 1977.

- Sandler, M., Carter, S. B., Hunter, K. R. & Stern, G. M. *Nature* **241**, 439-443 (1973).
- Davis, V. E. & Walsh, M. J. *Science* **167**, 1005-1007 (1970).
- Cohen, G. & Collins, M. *Science* **167**, 1749-1751 (1970).
- Cohen, G. *Biochem. Pharmacol.* **25**, 1123-1128 (1976) (and references cited therein.)
- Myers, R. D. & Melchior, C. L. *Science* **196**, 554-556 (1977).
- Wilson, M. L. & Coscia, C. J. *J. Am. chem. Soc.* **97**, 431-432 (1975).
- Battersby, A. R., Jones, R. C. F. & Kazlauskas, R. *Tetrahedron Lett.* 1873-1876 (1975).
- Watson, E., Wilk, S. & Roboz, J. *Analyt. Biochem.* **59**, 441-451 (1974).
- Landsberg, L., Berardino, M. B. & Silva, P. *Biochem. Pharmacol.* **24**, 1167-1174 (1975).
- Sourkes, T. L. *Nature* **229**, 413-414 (1971).
- Hornykiewicz, O. *Fedn Proc.* **32**, 183-190 (1973).
- Dougan, D., Wade, D. & Mearrick, P. *Nature* **254**, 70-72 (1975).

γ Endorphin, α endorphin and Met-enkephalin are formed extracellularly from lipotropin C fragment

A SERIES of peptides derived from lipotropin has been reported to possess morphinomimetic properties. The C fragment (or β endorphin, residues 61-91) was isolated from pituitary^{1,2} and shown to be present in brain³, the C' fragment (61-87) occurs in pituitary¹, γ endorphin (61-77) and α endorphin (61-76) were obtained after acid extraction of tissue of hypothalamic and pituitary origin⁴, and Met-enkephalin (61-65) was found in extracts of brain^{5,6}. Examination of the ability of these peptides to displace specifically bound ^3H -naloxone from brain opiate receptors showed that C fragment was much more potent than the shorter peptides⁷ and investigation of the antinociceptive properties showed that C fragment alone produces profound and long-lasting analgesia⁸⁻¹¹. In addition, various other central activities are exhibited by C fragment¹²⁻¹⁴. We report here that C fragment can be degraded by membrane bound enzymes from rat brain to form the smaller opiate-like peptides.

The use of iodine-labelled peptide, with the radiolabel on the NH_2 -terminal tyrosine, ensured that the substrates could be studied at near physiological concentration and products containing the NH_2 -terminal region of C fragment were readily identified. Membranes from rat brain were prepared by extensive washing of the synaptosomal fraction (P_2) described by Whittaker *et al.*¹⁵. Incubations of [^{125}I]C-fragment (100,000 d.p.m.; 2 Ci mmol⁻¹) with membrane preparations (0.42 mg per ml) were performed in 10^{-3} M bacitracin for 16 h at 37°C in 1 ml of 0.05 M sodium chloride-0.1 M sodium phosphate at pH 7.4 or 0.1 M ammonium acetate at pH 5 and the suspensions were gently agitated. The products were resolved by gel filtration on Sephadex G-50 in 50% (v/v) acetic acid and chromatography on thin layers of cellulose MN400 with *n*-butanol-pyridine-acetic acid-water (15 : 10 : 3 : 12; v/v) or *n*-propanol-0.2 M ammonium hydroxide (3 : 1; v/v). Shorter peptides (LPH 61-67 or 61-68, 0.34 mM) were incubated with membranes in similar conditions for 1 h and the mixtures were fractionated by

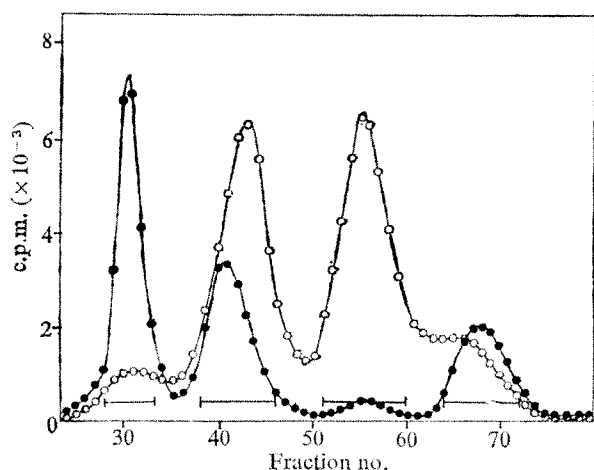


Fig. 1 Gel filtration of the products of digestion of [125 I] C fragment with membrane-bound proteases from brain. Brain digests were prepared as described in the text. After addition of an equal volume of acetic acid, membranes were removed by centrifugation. The supernatant was concentrated *in vacuo* at 40 °C and gel filtration carried out on a 150 \times 1 cm Sephadex G-50 column in 50% (v/v) acetic acid; fractions (1.8 ml), flow rate of 6 ml h $^{-1}$. ●, Elution profile obtained after digestion at pH 7.4; ○, profile obtained at pH 5.0. Fractions pooled as indicated by the horizontal bars were evaporated to small volume and portions were subjected to thin layer chromatography together with reference peptides¹⁶. Fractions 28–33 contained residual C fragment, fractions 38–46 contained only γ endorphin (pH 7.4) or α endorphin together with 61–73 (pH 5), fractions 52–60 contained Met-enkephalin (pH 7.4) and 61–68 (pH 5) and fractions 64–73 contained tyrosine.

gel filtration on Sephadex G-25 in 50% acetic acid and preparative electrophoresis on paper. Identification of the reaction products was by amino acid analysis.

At pH 7.4 in the presence of bacitracin (Fig. 1) the main product formed from C fragment was γ endorphin (61–77); there was in addition a small amount of Met-enkephalin (61–65). At lower pH values, C fragment was converted rapidly and in high yield to α endorphin (61–76) and Met-enkephalin. The heptapeptide (61–67) generated Met-enkephalin and the dipeptide Thr-Ser readily even at neutral pH.

The endopeptidase activity, assayed by release of Met-enkephalin from the heptapeptide 61–67, was associated with membranes but the enzyme could be detected in intact as well as lysed synaptosomal preparations. Moreover, the enzyme did not co-purify with the membrane components carrying the opiate receptors. When extensively washed synaptosomes were lysed and the membrane fragments resolved on sucrose density gradients, the membranes with affinity for 3 H-dihydromorphine were concentrated in the central fractions of the gradient whereas the endopeptidase activity was distributed uniformly. The results indicate that the endopeptidase is not an intrinsic component of the opiate receptor complex.

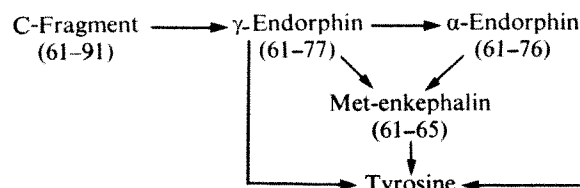
On incubation with striatal slices, [125 I]C-fragment underwent slow degradation with a half life of 3.2 h whereas the half life of [125 I] γ endorphin was 1.1 h. Residual peptide was determined by chromatography of the medium on Sephadex G-50 (Fig. 2). With C-fragment no stabilisation occurred on addition of bacitracin but the aminopeptidase inhibitor had a pronounced effect on the degradation of γ endorphin. The results show that the degradation of C fragment in striatum is not initiated by the attack of an aminopeptidase. The first step, as with the proteolysis catalysed by brain membranes, must involve attack by an endopeptidase.

The specificity of the endopeptidase was determined by examination of the soluble fraction of the incubation mixture. Gel filtration on Sephadex G-50 showed that the disappearance of C fragment was accompanied by the appearance of peptide fractions of lower molecular weight which retained the NH $_2$ -

terminal region of C fragment (Peaks I and II, Fig. 2). The formation of fraction I was markedly enhanced in the presence of bacitracin and ion-exchange chromatography showed that the major product was γ endorphin (Fig. 3). While the yield of γ endorphin was increased by bacitracin, continued incubation with the aminopeptidase inhibitor led to the formation of α endorphin in addition to γ endorphin. Thus, although C fragment is resistant to the action of aminopeptidases¹⁶ and carboxypeptidases¹⁷, γ endorphin is more susceptible to both enzymes.

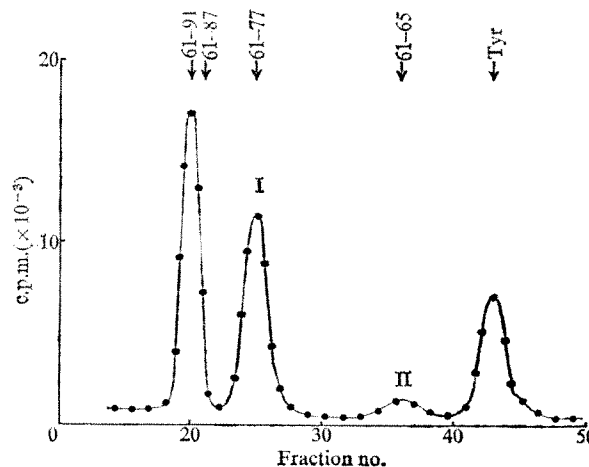
A small percentage of short peptides retaining the NH $_2$ -terminal section of C fragment was observed during the gel filtration on Sephadex G-50 (Peak II, Fig. 2). Resolution of this fraction showed that the major component was Met-enkephalin and there was also some hexa- or heptapeptide (Fig. 4). Even though the small peptides are susceptible to degradation by exopeptidases, their formation was not greatly enhanced by the addition of bacitracin. It seems that the endopeptidase cleavage of γ and α endorphin proceeds only with difficulty.

The degradation of C fragment by membrane-bound enzymes and by striatal slices takes place in discrete stages, forming γ endorphin, α endorphin and Met-enkephalin. The accumulation of these peptides is strongly favoured by the presence of bacitracin or by acid pH, conditions which prevent the loss of the NH $_2$ -terminal tyrosine.



The degradation seems to be mediated by extracellular enzymes and does not involve a tissue uptake system because

Fig. 2 Gel filtration of [125 I] C fragment incubated with striatal slices. Coronal slices (about 200 mg wet weight) through the corpus striatum were prepared by section of fresh rat brains 3.5 mm and 2.5 mm rostral to the anterior part of the optic chiasma. The slices were pre-incubated (45 min, 37 °C) in 3 ml of saline (NaCl, 134 mM; KCl, 5 mM; KH $_2$ PO $_4$, 1.25 mM; MgSO $_4$, 2.0 mM; CaCl $_2$, 1 mM; NaHCO $_3$, 16 mM; glucose, 10 mM) containing 10 $^{-4}$ M bacitracin. The slices were transferred and gently agitated at 37 °C in 3 ml of saline, or 3 ml of saline and 10 $^{-4}$ M bacitracin, containing 6 \times 10 6 c.p.m. of iodinated C fragment (about 2 Ci mmol $^{-1}$). After 4 h incubation an equal volume of glacial acetic acid was added to the supernatant and gel filtration was performed on Sephadex G-50 (150 \times 0.9 cm) in 50% (v/v) acetic acid. The eluate fractions (2.2 ml) were monitored for radioactivity. The arrows indicate the elution positions of authentic 125 I or 125 I-labelled peptides.



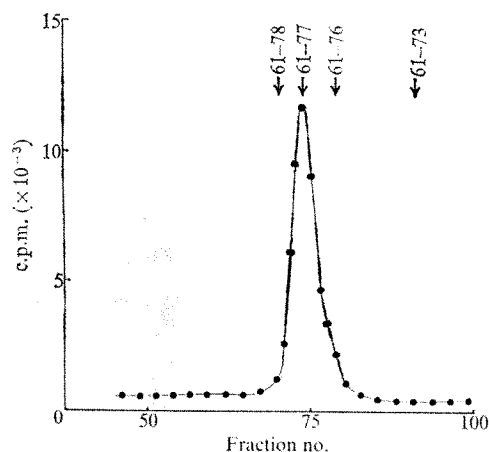


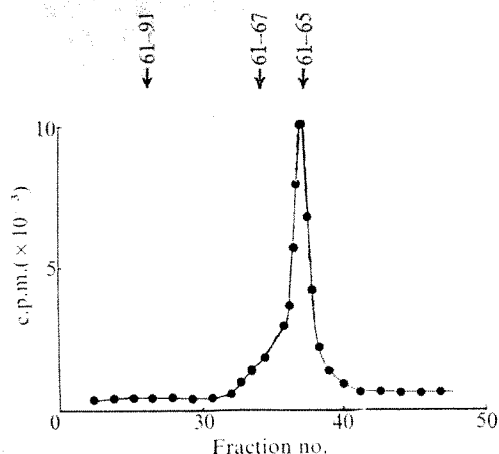
Fig. 3 Identification of γ -endorphin as the first degradation product formed from C fragment in striatal slices. The peptide fraction Peak I, obtained by gel filtration of [125 I] C fragment incubated with a striatal slice in the presence of bacitracin, was concentrated *in vacuo* and chromatographed together with [131 I] γ -endorphin on SP Sephadex C-25 in 0.05 M phosphate pH 4.5 with a linear gradient from 0 to 0.5 M NaCl (200-ml mixer). The elution positions of the 61-73, 61-76, 61-77 and 61-78 peptides were established by chromatography of standard mixtures containing 125 I- or 131 I-labelled peptides.

no enrichment of labelled peptide could be detected in the slices; but the results do not preclude the possibility that formation of Met-enkephalin and the endorphins might also occur intracellularly.

Our results show that the extracellular metabolism of C fragment takes place in a specific manner to form the series of opiate-like peptides that have been isolated from brain. This may mean that the shorter peptides that have been isolated are degradation products of C fragment rather than natural peptides. It is, however, too early to be certain about this since it has been reported that enkephalin is present in rat brain even after rapid killing by microwave irradiation¹⁸.

We thank Dr A. F. Bradbury for synthesising Met-enkephalin

Fig. 4 Identification of Met-enkephalin as the principal component of the small peptide fraction formed by degradation of C fragment in striatal slices. The peptide fraction Peak II, obtained by gel filtration of [125 I]C fragment incubated with a striatal slice in the presence of bacitracin, was evaporated *in vacuo* and chromatographed on Sephadex G-15 (150 \times 0.9 cm) in 50% (v/v) acetic acid, fraction size 1.4 ml. The column was calibrated with [125 I]C fragment, the heptapeptide (61-67) and Met-enkephalin (61-65).



and the heptapeptide 61-67, and D. E. Massey for technical assistance.

B. M. AUSTEN
D. G. SMYTH
C. R. SNELL

National Institute for Medical Research,
The Ridgeway, Mill Hill, London NW7, UK

Received 19 April; accepted 10 August 1977.

- ¹ Bradbury, A. F., Smyth, D. G. & Snell, C. R. in *Peptides: Chemistry, Structure and Biology* (eds Walter, R. & Meienhofer, J.), 609-615 (Ann Arbor, Ann Arbor, 1975).
- ² Bradbury, A. F., Smyth, D. G. & Snell, C. R. *CIBA Found. Symp.* No. 41, 61-75 (1976).
- ³ Bradbury, A. F., Feldberg, W. S., Smyth, D. G. & Snell, C. R. in *Opiates and Endogenous Opioid Peptides* (ed. Kosterlitz, H. W.) 9-17, (Elsevier/North Holland, Amsterdam 1976).
- ⁴ Guilleman, R., Ling, N. & Burgus, R. C. *r. hebd. Séanc. Acad. Sci., Paris Ser. D.* 282, 783-785 (1976).
- ⁵ Hughes, J. *et al. Nature* 258, 577-579 (1975).
- ⁶ Simantov, R. & Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.* 73, 2515-2519 (1976).
- ⁷ Bradbury, A. F., Smyth, D. G. & Snell, C. R., Birdsall, N. J. M. & Hulme, E. C. *Nature* 260, 793-795 (1976).
- ⁸ Feldberg, W. S. & Smyth, D. G. *J. Physiol. Lond.* 260, 30-31 (1976).
- ⁹ Van Ree, J. M. *et al. Nature* 264, 792-794 (1976).
- ¹⁰ Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. *Proc. natn. Acad. Sci. U.S.A.* 73, 2895-2898 (1976).
- ¹¹ Bradbury, A. F., Smyth, D. G., Snell, C. R., Deakin, J. F. W. & Wendlandt, S. *Biochem. biophys. Res. Commun.* 74, 748-754 (1977).
- ¹² Gispen, W. H., Wiegant, V. M., Bradbury, A. F., Hulme, E. C., Smyth, D. G. & Snell, C. R. *Nature* 264, 794-795 (1976).
- ¹³ Loh, H. H., Brase, D. A., Sampath-Khanna, S., Mar, J. B., Wei, E. L. & Li, C. H. *Nature* 264, 567-568 (1976).
- ¹⁴ Feldberg, W. S. & Smyth, D. G. *Br. J. Pharmac.* 60, 445-454 (1977).
- ¹⁵ Whittaker, V. P., Michaelson, I. A. & Kirkland, R. J. A. *Biochem. J.* 90, 293-303 (1964).
- ¹⁶ Austen, B. M. & Smyth, D. G. *Biochem. biophys. Res. Commun.* 76, 477-482 (1977).
- ¹⁷ Geisow, M. J. & Smyth, D. G. *Biochem. biophys. Res. Commun.* 75, 625-629 (1977).
- ¹⁸ Yang, H. Y., Hong, J. S. & Costa, E. *Neuropharmacology* 16, 303-307 (1977).

Identification of the herpes simplex virus DNA polymerase gene

THERE is at present much interest in the herpes viruses because they have been associated with human cancer and cause latent infections with recurrent disease. They are also of interest because they form a relatively simple but apt model for the processes of transcription and DNA replication in eukaryotic cells. We have studied the virus-induced proteins involved in DNA replication, and present here results that give definitive evidence that the virus-induced DNA polymerase is encoded in the virus genome, that locate the gene for virus DNA polymerase, and also show that the enzyme is essential for virus replication.

Keir and Gold¹ demonstrated that herpes simplex virus (HSV) induced a DNA polymerase and Keir *et al.*^{2,3} showed that the enzyme was stimulated by high salt concentrations and inhibited by antiserum to virus-infected cells. Weissbach *et al.*⁴ demonstrated partial purification of the enzyme and more recently, we have purified the enzyme to the point where a virus-induced protein of molecular weight 149,000 can be associated with this activity⁵. Aron *et al.*⁶ and Purifoy and Benyesh-Melnick⁷ showed that HSV mutants in several complementation groups were deficient in induction of the DNA polymerase and, further, that some of these mutants induced an enzyme which was temperature sensitive *in vivo* but not *in vitro* in crude extracts.

We have been studying the DNA polymerase induced by one of these temperature-sensitive mutants of HSV type 1 (HSV-1) which is designated tsD9 (ref. 8) and by a spontaneous ts⁺ revertant of this mutant, designated DR3 (ref. 9). The purification of the enzyme from HEp-2 cells infected with this mutant at the permissive temperature is shown in Fig. 1. The details of this purification scheme and its evaluation will be published elsewhere, but suffice to say here that the mutant enzyme behaves exactly as the wild-type enzyme during purification, both in chromatographic behaviour and in the amounts of the starting

enzyme recovered (10–15% overall yield). Enzyme was purified in precisely the same manner both from cells infected with the wild-type virus (HSV-1 strain KOS) and from cells infected with the revertant, DR3. All the enzymes were neutralised specifically by antiserum to virus-infected cells.

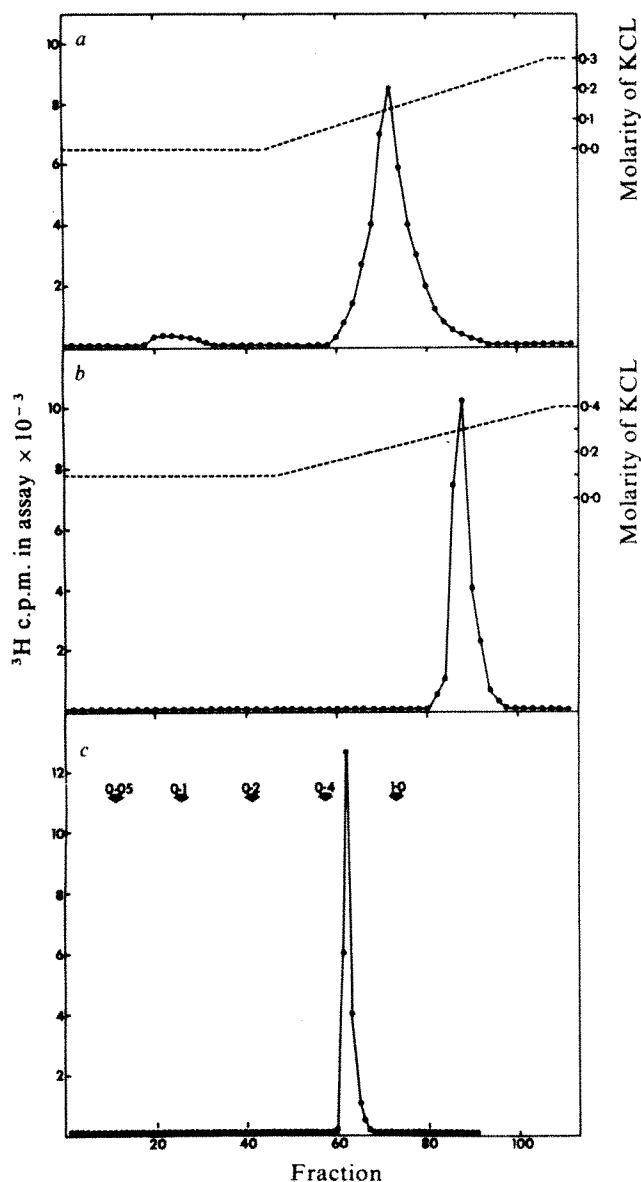


Fig. 1 Purification of virus-induced DNA polymerase from tsD9-infected cells. Enzyme was extracted from virus-infected cells as described previously¹³, using a high salt procedure. Total enzyme activity was extracted, dialysed against 50 mM Tris-HCl, pH 7.5, 20% glycerol, 0.2% NP40 and 0.5 mM dithiothreitol (DE buffer), and applied to a DEAE-cellulose column equilibrated in the same buffer. The enzyme was adsorbed to the column and then eluted with DE buffer containing 0–0.3 M KCl. DNA polymerase activity was detected in column fractions using our standard assays for the virus-induced enzyme containing 100 mM KCl in the reaction mixture⁷. The enzyme eluted at between 0.1 and 0.15 M KCl as shown (a). The partially purified enzyme was diluted in DE buffer and applied to a phosphocellulose column equilibrated in the same buffer and prewashed with 500 $\mu\text{g ml}^{-1}$ bovine serum albumin (BSA). The column was developed with DE buffer containing 0.1–0.4 M KCl and the enzyme eluted at 0.25–0.3 M KCl (b). The activity peak from this column was dialysed against DNA cellulose buffer (DNB, 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 20% glycerol, 0.2% NP40, 0.5 mM dithiothreitol, 1 mM EDTA) after adjusting the protein concentration to 500 $\mu\text{g ml}^{-1}$ with BSA. The dialysed enzyme was applied to a DNA-cellulose column equilibrated with DNB and eluted in stepwise fashion with 0.05, 0.1, 0.2, 0.4, and 1.0 M KCl in the same buffer. Purified enzyme eluted with 0.4 M KCl (c) and was then used for further experiments.

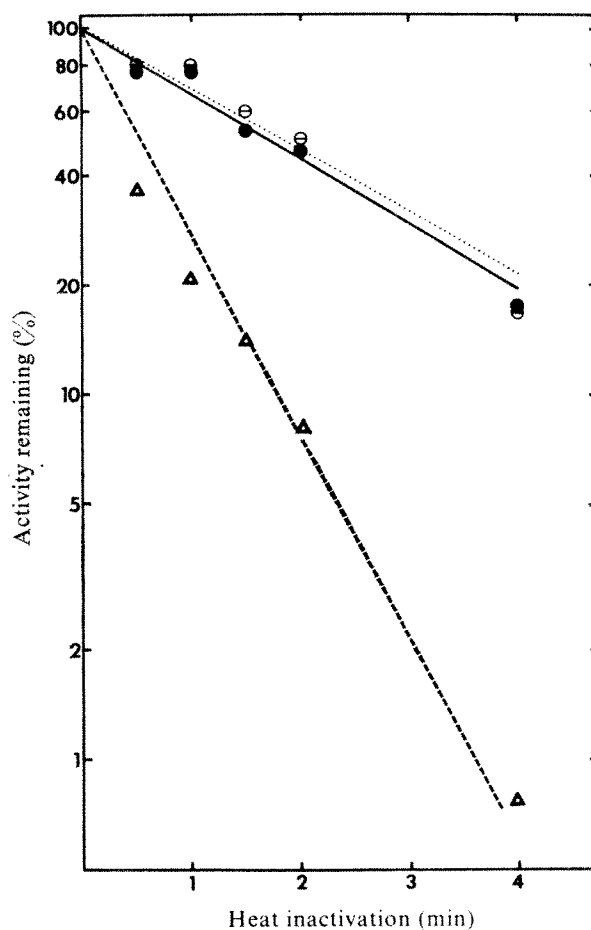


Fig. 2 Heat inactivation of purified enzymes. DNA polymerase purified from wild-type (●), mutant tsD9 (△), or revertant DR3 (○) infected cells was heated at 39 °C for various times in reaction mixture, then template DNA was added and the residual DNA polymerase activity determined using standard assay methods. The specific activity of the enzymes was between 4,000 and 5,000 units mg^{-1} (1 unit is defined as 1 nmol TTP incorporated h^{-1}). One hundred per cent activity corresponds to 70,000 c.p.m. in this assay for each enzyme.

The next step was to test the thermal inactivation of the purified enzymes from mutant, revertant, and wild-type virus-infected cells in the absence of DNA template. In these experiments, purified enzyme (specific activity approximately 4,500 units mg^{-1}) was incubated at 39 °C in reaction mixture without DNA for various periods, template was added and the residual DNA polymerase activity determined. The results presented in Fig. 2 demonstrate that DNA polymerase induced by wild-type virus and revertant is considerably more stable than that induced by the mutant. This result has been verified with batches of enzyme from several independent purifications. Thus, the temperature-sensitive mutation in tsD9 directly affects the DNA polymerase induced by the mutant, causing a loss in the stability of the enzyme; reversion of the mutation is accompanied by recovery of that stability.

HSV replication has been shown to be inhibited by the drug, phosphonoacetic acid¹⁶. Further mutants of the virus have been isolated which are resistant to the drug, and this resistance correlates with DNA polymerase activity in crude extracts *in vitro* (refs 11 and 12). TsD9 was not selected for phosphonoacetic acid resistance and yet is resistant to the drug (ref. 9). To confirm this observation and further to correlate the sensitivity or resistance of the enzyme to phosphonoacetate, it was essential to test

purified enzyme. This experiment has been done and the results are shown in Fig. 3. Purified DNA polymerase from wild-type or DR3 (revertant)-infected cells was extremely sensitive to the drug: 50% inhibition of the enzyme activity was observed at a drug concentration of $0.5\text{--}1\text{ }\mu\text{g ml}^{-1}$. With the purified mutant enzyme a 10–20-fold higher concentration of the inhibitor was required to inhibit the enzyme activity. Reversion of the temperature-sensitive lesion again resulted in restoration of wild-type characteristics to the mutant enzyme.

Our results demonstrate that the temperature-sensitive mutation in the HSV-1 tsD9 genome elicits altered characteristics of the virus-induced DNA polymerase, namely temperature sensitivity and phosphonoacetic acid resistance. Since tsD9 has been located on the virus genetic map between tsO22 and tsN20 (P. A. Schaffer, personal communication), our results enable the virus DNA polymerase gene to be positioned on the current genetic map. Marker rescue with restriction enzyme fragments of tsD9 will enable the location of the DNA coding for the enzyme on the physical map of virus DNA. Several other conclusions can be drawn from the data. First, the virus codes for the induced DNA polymerase, which is the first direct evidence for an animal virus specified DNA-dependent DNA polymerase. We suggest that this polymerase be designated pol H, to distinguish it simply from host cell enzymes. Second, the DNA polymerase activity is essential for virus DNA replication and growth, since tsD9 does not replicate DNA at the non-permissive temperature. Third, phosphonoacetate resistance, at least in this case, is conferred on the virus by changes in the structure of its DNA polymerase—

thus providing a useful method for selection of temperature-sensitive mutants in the viral enzyme.

We thank Ms Beth Gingerich and Mr Ira Wimberley for technical assistance. We are grateful to Drs P. Schaffer and G. Aron for the original stock of mutant tsD9, to Abbot Laboratories for the gift of the phosphonoacetic acid, and to Dr S. Kit for reading the manuscript. This study was supported by a grant from the NCI.

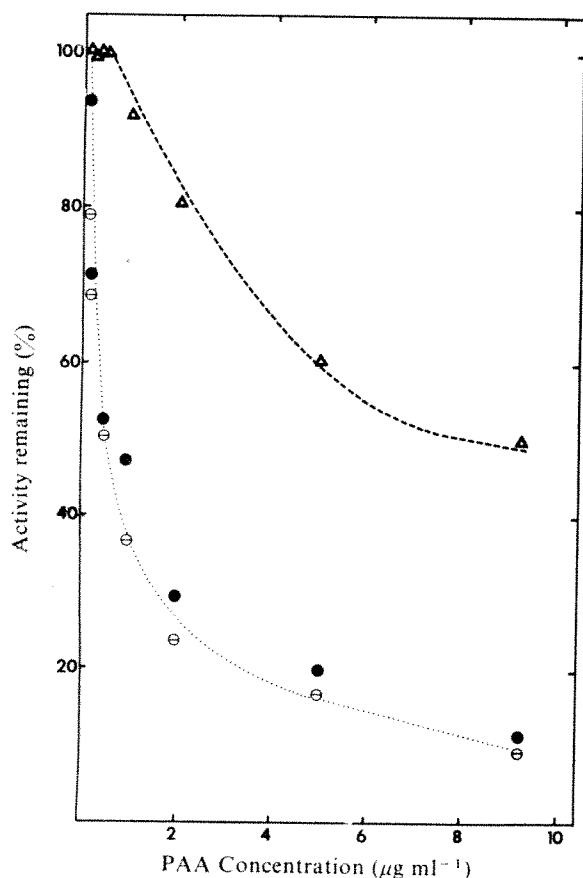
DOROTHY J. M. PURIFOY
ROBERT B. LEWIS
KENNETH L. POWELL

Department of Virology and Epidemiology,
Baylor College of Medicine,
Houston, Texas 77030

Received 6 June; accepted 15 August 1977.

1. Keir, H. M. & Gold, E. *Biochim. biophys. Acta* **72**, 263–276 (1963).
2. Keir, H. M., Subak-Sharpe, H., Shedden, W. I. H., Watson, D. H. & Wildy, P. *Virology* **30**, 154–157 (1966).
3. Keir, H. M., Hay, J., Morrison, J. M. & Subak-Sharpe, H. *Nature* **210**, 369–371 (1966).
4. Weissbach, A., Hong, S.-C. L., Aucker, J. & Muller, R. *J. biol. Chem.* **248**, 6270–6277 (1973).
5. Powell, K. L. & Purifoy, D. J. M. *J. Virol.* **24** (in the press).
6. Aron, G. M., Purifoy, D. J. M. & Schaffer, P. A. *J. Virol.* **16**, 498–507 (1975).
7. Purifoy, D. J. M. & Benyesh-Melnick, M. *Virology* **68**, 374–386 (1975).
8. Schaffer, P. A., Aron, G. M., Biswal, N. & Benyesh-Melnick, M. *Virology* **52**, 57–71 (1973).
9. Purifoy, D. J. M. & Powell, K. L. *J. Virol.* **24** (in the press).
10. Overby, L. R. *et al. Antimicrobial Agents & Chemother.* **6**, 360–365 (1974).
11. Hay, J. & Subak-Sharpe, J. H. *J. gen. Virol.* **31**, 145–148 (1976).
12. Honess, R. W. & Watson, D. H. *J. Virol.* **21**, 584–600 (1977).
13. Purifoy, D. J. M. & Powell, K. L. *J. Virol.* **19**, 717–731 (1976).

Fig. 3 Phosphonoacetic acid (PAA) sensitivity of purified enzymes. DNA polymerase purified from wild-type (●), mutant tsD9 (△), or revertant DR3 (○) infected cells was assayed in the presence of various concentrations of PAA. One hundred per cent activity corresponds to about 120,000 c.p.m. in this assay.



Sister chromatid exchange and chromatid interchange as possible manifestation of different DNA repair processes

SISTER chromatid exchange (SCE) is a symmetrical exchange between a newly duplicated chromatid and its sister¹. The demonstration that many agents that damage DNA also increase the frequency of SCE² suggests that SCE is a reflection of a basic DNA repair process, and perhaps a cytological manifestation of post-replication repair of a recombinational character^{2–6}. The question then arises as to the correlation between SCE and chromatid aberration, particularly chromatid interchange (CI), which also occurs as a consequence of exchange between newly duplicated chromatids^{7,8}. To investigate whether these two types of exchange are due to the same molecular event, I have studied the reaction of SCE and CI to caffeine and cycloheximide in endoreduplication mitoses of cultured human embryonic skin fibroblasts. I report here evidence suggesting that the events involved are separate.

Endoreduplication is an internal doubling of chromosomes resulting from two successive DNA synthesis periods without intervening cytokinesis. Sister chromosomes are paired to form a diplochromosome. When the cells are grown for two replication cycles in the presence of 5-bromodeoxyuridine (BUDR) so that chromosomes can be stained differentially to distinguish one chromatid unifarly with BUDR from its sister bifarly substituted, three types of exchange can be seen—twin SCE occurring in the first S phase, single SCE occurring in the second S phase and intradiplochromatid interchange (IDCI) which occurs between chromosomes in a diplochromosome (Fig. 1).

Fibroblast cultures established from skin fragments of 9–11-week-old human abortuses were used at passages 4–7. Confluent cultures were trypsinised and the cells were grown in Leibovitz's L-15 medium containing 15% foetal calf serum and 20 μM BUDR. Cells were fixed for chromosome

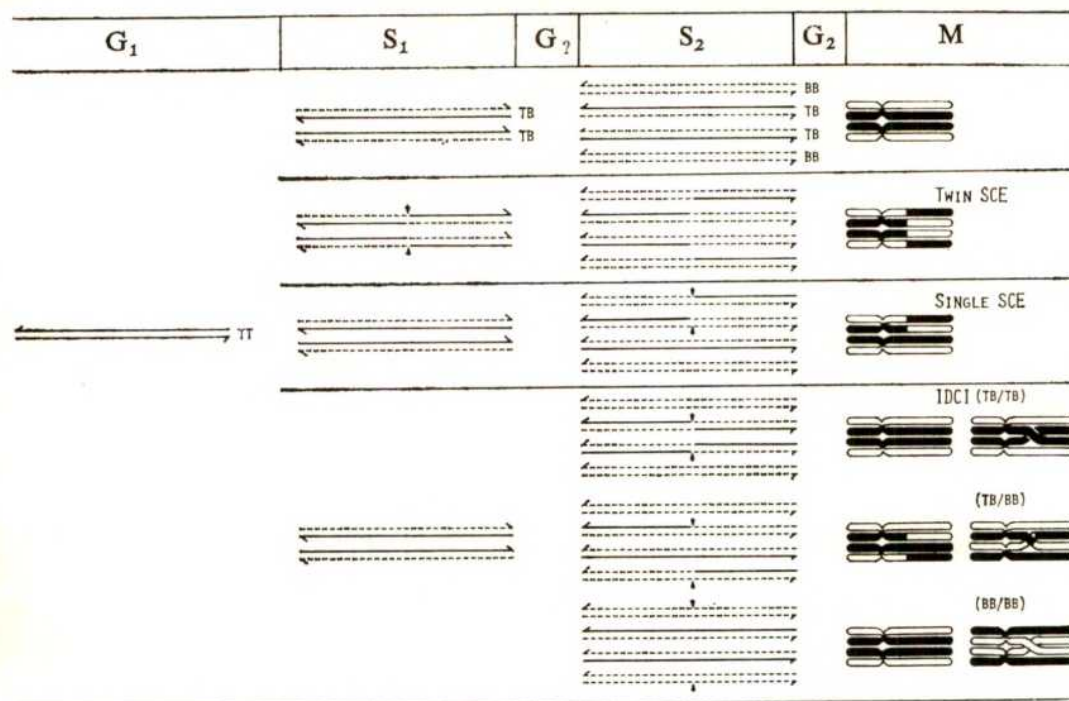
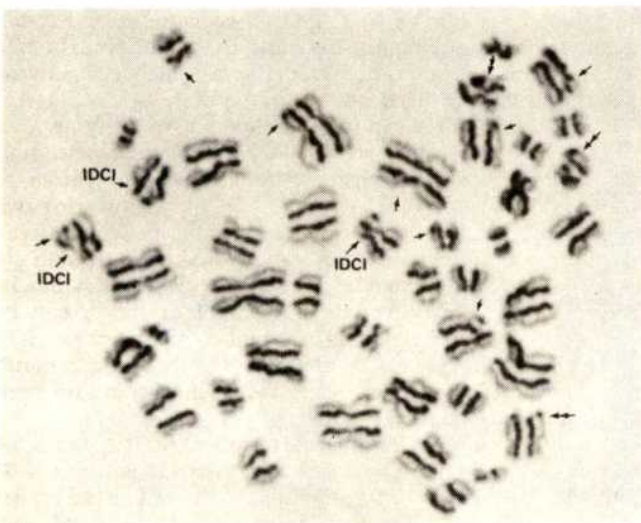


Fig. 1 Diagram of SCEs and IDCIs in diplochromosomes and orderly segregation of DNA strands during the replication cycle. The diagram is based on the unimere model of chromosomes, in which a chromatid contains a single DNA duplex. The new DNA strands always segregate outside the parental strands. When the cells are grown in the presence of BUdR for two replication cycles, except for disruption by exchanges, the chromatids of unifilar BUdR-substitution (TB) are on the inner side in diplochromosomes while those of bifilar BUdR-substitution (BB) are on the outer side. Broken lines represent BUdR-substituted DNA strands and solid lines unsubstituted grand-parental strands. Arrows show the sites of exchanges.

preparations 70 h after plating—mitotic arrest was achieved by a 4-h prefixation with colcemid. The effects of caffeine and cycloheximide were studied by treating cells for the last 24 h. In some cultures, 0.005% β -mercaptoethanol was added to increase the frequency of endoreduplication mitoses. Chromosome preparations were processed for differential chromatid staining according to the 'fluorescent plus Giemsa' technique of Perry and Wolff⁹, and endoreduplication mitoses (Fig. 2) were analysed for types and frequencies of SCEs and IDCIs.

Figure 3 shows that incubation in the presence of β -mercaptoethanol increased the frequency of twin and

Fig. 2 Endoreduplication mitosis with differential chromatid staining showing single SCEs (single arrows), twin SCEs (double arrows) and IDCIs. The cell was exposed overnight to a black-light fluorescent lamp (Toshiba FL20BL) in 33258 Hoechst $1 \mu\text{g ml}^{-1}$, incubated in distilled water for 2 h at 62 °C and stained with Giemsa. Bifilarly BUdR-substituted chromatids are stained light while unifilarly substituted ones are stained dark.



single SCEs but did not affect that of IDCIs. In the untreated cultures, either with or without β -mercaptoethanol, the rates of SCE in the first and second S phases were approximately the same, the number of twin SCEs per diplochromosome not being significantly different from the number of single SCEs per chromosome. Because the treatment for the last 24 h covers only the second replication cycle in my culture conditions¹⁰, twin SCEs related with the first S phase could not be influenced by caffeine and cycloheximide treatment during this interval. But exchange events relating to the second S phase, that is, single SCEs and IDCIs, were significantly affected by these compounds, although in different ways—caffeine reduced IDCIs but increased single SCEs, whereas cycloheximide had no effect on IDCIs but suppressed single SCEs. The involvement of chromatids in the formation of IDCIs was non-random. Of 565 IDCIs scored, 518 (91.7%) were exchanges between chromatids with uni- and bifilar substitution (TB/BB type) (Fig. 1), and the TB/TB and BB/BB types were unexpectedly infrequent, constituting only 7.8% and 0.5%, respectively. The IDCIs of TB/TB and BB/BB types between chromatids with identical staining properties, could only be recognised morphologically as X-shaped criss-cross exchanges. But among IDCIs of TB/BB type, 91.3% had the same configuration of overlapping chromatids. Thus the predominance of TB/BB exchanges cannot be explained by different rates of detection, but suggests some biological significance for the formation of IDCIs in these conditions. In the cells grown in the absence of BUdR, the frequency of morphologically identifiable IDCIs was 0.02 per diplochromosome, about a quarter of that in the presence of 20 μM BUdR, which apparently induces IDCI.

It is interesting that IDCIs can be suppressed by caffeine. Caffeine inhibits post-replication repair¹¹⁻¹³, which is thought to involve the formation of gaps in the daughter strand during replication on a damaged DNA template and the filling in of these gaps by *de novo* synthesis¹⁰. Similar processes and/or types of lesion are likely to be responsible for CI formation, possibly through a recombinational event. In eukaryotic cells, cycloheximide causes immediate inhibition of DNA replication¹⁷⁻¹⁹. Although the action of cycloheximide may be diverse²⁰⁻²¹, it is tempting to correlate the

cycloheximide-sensitive SCE with some components of the DNA replication process.

Although my study concerned chromatid exchange in diplochromosomes, it is possible that the process responsible for IDCI formation occurs during normal diploid mitosis. This would manifest as false SCE²²⁻²³ when occurring between sister chromatids and would give rise to CI and other chromatid exchange aberrations if chromosome segments involved are closely positioned in the nucleus and replicate at a similar time. In spite of the general association between SCEs and chromatid aberrations in their response to DNA-damaging agents, there is evidence against a correlation between two phenomena. Among human genetic diseases known to be characterised by a high frequency of spontaneous chromatid aberrations, only Bloom's syndrome involves apparent association of the two, showing an abnormally high rate of spontaneous SCE²⁴. But in

Fanconi's anaemia²⁵, ataxia telangiectasia²⁶ and incontinentia pigmenti (my unpublished data) the rate of SCE is normal. Moreover, in Fanconi's anaemia²⁵ and xeroderma pigmentosum²⁷, sensitivity to SCE caused by chemical mutagens does not correlate with the sensitivity to chromatid aberration formation, whereas IDCI formation does²⁸. Also the effects of caffeine on chemical induction of SCE differ in plants²⁹ and Chinese hamster cells⁴. These lines of evidence reinforce my conclusion that there is a two-step, replication-mediated, repair mechanism and that SCE and CI are cytological manifestations of them. The relative potential of each process may vary with the types of DNA damage, repair systems and organisms involved.

This work was supported by grants from the Ministry of Education, Science and Culture, and the Ministry of Health and Welfare of Japan. I thank Dr H. Loewe, Hoechst, Frankfurt, for fluorochrome 33258.

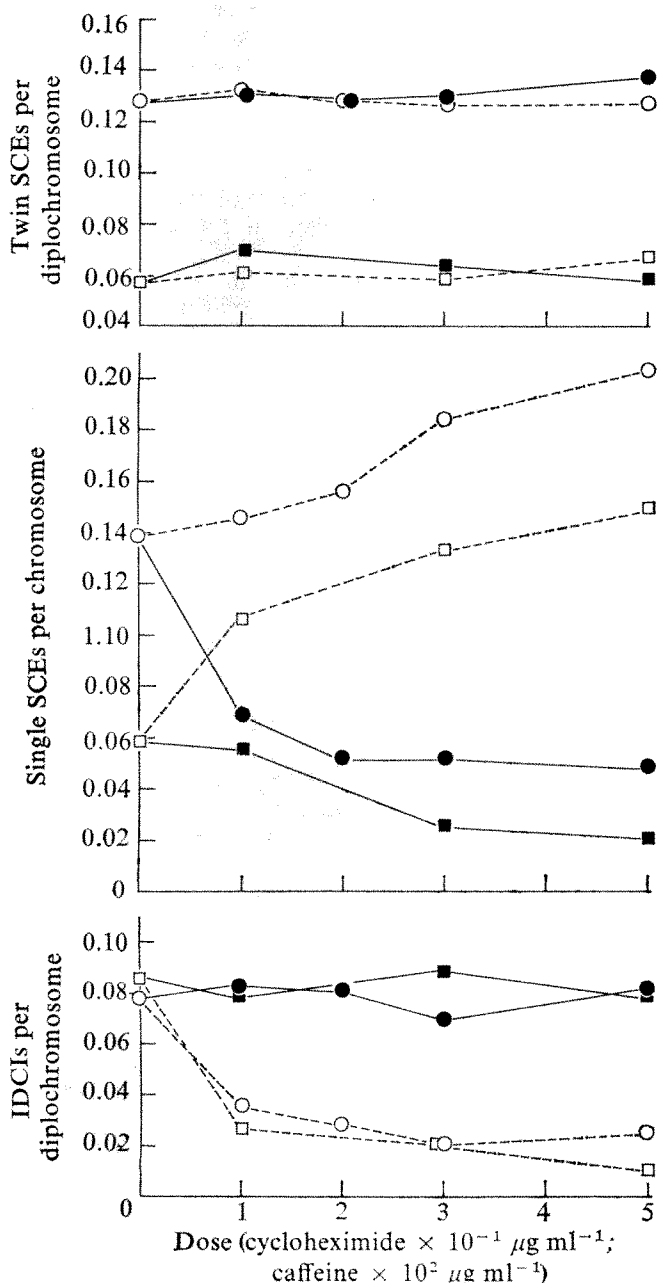
M. S. SASAKI

Department of Cytogenetics,
Medical Research Institute,
Tokyo Medical and Dental University,
Yushima, Bunkyo-ku, Tokyo 113, Japan

Received 17 February; accepted 2 August 1977.

1. Taylor, J. H., Woods, P. S. & Hughes, W. L. *Proc. natn. Acad. Sci. U.S.A.* **43**, 122-127 (1957).
2. Perry, P. & Evans, H. J. *Nature* **258**, 121-125 (1975).
3. Kato, H. *Expl Cell Res.* **82**, 383-390 (1973).
4. Kato, H. *Expl Cell Res.* **85**, 239-247 (1974).
5. Wolff, S., Bodycote, J. & Painter, R. B. *Mutat. Res.* **25**, 73-81 (1974).
6. Latt, S. A. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3162-3166 (1974).
7. Evans, H. J. in *Recovery and Repair Mechanism in Radiobiology* 111-133 (Brookhaven National Laboratory Associated Universities Inc., New York, 1967).
8. Kihlman, B. A. in *Advances in Cell and Molecular Biology* 59-107 (ed. DuPraw, E. J.) (Academic, New York and London, 1971).
9. Perry, P. & Wolff, S. *Nature* **251**, 156-158 (1974).
10. Sasaki, M. S. *Mutat. Res.* **29**, 433-448 (1975).
11. Cleaver, J. E. & Thomas, G. H. *Biochem. biophys. Res. Commun.* **36**, 203-208 (1969).
12. Fujiwara, Y. *Expl Cell Res.* **75**, 483-489 (1972).
13. Trosko, J. E. & Chu, E. H. Y. *Chem. Biol. Interaction* **6**, 317-332 (1973).
14. Lehmann, A. R. & Kirk-Bell, S. *Mutat. Res.* **26**, 73-82 (1974).
15. Nilsson, K. & Lehmann, A. R. *Mutat. Res.* **30**, 255-266 (1975).
16. Lehmann, A. R. *J. molec. Biol.* **66**, 319-337 (1972).
17. Hyodo, M., Koyama, H. & Ono, T. *Expl Cell Res.* **67**, 461-463 (1971).
18. Fujiwara, Y. *Cancer Res.* **32**, 2089-2093 (1972).
19. Gautzchi, J. R. & Kern, R. M. *Expl Cell Res.* **80**, 15-26 (1973).
20. Pohjanpelto, P. *Expl Cell Res.* **102**, 138-142 (1976).
21. Ceccarini, C. & Eagle, H. *In Vitro* **12**, 346-351 (1976).
22. Wolff, S. & Bodycote, J. *Mutat. Res.* **29**, 85-91 (1975).
23. Kato, H. *Chromosoma* **59**, 179-191 (1977).
24. Chaganti, R. S. K., Schonberg, S. & German, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4508-4512 (1974).
25. Latt, S. A., Stetten, G., Juergens, L. A., Buchanan, G. R. & Gerald, P. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4066-4070 (1975).
26. Galloway, S. M. & Evans, H. J. *Cytogenet. Cell Genet.* **15**, 17-29 (1975).
27. Wolff, S., Rodin, B. & Cleaver, J. E. *Nature* **265**, 347-349 (1977).
28. Sasaki, M. S. *Mutat. Res.* **46**, 152-153 (1977).
29. Kihlman, B. A. *Chromosoma* **51**, 11-18 (1975).

Fig. 3 Effects of caffeine and cycloheximide on frequencies of SCE and IDCI. Experiments were done in the presence (circles) or absence (squares) of β -mercaptoethanol. Solid symbols, cycloheximide, open symbols, caffeine. Each point is based on 12-25 endoreduplicated mitoses.



Long-term persistence of O^6 -methylguanine in rat brain DNA

THE carcinogenic potency of alkylating agents correlates with the extent of their reaction at oxygen atoms in DNA bases¹. O^6 -Alkylguanine is amongst those derivatives most likely to be involved in the initiation of malignant transformation since it leads to mispairing *in vitro* and thus can be considered promutagenic^{2,3}. Repair excision of O^6 -alkylguanine from DNA has been demonstrated in bacteria⁴ and mammalian cells⁵, and seems to be initiated by a specific *N*-glucosidase⁶. In the intact animal, the persistence of O^6 -alkylguanine in DNA of different tissues varies considerably. In rats, the induction of neural^{7,8} and renal⁹ neoplasms correlates with a deficient excision capacity of the respective target tissues. O^6 -Alkylguanine produced by a single injection of the neuro-oncogenic agents *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea (MNU), is removed

from brain DNA significantly more slowly than from that of liver and kidney, the half life in cerebral DNA being in the range of 9–11 d for both *O*⁶-ethyl- and *O*⁶-methylguanine^{7,10}. These data are, however, based only on observations for up to 10 d. We report here that the decay curve for *O*⁶-methylguanine in brain DNA consists of different components and that this modified base may persist in cerebral DNA for a considerable fraction of the animal's life.

Adult female BD-IX rats (100–120 g) received a single intravenous injection of *N*-[³H]-methyl-*N*-nitrosourea (25.5 mCi mmol⁻¹) at a dose of 10 mg per kg body weight. After time intervals ranging from 4 h to 184 d, DNA was isolated by phenol extraction¹¹ from the combined organs of two animals. Following hydrolysis in 0.1 M HCl (37 °C, 24 h) purine bases were separated on Sephadex G-10 columns (1 × 90 cm) using 0.05 M ammonium formate (pH 6.2) as eluent. The amounts of methylated purines were expressed as a fraction of the parent base, assuming that the specific radioactivity of the methyl group was identical to that of the injected carcinogen¹².

The persistence of *O*⁶-methylguanine in DNA of various rat organs is shown in Fig. 1. Excision from hepatic DNA was faster than from that of any other tissue, *O*⁶-methylguanine concentrations being below the level of detectability within 7 d. In lung and kidney DNA, the limit of detection was reached by 28 and 84 d respectively. In brain, the rate of excision was considerably slower. Following a rapid decrease during the first hours after injection, there is a distinct slope extending from 12-h to 14 d. During this period, *O*⁶-methylguanine values were reduced from 78% to 50% of the initial (4 h) concentration. Subsequently, the rate of removal decreased greatly. In contrast to the other organs and to the initial part of the decay curve for the nervous system, this latter component was linear. At 184 d, 25% of the initial concentration was still present in cerebral DNA. Extrapolation of these data suggests that complete removal of *O*⁶-methylguanine would require approximately 1 yr.

We can rule out the possibility that tissue-dependent variations in cell turnover significantly account for the observed differences in the rate of loss of *O*⁶-methylguanine from DNA. 7-Methylguanine which is removed non-enzymatically from DNA by hydrolysis, shows a similar decay in both liver and brain (Fig. 2).

The dose of MNU used in this study (10 mg per kg) represents approximately 10% of the 50% lethal dose (LD₅₀) and is not sufficient to induce tumours. Given

Fig. 1 Persistence of *O*⁶-methylguanine in DNA of brain (●), kidney (△), lung (▽), and liver (○). Female BD-IX rats received a single intravenous injection of *N*-[³H]-methyl-*N*-nitrosourea (10 mg kg⁻¹) and were killed at time intervals ranging from 4 h to 184 d.

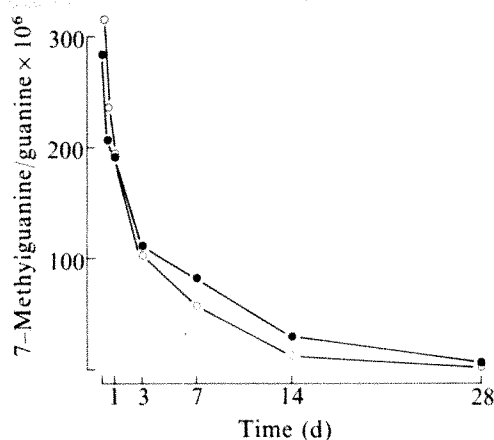
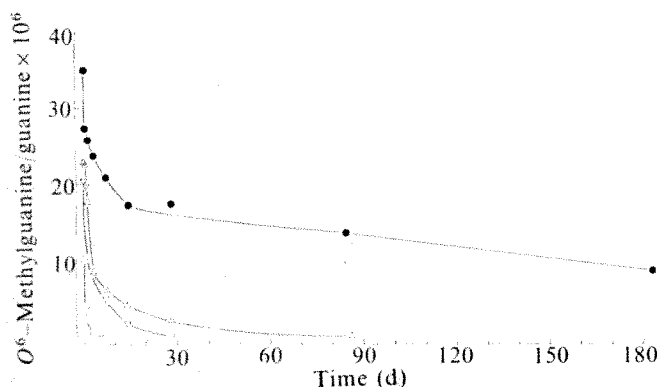


Fig. 2 7-Methylguanine concentrations in DNA of rat brain (●) and liver (○) following a single injection of ³H-MNU. Experimental details as in Fig. 1.

repeatedly, however, it will produce primarily tumours of the nervous system. In contrast to other organs, such multiple doses lead to a linear increase of *O*⁶-methylguanine in brain DNA¹¹. It now seems that this additive effect is based on the linear (slow) segment of the excision curve. It is possible, however, that different excision kinetics apply to alkylated bases resulting from environmental exposure to very small doses. In rat liver, the rate of excision has indeed been shown to be inversely related to the initial amount of *O*⁶-methylguanine produced^{13,14}.

The different components of the *O*⁶-methylguanine removal curve in rat brain may represent DNA fractions within the chromatin complex having a different accessibility to repair enzymes¹⁵. Alternatively, the different slopes may reflect the repair capacity of different brain cell populations, for example neuronal versus glial cells. The latter possibility is being investigated. If the persistence of *O*⁶-alkylguanine in DNA leads to mutational events responsible for malignant transformation, the slow component should represent glial DNA, since the tumours induced by MNU and related carcinogens are almost exclusively of glial origin.

This work was supported by the Deutsche Forschungsgemeinschaft.

PAUL KLEIHUES

Pathologisches Institut der Universität,
Abteilung Neuropathologie,
78 Freiburg i. Br., West Germany

JOACHIM BUEHLER

Max-Planck-Institut für Hirnforschung,
5 Köln 91, West Germany

Received 16 June; accepted 3 August 1977.

- Lawley, P. D. *Mutat. Res.* **23**, 283–295 (1974).
- Gerchman, L. L. & Ludlum, D. B. *Biochim. biophys. Acta* **308**, 310–316 (1973).
- Loveless, A. *Nature* **223**, 206–208 (1969).
- Lawley, P. D. & Orr, D. J. *Chem.-Biol. Interact.* **2**, 154–157 (1970).
- Kirtikar, D. & Goldthwait, D. A. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2022–2026 (1974).
- Lindahl, T. *Nature* **259**, 64–66 (1976).
- Goth, R. & Rajewsky, M. F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 639–643 (1974).
- Kleihues, P. & Margison, G. P. *J. natn. Cancer Inst.* **53**, 1839–1841 (1974).
- Nicoll, J. W., Swann, P. F. & Pegg, A. E. *Nature* **254**, 261–262 (1975).
- Kleihues, P., Cooper, H. K., Bücheler, J., Kolar, G. F. & Diessner, H. *Natn. Cancer Inst. Monogr.* (in the press).
- Margison, G. P. & Kleihues, P. *Biochem. J.* **148**, 521–525 (1975).
- Lawley, P. D. & Shah, S. A. *Chem.-Biol. Interact.* **7**, 115–120 (1973).
- Kleihues, P. & Margison, G. P. *Nature* **259**, 153–155 (1976).
- Pegg, A. E. *J. natn. Cancer Inst.* **58**, 681–687 (1977).
- Trosko, J. E. & Chu, E. H. Y. *Adv. Cancer Res.* **21**, 391–424 (1975).

Daunorubicin and adriamycin facilitate actinomycin D binding to poly(dA-dT)·poly(dA-dT)

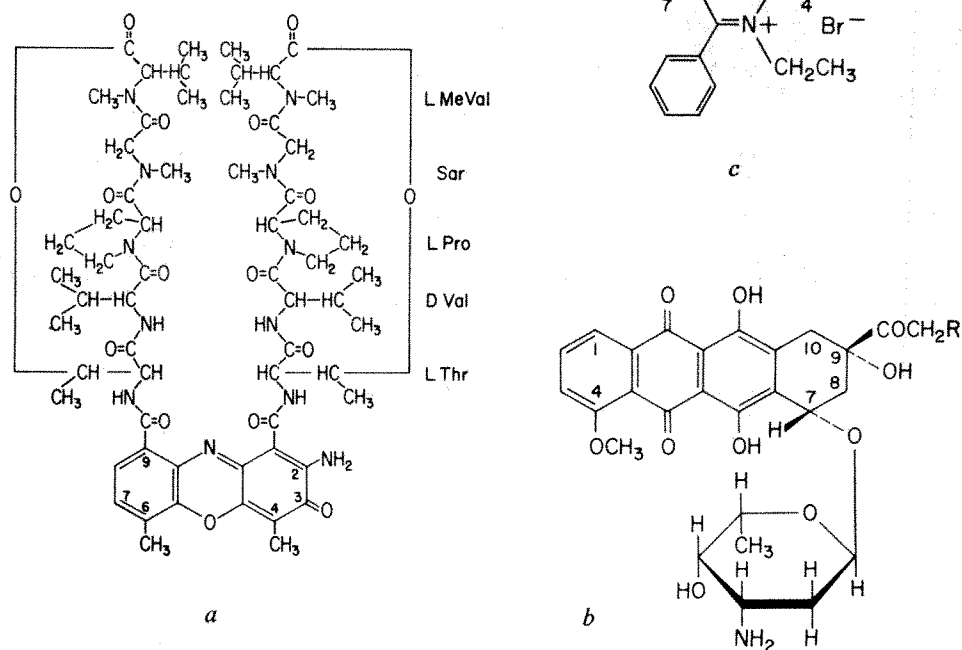
ACTINOMYCIN D (Fig. 1a) binds to DNA by intercalation of the phenoxazone ring between adjacent base pairs of the double helix¹⁻⁴. There is a general requirement for a guanine base at the actinomycin D binding site on DNA, as illustrated by the observation that actinomycin D does not intercalate into double-stranded (ds) poly(dA-dT)·poly(dA-dT) (refs 1-4). Daunorubicin, adriamycin (Fig. 1b) and ethidium bromide (Fig. 1c) also intercalate into dsDNA, but in contrast to actinomycin D, these drugs do not show any requirement for a particular base at the intercalation site^{5,6}. Both actinomycin D and ethidium bromide do, however, show definite preferences for binding to certain sequences of nucleic acid bases at the intercalation site⁷⁻¹¹. In this report we show that daunorubicin and adriamycin facilitate strong binding of actinomycin D to poly(dA-dT)·poly(dA-dT) and we discuss the implications of this new observation.

Circular dichroism (CD) spectroscopy is a particularly sensitive technique for monitoring the formation of a complex between drugs and nucleic acids. Both daunorubicin and actinomycin D are optically active and give rise to circular dichroism spectra which undergo characteristic changes when these drugs bind to DNA, synthetic polynucleotides, or oligonucleotides (refs 6, 12-19 and our unpublished observations). The intercalated complex of actinomycin D with DNA is characterised by a large negative band in the 440-490-nm region, whereas actinomycin D in dilute aqueous solution or in solution with poly(dA-dT)·poly(dA-dT) has only a negligible band in this region (Fig. 2). Daunorubicin and adriamycin exhibit a broad weak positive CD band in the 440-480-nm region,

which is intensified following binding to DNA or poly(dA-dT)·poly(dA-dT) (Fig. 2). The circular dichroism spectra of poly(dA-dT)·poly(dA-dT) with daunorubicin or actinomycin D, and of an equimolar mixture of the two drugs with poly(dA-dT)·poly(dA-dT) are also shown in Fig. 2. The appearance of the strong negative band in the 450-490-nm region of the spectrum of the equimolar mixture of the two drugs with poly(dA-dT)·poly(dA-dT) results from the simultaneous binding of these two drugs to poly(dA-dT)·poly(dA-dT). The intensification of the 380-nm band of actinomycin D when it binds to poly(dA-dT) (which is readily apparent in the difference spectrum shown in Fig. 2) also indicates that daunorubicin cooperatively facilitates the binding of actinomycin D to poly(dA-dT)·poly(dA-dT). We believe that this is the first example of co-operative binding between two intercalating drugs. Ethidium bromide intercalates into poly(dA-dT)·poly(dA-dT), but circular dichroism spectra indicate that ethidium bromide does not facilitate the binding of actinomycin D to poly(dA-dT)·poly(dA-dT), nor does the addition of actinomycin D significantly influence the binding of ethidium bromide to this polynucleotide (T.R.K., M.A.Y. & R. V. Kastrup, in preparation). In related experiments, we have used ³H-actinomycin D in equilibrium dialysis experiments, from which we estimate that the association constant for the binding of actinomycin D to poly(dA-dT)·poly(dA-dT) in the presence of daunorubicin is $\sim 4 \times 10^5 \text{ M}^{-1}$ (T.R.K., W. E. Moehle & K. G. Rao, in preparation).

These results raise several important questions. First, how does daunorubicin (or adriamycin) enhance the binding of actinomycin D to poly(dA-dT)·poly(dA-dT)? Intercalation of these drugs unwinds the DNA helix¹⁻⁶ and it is likely that the distortions induced in the double helix may be transmitted over several base pairs. The amino sugars of daunorubicin and adriamycin, and the cyclic pentapeptides of actinomycin D become located along the grooves of the double helix when the planar portions of the drugs (Fig. 1) intercalate between the base pairs. The

Fig. 1 The chemical structures of a, actinomycin D; c, ethidium bromide; and b, daunorubicin (also frequently called daunomycin) and adriamycin.



R = H Daunorubicin

R = OH Adriamycin

changes in the double helical geometry of poly(dA-dT)·poly(dA-dT) which accompany daunorubicin binding are presumably responsible for the facilitation of actinomycin D binding to an adjacent region of poly(dA-dT)·poly(dA-dT).

Thus the present results show that when daunorubicin binds to poly(dA-dT)·poly(dA-dT) a change occurs in the conformation of the polynucleotide at an adjacent region of

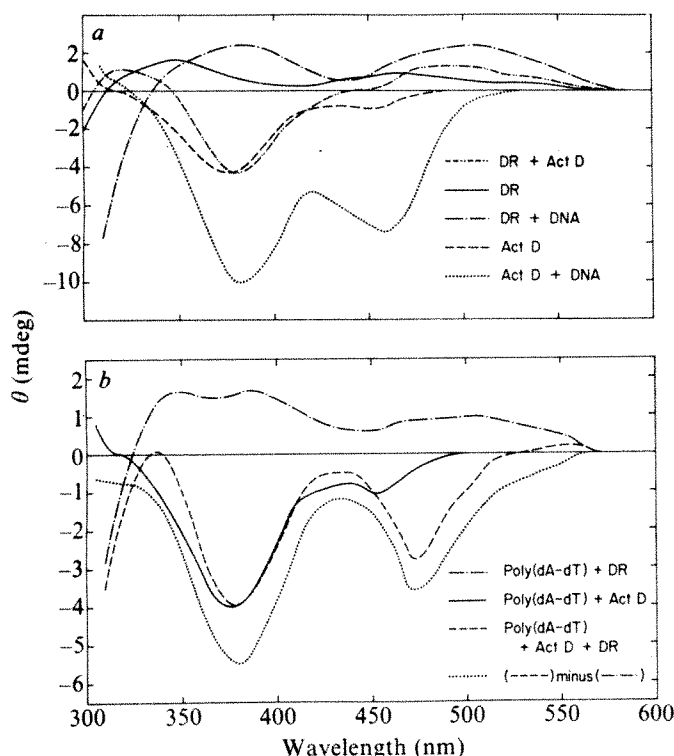


Fig. 2 Circular dichroism spectra of solutions of: *a*, 8.5×10^{-6} M daunorubicin (DR) and actinomycin D (Act D) alone and in the presence of 8.5×10^{-6} M DNA; *b*, 8.5×10^{-6} M daunorubicin plus 8.5×10^{-6} M poly(dA-dT)·poly(dA-dT); 8.5×10^{-6} M actinomycin D plus 8.5×10^{-6} M poly(dA-dT)·poly(dA-dT); 8.5×10^{-6} M actinomycin D plus 8.5×10^{-6} M daunorubicin plus 8.5×10^{-6} M poly(dA-dT)·poly(dA-dT). The curve was calculated by subtracting the poly(dA-dT)·poly(dA-dT)+DR spectrum from the poly(dA-dT)·poly(dA-dT)+DR+Act D spectrum which, to a first approximation, is an estimate of the circular dichroism spectrum of actinomycin D when bound to poly(dA-dT)·poly(dA-dT). All spectra were recorded on a Jasco J-40 circular dichroism instrument in a 4-cm path length cell at 20 °C. All solutions contained 10 mM potassium phosphate buffer, pH 7.0. Essentially similar results were obtained at lower drug-to-phosphate ratios, as well as in the presence of 0.1 M NaCl. The spectra in which adriamycin were used in place of daunorubicin gave qualitatively similar results. 7-Amino-actinomycin D has also been used in place of actinomycin D with qualitatively similar results. The circular dichroism spectra in which ethidium bromide is used in combination with actinomycin D and poly(dA-dT)·poly(dA-dT) do not show the appearance of the large negative band in the 440–480-nm region. The circular dichroism spectrum (*a*) of a daunorubicin and actinomycin D solution (8.5×10^{-6} M in each drug) is not equal to the sum of the circular dichroism spectra of the individual drugs (data not included) at all wavelengths, which shows that a small fraction of the daunorubicin and actinomycin D molecules form a heterodrug complex. These small effects do not influence the present results, however, or the interpretation of the data presented here because >90% of the drugs are bound to poly(dA-dT)·poly(dA-dT) when the polynucleotide is present, and thus the concentrations of the free drugs are $<1 \times 10^{-6}$ M in the presence of the polynucleotides, which essentially eliminates the formation of drug–drug aggregates. We have also studied the binding of actinomycin D and daunorubicin to poly(dA), poly(dT), and a poly(dA)·poly(dT) duplex, where we observed the synergistic binding of the two drugs only in the presence of the ds polynucleotide, poly(dA)·poly(dT). No cooperative binding of the drugs was observed when the ribopolynucleotide poly(A-U)·poly(A-U) was used, which is not surprising because actinomycin D does not bind to polyribonucleotides^{1–4} and daunorubicin has been previously reported to bind only weakly to polyribonucleotides⁶.

the double helix which results in an increase in the stability of the actinomycin D–poly(dA-dT)·poly(dA-dT) complex. This transmission of the distortions of the nucleic acid conformation along the double helix may also be an important component in the selective recognition of nucleic acid sequences (as for example, in promoter–operator complexes), and is related to the transmission of thermal stability from the G-C region to the A-T region which has been observed in block oligonucleotides such as d(C₁₅A₁₅)-d(T₁₅G₁₅) (refs 20–22).

Second, do daunorubicin and actinomycin D (or adriamycin and actinomycin D) both intercalate into poly(dA-dT)·poly(dA-dT)? The shape of the circular dichroism spectra in Fig. 2 suggests that both chromophores are intercalated, but nuclear magnetic resonance experiments on complexes of these drugs with oligonucleotides are required to verify the geometry of the complexes^{8,23,24}.

Third, will these drugs bind cooperatively to DNA *in vivo*, and more important, will they exhibit a synergistic physiological activity if used in combination? *In vivo*, the primary mode of action of the intercalating drugs has been shown to be interference with the nucleic acid polymerases (see refs 25 and 26). In separate experiments (A. H. McHale, S. S. Holcomb, R. Josephson & T.R.K., in preparation) we have observed that actinomycin D does not inhibit the activity of *E. coli* DNA polymerase when poly(dA-dT)·poly(dA-dT) is used as a template, whereas daunorubicin and adriamycin give rise to the anticipated dose–response curves for the inhibition of this enzyme as a function of the drug concentration^{1–4,6,25,26}. The presence of actinomycin D does, however, affect the daunorubicin inhibition of poly(dA-dT)·poly(dA-dT) synthesis, which also illustrates the synergistic interaction of the two drugs when both are bound to poly(dA-dT)·poly(dA-dT). We have been unable to find reports of clinical studies involving the combined use of either daunorubicin or adriamycin with actinomycin D either by themselves or with other drugs. The results reported here suggest that combinations of these drugs should be explored clinically.

We thank R. V. Kastrup and K. G. Rao for assistance with parts of this research. This work was supported by research grants and a Research Career Development Award (to T.R.K.) from the National Cancer Institute. T.R.K. is an Alfred P. Sloan Fellow.

THOMAS R. KRUGH
MICHAEL A. YOUNG

Department of Chemistry,
University of Rochester,
Rochester, New York 14627

Received 31 May; accepted 15 August 1977.

- Meienhofer, J., Atherton, E. *Adv. appl. Microbiol.* **13**, 203–300 (1976).
- Sobell, H. M. *Prog. Nucl. Acids Res.* **13**, 140–153 (1973).
- Goldberg, I. H. & Friedman, P. A. *Rev. Biochem.* **40**, 775–810 (1971).
- Wells, R. D. in *Progress in Molecular and Subcellular Biology*, 2 (ed. Hahn, F. E.) 21–32 (Springer-Verlag, New York, 1971).
- LePecq, J.-B. *Meth. Biochem. Anal.* **20**, 41 (1971).
- Henry, D. W. in *Cancer Chemotherapy* (ed. Sartorelli, A. C.) *Am. Chem. Soc. Symp. Ser.* **30**, 15–57 (1976).
- Krugh, T. R. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1911–1915 (1972).
- Krugh, T. R. & Neely, J. W. *Biochemistry* **12**, 4418–4425 (1973).
- Krugh, T. R. & Reinhardt, C. G. *J. molec. Biol.* **97**, 133–162 (1975).
- Reinhardt, C. G. thesis, Univ. Rochester (1977).
- Chen Chiao, Y.-C. & Krugh, T. R. *Biochemistry* **16**, 747–755 (1977).
- Hollstein, U. *Chem. Rev.* **74**, 625–652 (1974).
- Krugh, T. R., Wittlin, F. N. & Cramer, S. P. *Biopolymers* **14**, 197–210 (1975).
- Crothers, D. M., Sabol, S. L., Ratner, D. I. & Muller, W. *Biochemistry* **7**, 1817–1823 (1968).
- Yamaoka, K. & Ziffer, H. *Biochemistry* **7**, 1001–1008 (1968).
- Homer, R. B. *Archs Biochem. Biophys.* **129**, 405–407.
- Quadrifoglio, F. & Crescenzi, V. *Biophys. Chem.* **2**, 64–69 (1974).
- Arcamone, F. *et al.* in *International Symposium on Adriamycin* (eds. Cart, S. K. *et al.*) 9–22 (Springer-Verlag, New York, 1972).
- Auer, H. E., Pawlowski-Konopnicki, B. E. & Krugh, T. R. *FEBS Lett.* **73**, 167–170 (1976).
- Burd, J. F., Wartell, R. M., Dodgson, J. B. & Wells, R. D. *J. biol. Chem.* **250**, 5109–5113 (1975).
- Burd, J. F., Larson, J. E. & Wells, R. D. *J. biol. Chem.* **250**, 6002–6007 (1975).
- Early, T. E., Kearns, D. R., Burd, J. F., Larson, J. E. & Wells, R. D. *Biochemistry* **16**, 541–551 (1977).
- Patel, D. J. *Biochemistry* **13**, 2396–2402 (1974).
- Reinhardt, C. G. & Krugh, T. R. *Biochemistry* **16**, 2890–2895 (1977).
- Di Marco, A. & Arcamone, F. *Arzneimitt. Forsch.* **25**, 368–375 (1975).
- Bachur, N. R. in *Cancer Chemotherapy* (ed. Sartorelli, A. C.) *Am. Chem. Soc. Ser.* **30**, 58–70 (1976).

reviews

Imaginative immunology

Maria de Sousa

B and T Cells in Immune Recognition. Edited by F. Loor and G. E. Roelants. Pp. xviii + 504. (Wiley-Interscience: New York and London, 1977.) £18.50; \$37.50.

A RATHER unimaginative title for a rare, imaginative immunology book.

The editors, Francis Loor and Georges Roelants, members of the staff of the Basel Institute of Immunology for long enough to reflect its eclectic atmosphere in their choices, bring together twenty chapters that leave out little that is relevant to those concerned with the way cellular immunology is going.

No complement, gut immunology or parasite diseases. Otherwise, those interested in autoimmunity, immunodeficiency, lymphoproliferative malignancy, HLA-associated disease, cell-mediated cytotoxicity, the major histocompatibility complex (MHC) its role in lymphocyte activation and its relevance to transplantation in man, will find competent chapters by Fye, Moutsopoulos and Talal (autoimmunity), Cooper and Seligman (immunodeficiency and lymphoid malignancy), Ryder and Sveigaard (HLA-associated disease), Dausset and Fradelizi (MLR genes and transplantation), Cerottini and Brunner (cell-mediated cytotoxicity) and Bluestein (MHC and lymphocyte activation).

Those wondering whether immunology still has anything to do with the understanding and therapy of cancer will find a concise review of the current state of the clinical and experimental art by Mitchison.

But there is also information for the developmental immunologist. From the wider biological world of LeDouarin, who candidly admits that "the actual immunological function of the thymus is well documented only for Aves and Mammals; only little information has been produced concerning the thymus equivalent organs found in other vertebrates". From the ontogeny perspective of Owen who concludes his chapter on "Ontogenesis of lymphocytes" with the remark that "many basic questions of immunology are essentially problems of lymphocyte differentiation", a remark that gains reality as one wanders through Beverley's chapter on "lymphocyte heterogeneity".

The role of thymic hormones is reviewed by Trainin, Small and Kook. But should one forget that immunology is only T or B cells, an excellent chapter by Roelants reminds us of "the regulatory role of macrophages in immune recognition". This is not the only healthy reminder of the variety of present day immunology.

Lymphocytes "go round", a frequently forgotten event competently reviewed by Sprent in a chapter on "migration and lifespan of lymphocytes"; the chapter by Schreier and Nordin entitled "An evaluation of the immune response *in vitro*" is certain to become a classical chapter of twentieth century immunological literature. This is the only chapter in the book from which the editors requested original data (we are told in the preface). Their intention was to illustrate how cautious one must be with *in vitro* experiments on the immune response. In it, one learns how varying rocking, feeding and adding mercaptoethanol to cell cultures placed in different dishes, leads five different investigators to being equally right in their five different conclusions.

Humour is not restricted to this latter chapter. Parkhouse and Abney's chapter on "Biochemical approaches to receptors for antigen on B and T

lymphocytes" has both humour and sufficient facts and arguments to help readers find their way through an area of much controversy, separately reviewed by Goodman in the chapter on "The specificity repertoire and antigen receptors of T and B lymphocytes".

It is the reviewer's own bias and interest to find evidence of roots of future in present day texts, that leads me to conclude by stressing the importance of the four most enlightening chapters: Loor's on "Structure and dynamics of the lymphocyte surface", Klaus' on "B cell maturation", Nabholz and Miggiano's on "The biological significance of the MLR", and Bretscher's closing chapter integrating T and B lymphocytes in immune activation.

Some minor printing errors: One wonders, for instance if Avron Mitchison (p 338) did mean to say that "modern immunology starts with Foley and Prehn and Main", knowing with all due respect, who else (including himself) was around at the time.

A rare and imaginative immunology book indeed. □

Maria de Sousa is Head of the Cell Ecology Laboratory at the Sloan-Kettering Institute for Cancer Research, New York.

Quark binding

Quark Confinement and Field Theory. Edited by D. R. Stump and D. H. Wein-garten. Pp. 253. (Wiley: London, New York and Tokyo, 1977.) £18.70; \$31.70.

THIS book offers a collection of papers presented at the workshop on quark binding held at the University of Rochester in June, 1976. The papers represent a cross section of current work on quark confinement and field theory.

Various approaches to the study of collective phenomena, and classical solutions to Yang-Mills field equations are found in papers by Y. Nambu, T. Eguchi, K. Huang and D. Stump, and F. Wilczek. In an attempt to understand a different class of non-perturbative phenomena, N. Snyderman and G. Guralnik discuss dynamical symmetry breaking.

In two closely related papers, E. Poggio and J. Carrazzone give summaries of their analyses of high orders in perturbation theory for Yang-Mills theories. The stumbling block of this method is, of course, a lack of knowledge about the infrared behaviour of the effective quark-gluon coupling strength. In another paper, P. Olesen offers a description of some aspects of this behaviour using a clever application of the renormalisation group.

Theoretical descriptions of particles produced in e^+e^- annihilation are offered in three papers. V. Matveev presents a phenomenological hydrodynamic model of collective resonances, a model which he suggests may have applications in the description of e^+e^- production of resonances. A more conventional model is used to describe charmonium by T. Yan. Yan presents

an excellent analysis using an effective field theory with a linear potential. A phenomenological model which attempts to describe charmed particle decays is presented by V. Mathur.

Each of the remaining three papers deals with areas with little connection to other work presented at the conference. In a discussion of covariance problems in two dimensions, C. Hagen questions the validity of light cone field theory. Deviations from scaling are analysed by H. Politzer, using the renormalisation group and the Wilson operator product expansion. An unconventional model involving massive gluons and unconfined colour is presented by R. N. Mohapatra as a possible explanation of high γ anomalies and dimuon events. (It should be noted

that recent experimental work, not yet made public at the time of publication of this collection, offers no evidence for the phenomena this paper attempts to explain.)

This book contains some excellent reports on theoretical work on the quark binding problem and field theory, and would be appropriate for the shelves of physics libraries. For those who are actively working in these areas, however, purchase of this book seems an expensive way to acquire papers which are readily available either as reprints or as published articles.

Larry McLerran

Larry McLerran is a research associate in the Laboratory for Nuclear Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Fungal symbiosis

The Biology of Symbiotic Fungi. By Roderic Cooke. Pp. xi+282. (Wiley: London and New York, 1977.) \$21; £10.75.

THE word *Symbiotic* in the title of this book is used in a much broader sense than most botanists have used it in recent years. Instead, the author adopts the older usage of de Bary, recently introduced again by D. H. Lewis, using the term symbiosis to include any association between two organisms which involves long-term intimate contact. This includes, therefore, both antagonistic (parasitic) relationships as well as the mutualistic relationships most commonly considered by botanists to be symbiosis. The author recognises that some relationships exist which cannot with certainty be classified as antagonistic or mutualistic, and these he calls neutral. Neutral symbioses are particularly important in associations of fungi with animals.

Symbiotic relationships between fungi and animals, fungi and higher plants, fungi and algae (lichens) and between fungi and other fungi, are all considered. As explained above, these are classified as antagonistic, neutral or mutualistic relationships; further, the fungi are divided on nutritional grounds, again following D. H. Lewis, into necrotrophic and biotrophic species. In this connection it may be mentioned that the author recognises—and this will be welcomed by many plant pathologists—that quite a number of plant-pathogenic fungi are not easily characterised as necrotrophs or biotrophs; some seem to start their association with their host plant as

biotrophs, finishing up as necrotrophs, and these are classified in this book as a distinct group, hemibiotrophs.

A very wide range of possible associations are thus considered; examples are discussed in each category with particular reference to nutritional and other physiological relationships between the two symbiotic associates. Five chapters are devoted to symbioses with animals and six chapters to symbioses between fungi and plants, including the lichen symbiosis. Since much more is known about fungus-plant symbioses than fungus-animal symbioses, one might expect that the inevitably more 'concentrated' material which appears in the chapters dealing with plants would lead to a degree of imbalance. This is not the case; the book gives a strong impression of unity, and this is all the more valuable in that it covers a range of topics which have never before been brought systematically together.

Indeed, it is striking how similar, in many respects, the fungi involved in biotrophic associations with animals are to those similarly involved with plants. To take one example, it seems to be very common among biotrophs in both situations that they are difficult to culture axenically. This characteristic may become less noticeable as cultural techniques improve, but at present it is quite striking.

This is undoubtedly a valuable book covering a wide field. It will be of interest not only to mycologists, but also to all botanists and zoologists interested in fungal symbiosis. Not many students will be able to afford it—let's hope that it is widely bought by departmental libraries.

P. W. Brian

P. W. Brian has just retired as Professor of Botany at the University of Cambridge, UK.

Introducing cryobiochemistry

Cryobiochemistry: An Introduction. By Pierre Douzou. Pp. x+286. (Academic: London and New York, 1977.) £12.60; \$24.65.

PROFESSOR DOUZOU'S book on the theory and practice of low temperature biochemistry appears at a time of increasing interest in the technique, and when the first reports of it being used at its full power are beginning to appear. The aim of the technique is no less than the thermal resolution of the individual steps in biochemical processes, especially enzyme reactions, and the stabilisation of intermediates for timescales sufficient for their detailed examination by the more powerful analytical techniques. This is an exciting prospect for the molecular biochemists, and this book is doubly welcome, because not only has Professor Douzou, as the major pioneer of the field, given us the gift of his ten years of experience and development of the subject, but he has also presented it in a well written and clearly organised text.

The major experimental problem of ensuring that even in solutions of mixed solvents at temperatures down to -100°C the proteins are both structurally intact and functionally, potentially competent, is dealt with at length. The book also provides invaluable guidance in choosing solvent systems, the pH, dielectric constant and viscosity at subzero temperatures of which are closely matched with those of the normal room temperature environment. In this respect, the book fulfills the function of a laboratory handbook on low temperature procedures, with many specific examples, drawings of special apparatus and tables of physical properties of mixed solvent systems. At the same time the book reveals its origins as a lecture course, in beginning each section at the most elementary level and taking the reader step by step, from the properties of mixed solvents, through their effects on enzyme activity, to the study of biological molecules at subzero temperatures.

This is a first-class introduction in both the theoretical and practical sense to what is bound to become an increasingly important technique in biochemistry.

C. C. F. Blake

C. C. F. Blake is Lecturer in Molecular Biophysics at the University of Oxford, UK.

Hartree-Fock method

The Hartree-Fock Method for Atoms: A Numerical Approach. By C. Froese Fischer. Pp. 308. (Wiley Interscience: London and New York, 1977.) £17.20; \$29.

THE Hartree-Fock (HF) method means different things to different people. The one point in common is that a wave function Φ for a many-particle system is constructed from anti-symmetrised products of one-particle functions ϕ , and that the latter are fully optimised. In many-body theory, as used in nuclear and solid-state calculations, Φ is taken to be a single determinant.

In applications of HF theory to non-relativistic atomic structure calculations, which is the subject of this book, the condition is invariably imposed that Φ must be an eigen function of total spin and total orbital angular momentum operators. In all but the simplest cases, this condition implies that Φ must be a linear combination of several determinants. The HF approximation for atoms is sometimes taken to imply the restriction that Φ is constructed

from functions ϕ , all belonging to the same configuration (although the exact meaning of this statement is not always clear, as is illustrated by Froese Fischer's discussion of the $1s2s^2$ 'S state'). In 1939, Hartree, Hartree and Swirles showed that this restriction need not be made. They introduced the multi-configuration method (MCHF), which has been used extensively in recent years, and provides one of the most powerful known methods for calculating accurate atomic wave functions. A great deal of skill has been devoted to the development of the numerical and computational techniques required for this work, and they are clearly described in this book, which puts particular emphasis on methods using numerical integration of the equations (as opposed to those using expansions in terms of basis functions).

The book gives a lucid account of the foundations of atomic HF theory, a detailed account for the specialist of modern MCHF work, and concludes with a useful discussion of available computer programs for atomic structure calculations.

M. J. Seaton

M. J. Seaton is Professor of Physics at University College, London, UK

Dielectric relaxation

Dielectric Spectroscopy of Polymers. By Peter Hedvig. Pp. 430. (Adam Hilger: Bristol; Akademiai Kiado: Budapest, 1977.) £17.50.

IN the past decade, an attempt has been made in many laboratories to unify the study of the dielectric, mechanical and nuclear magnetic resonance (NMR) relaxations in solid polymers, into a coherent spectroscopy. The underlying desire in this attempt was to put forward an interpretation on the molecular level for the observed relaxations. Although such a unified approach has not yet been achieved, Peter Hedvig's book is an excellent up-to-date review on efforts towards this aim.

This first two chapters deal in a concise and precise way with the principles of dielectric spectroscopy and structural transitions and molecular mobilities in solid polymers. Transitions in the glassy state involving local molecular motion are discussed using the potential barrier theory. Cryogenic relaxation phenomena are presented together with the molecular level interpretation. Transitions in the crystalline phase involving defects and orientation effects are discussed, well illustrated and supported by an extensive list of references.

These are followed by three chapters

which present both the experimental techniques and results of dielectric, mechanical and NMR spectroscopies together with dilatometry and differential thermal analysis. The depolarisation technique and the new time domain method (Fourier method) are presented in detail. Dielectric spectra of both pure polymers and polymer compounds are examined both from the chemical structural point of view as well as from the point of view of 'physical' phenomena such as plasticisers and filler effects.

In the last two chapters the study of crosslinking and ageing processes by dielectric spectroscopy is presented. These chapters are unique and original in the literature, and are of special interest to those involved in the technological aspects of dielectric spectroscopy of solid polymers.

In the Appendix the relaxation maps of 31 polymers are presented. This collection is of value to the experimentalist working in dielectric and mechanical spectroscopy fields.

All in all, the book is informative, well balanced, well illustrated and has few errors. It is strongly recommended both for the experimentalist in the field of dielectric relaxation as well as for the advanced student of that domain.

Shimon Reich

Shimon Reich is a Senior Scientist in the Polymer Department at the Weizmann Institute of Science, Rehovot, Israel.

Metabolic yearbook

The Year in Metabolism, 1975-1976. Edited by Norbert Freinkel. Pp. 353. (Plenum Medical: New York and London, 1977.) \$27.

THIS is the first of a new series of annual publications in which the previous year's developments in selected broad areas of metabolism related to human disease are reviewed. The following topics were chosen: hormone receptors, cyclic nucleotides and control of cell function; diabetes mellitus; glucagon; body fuel metabolism; obesity; lipid and protein metabolism; amino and mono amino organic acids; purine and pyrimidine metabolism; vitamins and minerals; urinary stones; the metabolic aspects of ethanol.

The topics chosen are appropriate because there is updating to be done in each case, and in some cases the authors have had to be selective within their chosen field; for example, chapter 9, "What's New—Vitamins and Minerals", deals only with vitamins C and D, although the author indicates that there may be changes in the future.

I found little of substance to criticise in this book but I am left in some doubt as to its most appropriate target. The honours student in biochemistry will find it much too clinically orientated, and the undergraduate medical student as well as almost all postgraduate medical students will find it much too detailed in a few narrow spheres. It is, in my opinion, a book for specialist metabolic physicians and medical research workers, as well as others who wish to read an up-to-date account of one of the topics covered. It will be invaluable for them.

The real test of this publication's success will be the extent to which the impetus which has brought it into being can be maintained. This implies that each chapter should be re-written for each new edition. If this can be done, it may well provide a needed intermediate between the very condensed but comprehensive publications of the *Annual Reviews* type, and several very good, well-edited and up-to-date large textbooks on the subject, with all of which it will be in competition.

R. W. E. Watts

R. W. E. Watts is Head of the Division of Inherited Metabolic Diseases at the MRC Clinical Research Centre, Harrow, UK.

Recent scientific and technical books

Mathematics

CONTI, R. Linear Differential Equations and Control. (Istituto Nazionale di Alta Matematica. *Publicationes Mathematicae*, Vol. 1.) Pp.174. (London and New York: Academic Press, 1976.) £6; \$11.75.

DA PRATO, G. Applications Croissantes et Équations d'Évolution dans les Espaces de Banach. (Istituto Nazionale di Alta Matematica. *Publicationes Mathematicae*, Vol. 2.) Pp.146. (London and New York: Academic Press, 1976.) £6; \$11.75.

JACOBS, D. (edited by). The State of the Art in Numerical Analysis. (Proceedings of the Conference held at the University of York, April 12th-15th, 1976, organised by The Institute of Mathematics and Its Applications.) Pp.xix+978. ISBN-0-12-378650-9. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £20; \$39.

MICHEL, Anthony N., and MILLER, Richard K. Qualitative Analysis of Large Scale Dynamical Systems. (Mathematics in Science and Engineering: A Series of Monographs and Textbooks, Vol. 134.) Pp.xv+289. ISBN-0-12-493850-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$22.50; £15.95.

SPENCER, A. J. M., PARKER, D. F., BERRY, D. S., ENGLAND, A. H., FAULKNER, T. R., GREEN, W. A., HOLDEN, J. T., MIDDLETON, D., and ROGERS, T. G. Engineering Mathematics. Pp.xi+400. ISBN-0-442-30206-1. (v.2). (New York and London: Van Nostrand Reinhold Company, Ltd., 1977.)

Astronomy

BOHME, S., ESSER, U., FRICKE, W., GUNTZEL-LINGNER, U., HEINRICH, I., KRAHN, D., SCHMADEL, L. D., and ZECH, G. (edited by). Astronomy and Astrophysics Abstracts. Vol. 18: Literature 1976, Part 2. (A Publication of the Astronomisches Rechen-Institut Heidelberg. Member of the Abstracting Board of the International Council of Scientific Unions.) Pp.viii+859. ISBN-3-540-08319-7. (Berlin and New York: Springer-Verlag, 1977. Published for Astronomisches Rechen-Institut.) DM86; \$37.90.

Physics

BENNETT, Jr., W. R. The Physics of Gas Plasmas. (Documents on Modern Physics.) Pp.ix+214. ISBN-0-677-03320-6. (New York, London and Paris: Gordon and Breach Science Publishers, Inc., 1977.) £18.

CARMELI, MOSCHE. Group Theory and General Relativity. (International Series in Pure and Applied Physics) Pp.xii+391. ISBN-0-07-009986-3. Berkshire, England: McGraw-Hill, 1977. £16.85.

FURRER, A. (edited by). Crystal Field Effects in Metals and Alloys. Pp.xiv+365. ISBN-0-306-31008-2. (New York and London: Plenum Press, 1977.) \$45.

LANDOLT-BORNSTEIN. Numerical Data and Functional Relationships in Science and Technology. Zahlenwerte und Funktionen aus Naturwissenschaften und Technik. New Series/Neue Serie. Editor in Chief/Gesamtherausgeber: K. H. Hellwege. Group II: Atomic and Molecular Physics/Atom- und Molekularphysik. Vol. 9. Supplement and Extension to Vol. I/Ergänzung und Erweiterung zu Band I. Magnet Properties of Free Radicals/Magnetische Eigenschaften Freier Radikale. Part A/, Teil A: Atoms, Inorganic Radicals and Radicals in Metal Complexes/Atome, Anorganische Radikale und Radikale in Metallkomplexen. By/Von C. Daul, H. Fischer, J. R. Morton, K. F. Preston and A. v. Zelewsky. Editors/Herausgeber: H. Fischer and K. H. Hellwege. Pp.x+341. ISBN-3-540-08019-8. (Berlin and New York: Springer-Verlag, 1977.) DM350; \$154.

LOVESEY, S. W., and SPRINGER, T. (edited by). Dynamics of Solids and Liquids by Neutron Scattering. (Topics in Current Physics, Vol. 3.) Pp.xi+379. ISBN-3-540-08156-9. (Berlin and New York: Springer-Verlag, 1977.) DM72; \$31.70.

MARON, E., FRIESEM, A. A., and WEINER-AVNEAR, E. (editors). Applications of Holography and Optical Data Processing. (Proceedings of the International Conference, Jerusalem Aug. 23-26, 1976.) Pp.xvi+723. ISBN-0-08-021625-0. (Oxford, New York, Toronto, Sydney, Paris and Frankfurt: Pergamon Press, 1977.)

OPPENHEIM, Irwin, SHULER, Kurt E., and WEISS, George H. Stochastic Processes in Chemical Physics: The Master Equation. Pp.viii+561. ISBN-0-262-15017-4. (Cambridge, Mass. and London: The MIT Press, 1977.) £14.

USCINSKI, B. J. The Elements of Wave Propagation in Random Media. Pp.xxvi+153. ISBN-0-07-066650-4. (New York and London: McGraw-Hill International Book Company, 1977.) £13.35.

Chemistry

BOSCHKE, F. L. (managing editor). Inorganic Biochemistry. II. (Topics in Current Chemistry, Vol. 69.) Pp.ix+204. ISBN-3-540-08157-7. (Berlin and New York: Springer-Verlag, 1977.) DM84; \$37.

ELIAS, HANS-GEORGE. Macromolecules: Synthesis and Materials. Pp.xxx+1131. ISBN-0-471-99488. (London, New York and Toronto: John Wiley & Sons, 1977.) £24.00 \$42.50.

EPIOTIS, N. D., CHERRY, W. R., SHAIK, S., YATES, R., and BERNARDI, F. Structural Theory of Organic Chemistry. (Topics in Current Chemistry, Vol. 70.) Pp.viii+250. ISBN-3-540-08099-6. (Berlin and New York: Springer-Verlag, 1977.) DM90; \$41.40.

NIKETIC, S. R., and RASMUSSEN, K. The Consistent Force Field: A Documentation. (Lecture Notes in Chemistry, Vol. 3.) Pp.ix+212. ISBN-3-540-08344-8. (Berlin and New York: Springer-Verlag, 1977.) DM28.80; \$12.70 (No pub. date).

PIZEY, J. S. Synthetic Reagents: Lithium Aluminium Hydride. Pp.234. ISBN-85312-081-1. (Chichester: Ellis Horwood, Ltd., 1977. World Distributors: John Wiley and Sons, Inc., New York, London, Sydney and Toronto.) £9; \$17.10.

RANBY, B., and RABEK, J. F. ESR Spectroscopy in Polymer Research. (Polymers/Properties and Applications, Vol. 1.) Pp. xiv+410. ISBN-3-540-08151-8. (Berlin and New York: Springer-Verlag, 1977.) DM112; \$49.30.

ROBERTS, John D., and CASERIO, Marjorie C. Basic Principles of Organic Chemistry. Second edition. Pp.xix+1596. ISBN-0-8053-8329-8. (Menlo Park, Calif. and London: W. A. Benjamin, Inc., 1977.) \$24.95.

SVAROVSKY, Ladislav (edited by). Solid-Liquid Separation. (Chemical Engineering Series.) Pp.xii+333. ISBN-0-408-70795-X. (London and Boston, Mass.: Butterworths, 1977.) £21.

Technology

BLANC, J. P., and MONRO, Mary (edited by). Design and Construction of Offshore Structures. (Proceedings of the Conference held on 27-28 October 1976.) Pp.177. ISBN-0-7277-0041-3. (London: Thomas Telford Limited, 1977. Published for the Institution of Civil Engineers.) £16.

HERMAN, Stewart W., CANNON, James S., and MALEFATTO, Alfred J. ORR, Leonard H. (edited by). Energy Futures: Industry and the New Technologies. Pp.xvi+651. ISBN-0-88410-613-6. (Cambridge, Mass.: Ballinger Publishing Company, a Subsidiary of J. B. Lippincott Company, 1977. Distributed in UK by John Wiley and Sons, Ltd., Chichester.) £30.15; \$49.50.

LYNCH, A. J. With contributions by BUSH, P. D. et al. Mineral Crushing and Grinding Circuits: Their Simulation, Optimisation, Design and Control. (Developments in Mineral Processing, 1.) Pp.xii+342. ISBN-0-444-41528-9. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.122; \$49.50.

OWER, E., and PANKHURST, R. C. The Measurement of Air Flow. 5th edition (in SI Units). Pp.xi+362. ISBN-0-08-021281-6. (Oxford and New York: Pergamon Press, 1977.) £6.25.

PILLER, Horst. Microscope Photometry. Pp.viii+253. ISBN-3-540-08094-5. (Berlin and New York: Springer-Verlag, 1977.) DM68; \$30.

WARNER, F. L. Microwave Attenuation Measurement. Pp.xix+338. ISBN-0-901223-79-4. (Stevenage, Herts.: Peter Peregrinus, Ltd., 1977. Published on behalf of the Institution of Electrical Engineers.) UK £14; Overseas £16.40 (excluding Americas.)

WELCH, Ann., WELCH, Lorne, and IRVING, Frank. New Soaring Pilot. Third edition, revised and enlarged. Pp.xiv+412+10 plates. ISBN-0-7195-3302-3. (London: John Murray (Publishers), Ltd., 1977.) £7.50 net.

Earth Sciences

COLEMAN, Robert G. Ophiolites: Ancient Oceanic Lithosphere? (Minerals and Rocks, Vol. 12.) Pp.ix+229. ISBN-3-540-08276-X. (Berlin and New York: Springer-Verlag, 1977.) DM68; \$30.

GEYER, Richard A. (edited by). Submersibles and Their Use in Oceanography and Ocean Engineering. (Elsevier Oceanography Series, 17.) Pp.xxi+383. ISBN-0-444-41545-9. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.120; \$49.

HALLAM, A. (consultant editor). Planet Earth: an Encyclopedia of Geology. Pp.319. ISBN-0-7290-0055-9. (Oxford: Elsevier-Phaidon, an imprint of Phaidon Press, Ltd., 1977.) £7.95.

HOLMQUIST, R., (Scientific Editor) and STICKLAND, A. C., (Executive Editor) Life Sciences and Space Research Vol. XV. Pp.ix+316. ISBN-0-08-021635-8. (Oxford, New York, Toronto, Sydney, Paris and Frankfurt: 1977.) £28.

KAUFFMAN, Erle G., and HAZEL, Joseph E. (edited by). Concepts and Methods of Biostatigraphy. Pp.xiii+658. ISBN-0-470-99006-6. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$44.45; £26.25.

LOVELL, J. P. B. The British Isles Through Geological Time: a Northward Drift. Pp.40. ISBN-0-04-554003-9. (London, Boston, Mass. and Sydney: George Allen and Unwin (Publishers), Ltd., 1977.) £1.95.

PEACEMAN, Donald W. Fundamentals of Numerical Reservoir Simulation. (Developments in Petroleum Science, 6.) Pp.xiii+176. ISBN-0-444-41578-5. (Amsterdam, Oxford and New York: Elsevier Scientific Publishing Company, 1977.) Dfl.98; \$39.95.

TARLING, D. H., and TARLING, M. P. Continental Drift: a Study of the Earth's Moving Surface. Second edition. Pp.154+5 photographs. ISBN-0-14-02-1533-6. (Harmondsworth, Middx.: Penguin Books, Ltd., 1977.) 80p.

PONNAMPERUMA, Cyril (edited by). Chemical Evolution of the Early Precambrian. Pp.xi+221. ISBN-0-12-561360-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$13.50; £9.60.

Biology

ALAYEV, Yu. G. Nekton. Pp.vi+435. ISBN-90-6193-560-1. (The Hague: Dr W. Junk b.v., Publishers, 1977.) Dutch guilders 120.

ATASSI, M. Z. (edited by). Immunochemistry of Proteins, Vol. 2. Pp.xx+438. ISBN-0-306-36222-8 (v.2). (New York and London: Plenum Press, 1977.) \$53.40.

BOGORAD, L., and WEIL, J. H. (edited by). Nucleic Acids and Protein Synthesis in Plants. (NATO Advanced Study Institutes Series, Series A: Life Sciences, Vol. 12.) Pp.xi+417. ISBN-0-306-35612-0. (New York and London: Plenum Press, 1977. Published in cooperation with NATO Scientific Affairs Division.) \$47.40.

BRONNER, Felix, and KLEINZELLER, Arnold (edited by). Current Topics in Membranes and Transport, Vol. 9. Pp.xiii+394. ISBN-0-12-153309-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$36; £25.55.

CHAPPELL, J. B. ATP. (Carolina Biology Readers, 50.) Pp.32. ISBN-0-89278-250-1. (Burlington, North Carolina: Scientific Publications Division, Carolina Biological Supply Company, 1977.)

COTTELL, Glen A., and USHERWOOD, Peter N. R. (general editors). Synapses. (The Proceedings of an International Symposium held under the auspices of The Scottish Electrophysiological Society, 29 March - 2 April 1976.) Pp.xviii+384. ISBN-0-216-90326-2. (Glasgow and London: Blackie and Son, Ltd., 1977.) £13.95.

DROOP, M. R., and JANNASCH, H. W. (edited by). Advances in Aquatic Microbiology, Vol. 1. Pp.viii+378. ISBN-0-12-003001-2. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £14.50; \$31.75.

ECHTERNACHT, Arthur C. How Reptiles and Amphibians Live. Pp.142. ISBN-0-7290-0023-0. (Oxford: Elsevier-Phaidon, an imprint of Phaidon Press, Ltd., 1977.) £4.50.

Psychology

BUTTERWORTH, George (edited by). The Child's Representation of the World. Pp.xii+239. ISBN-0-306-31025-2. (New York and London: Plenum Press, 1977.) \$27.

EYSENCK, H. J. Psychology is About People. Pp.xvii+331. ISBN-0-14-02-1980-3. (Harmondsworth, Middx.: Penguin Books Ltd., 1977.) 90p.

IZARD, Cartoll E. Human Emotions. (Emotions, Personality, and Psychotherapy.) Pp.xvi+495. ISBN-0-306-30986-6. (New York and London: Plenum Press, 1977.) \$30.

PLOG, Stanley C., and AHMED, Paul I. (edited by). Principles and Techniques of Mental Health Consultation. (Current Topics in Mental Health.) Pp.xiv+234. ISBN-0-306-30963-7. (New York and London: Plenum Medical Book Company, 1977.) \$23.40.

VAN PRAAG, H. M., Depression and Schizophrenia: A Contribution to their Chemical Pathologies. Pp.260. ISBN-0-89335-002-8. (London: SP Medical & Scientific Books, 1977.) £15.00.

WHITAKER, Haiganoosh, and WHITAKER, Harry A. (edited by). Studies in Neurolinguistics, Vol. 3. (Perspectives in Neurolinguistics and Psycholinguistics.) Pp.xiv+322. ISBN-0-12-746303-8 (v.3). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$18.50; £13.13.

History of Science

BURKHARDT, Jr. Richard W. The Spirit of System: Lamarck and Evolutionary Biology. Pp.285. ISBN-0-674-83317-1. (Cambridge, Mass. and London: Harvard University Press, 1977.) £11.55.

obituary

Wernher von Braun

ONLY occasionally may it be claimed that a single individual has exerted a major influence on a global scale during his lifetime. For von Braun, who died in America on 16 June 1977, the claim may be justified since his work was of critical importance in war and peace throughout the turbulent years of the middle decades of this century. There was, too, a uniqueness about von Braun—that he exerted these influences in two nations divided for half of his life by two World Wars. For although his later triumphs belonged to America, who claimed his citizenship for the last 20 years of his life, he was a German by birth who created a devastating weapon of war in support of Hitler's regime.

Wernher von Braun was born in Wirsitz, Germany on 23 March 1912, the second of the three sons of Baron Magnus von Braun and Baroness Emmy von Braun (née von Quistorp). The ancestral family estates and his early environment might well have led Wernher to a traditional cultural life, but for the circumstance that his mother was an exceptional person. The Baroness was an amateur astronomer who presented Wernher with a telescope as his confirmation gift. We have von Braun's own authority for the statement that it was this event and his early reading of an article in an astronomical magazine describing an imaginary trip to the Moon which fired his determination to join the Verein für Raumschiffahrt (VfR—Society for Space Travel) in 1929. He was then a student at the Berlin Institute of Technology and the VfR, with Hermann Oberth as President, although founded only two years previously, was a thriving concern with over 500 members.

This VfR began the real German experimental work on rocket flight from the *Raketenflugplatz* at Reinickendorf on the outskirts of Berlin. Von Braun participated in the establishment of this 'rocket flying field' from which in 1931 the VfR claimed to have launched a hundred liquid-fuel rockets and made three hundred static firing tests. Altitudes of a mile were achieved but in spite of these successes the VfR almost immediately suffered from the economic depression in Germany. Their difficulties were enhanced by the increasing objections



Von Braun, arm fractured in a car accident, surrenders to the US Army in 1945. With him, L to R: Maj-Gen Walther Dornberger (Peenemünde Commander) and scientists Lt-Col Herbert Axter and Hans Lindenberg.

of the Berlin police to rocket flights within the city limits.

Ironically it was this rapid decay of the VfR which determined von Braun's future, for, in its desperation the group sought support from the German army. In the summer of 1932 a demonstration of a *Repulsor* (the liquid-fuel rocket of the successful 1931 tests) was given at the army proving grounds at Kummersdorf. This demonstration did not save the VfR—by 1933 it was bankrupt and was finally extinguished by Hitler and the Gestapo—but the army had been greatly impressed by von Braun and invited him to do the experimental work for his doctor's thesis (he graduated in 1932) on rocket combustion phenomena at Kummersdorf.

The army's rocket work was under Captain (later General) Walter Dornberger and his acquisition of von Braun was to prove an event of major significance. Two years later his rockets were reaching an altitude of 6,500 ft. and in 1937 the army rocket group of 80 personnel with von Braun as technical director moved to Peenemünde on the Baltic coast. There, under pressure

from the army to develop a missile with a range of 150 to 200 miles and capable of carrying a one ton warhead, von Braun and his team created modern rocket technology. At last in October 1942 when a test rocket reached an altitude of 50 miles and travelled 120 miles, Hitler ordered top priority for the work.

The attacks against London with the V1 (the buzz bomb) began on 12 June 1944. On 9 September 1944, three days after the Chiefs of Staff reported that all the V1 launching sites had been captured, the world's first ballistic rocket, the V2, fell on London. These attacks continued until 27 March 1945 when all the launching sites had been captured. These V1 and V2 missiles of von Braun had killed 8,938 people and injured 24,504 mainly in the London region.

For von Braun it was an immense achievement—8,000 V1's had been launched against London alone, 1,115 V2's fell on southern England and the Germans claimed another 2,000 had been launched against targets on the continent. The V2 as a ballistic missile was far in advance of anything conceivable elsewhere in the world. Weighing 12 tons at take-off the device was 40 ft long, 5.5 ft in diameter and could carry a one ton warhead 180 to 200 miles. The engine, using a turbo-pump-fed liquid oxygen and alcohol mixture and generating a sea-level thrust of 56,000 pounds worked for a minute after which the trajectory was entirely ballistic. The V2 had an inertial guidance system with two free gyroscopes, levelling pendulums and an integrating gyro-accelerometer. The rocket climbed 60–70 miles and five minutes after lift-off dropped on the target with a speed of 3,500 miles per hour.

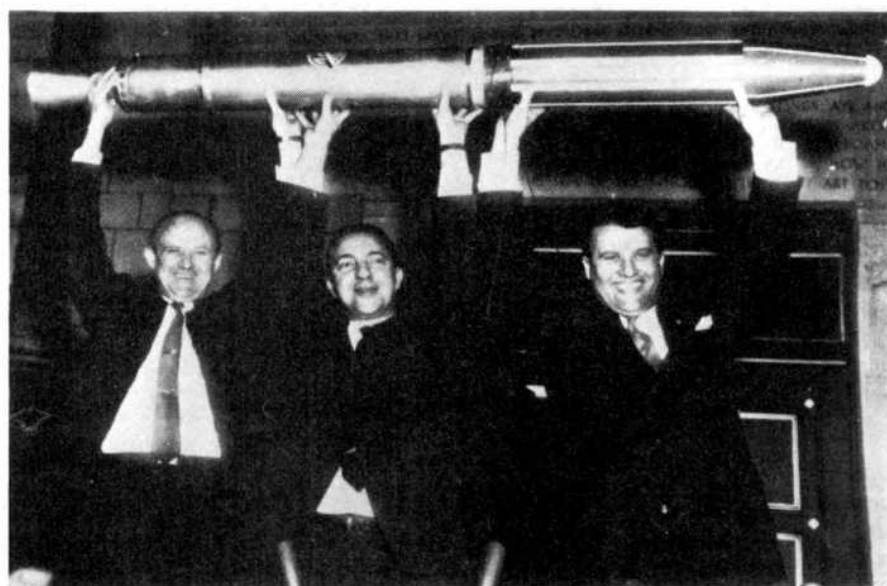
Familiarity with contemporary rockets should not be allowed to obscure the monumental nature of von Braun's technological achievement in the development of the V2. It was a weapon which many experts in the Allied countries believed to be impossible to achieve and against which there was no defence. The effect of the V2 on the outcome of the War could well have been decisive if Hitler had not diverted priorities from Peenemünde to the Luftwaffe which curtailed von Braun's

efforts in the period from 1940 until the end of 1942. There were also internal disputes when Himmler attempted to take control of the project—an event which led to von Braun's arrest by the Gestapo and his imprisonment early in 1944 from which he was released after two weeks only by the personal intervention of Hitler.

The second phase of von Braun's career in America culminating in the development of the Saturn rockets which launched men to the moon has received great publicity and is far better documented than the remarkable episodes in 1945 surrounding his escape from Peenemünde and his surrender to the American army. In January 1945 when it was clear to him that Hitler's Reich was about to collapse, he met secretly with his top staff members to decide whether to obey the directive to stand firm at Peenemünde and fall into the hands of the advancing Russians or escape to the south and surrender to the Western Allies. The almost unanimous decision to escape was assisted by an enormous bluff. 5,000 employees and their families together with vast amounts of literature and equipment set out from Peenemünde in February 1945 in railway trains, trucks and cars blazoning prominent signs indicating a purely mythical "Project for special disposition." Carrying one of several contradictory orders implying that rocket research was to continue in the Harz mountains, the von Braun convoy forced its way through road blocks, the SS and Gestapo and eventually reached Bleicherode in the Harz mountains. 12,000 tons of equipment shipped from Peenemünde and earmarked to follow von Braun to the Harz mountains was eventually captured by the Russians in Lubeck.

At Bleicherode von Braun found himself faced with the SS General Kammler who had earlier tried to take over Peenemünde, but Kammler thought he could bargain better with the approaching Allies if he used von Braun and his team as hostages and he placed them in a prison camp at Oberammergau. Eventually von Braun managed to disperse his group into the surrounding mountains and it was from that region of the Bavarian Alps that he surrendered to the Americans early in May 1945. They had acquired one of the great technical prizes in history for, apart from the Peenemünde team, the huge underground V2 assembly plant at Niedersachswerfen was captured intact.

When he arrived at Bleicherode, von Braun hid 14 tons of documents about the Peenemünde developments in a tunnel near Dörten at the northern edge of the Harz mountains. These and large amounts of the V2 material



3 February 1958. Holding a model of the successful Explorer satellite launch vehicle. L to R: William Pickering, Director of the Jet Propulsion Laboratory; James Van Allen; von Braun.

(enough for about 100 rockets) were removed from the region only days before the Russian army moved in. Sixteen Liberty ships transported the cargo from Antwerp to New Orleans and within a year the Americans were gaining invaluable experience and commencing their upper atmosphere researches by launching the reassembled rockets from White Sands, New Mexico.

These complex and urgent movements were under the command of the US General Holger N. Toftoy, Chief of the Ordnance Technical Intelligence team in Paris. It was Toftoy who recommended that von Braun and his technical staff should be brought to the United States. On 23 July he was ordered to arrange the move but only for 100 of the staff and in early August 1945 he met von Braun at Witzenhausen to offer him a one year contract in the US under Ordnance Corps custody. Before finally leaving for the US von Braun spent two weeks in England for meetings with Sir Alwyn Crow and other members of the Ministry of Supply who had been involved in the attempts to develop British rockets during the war.

His American life began on 29 September 1945 when, with seven other members of the team, he was met at Fort Banks in Boston harbour by Col. James P. Hamill who was to be his C.O. for many years. Soon, von Braun was at Fort Bliss in El Paso, Texas. Gradually his team arrived and by February 1946 they were complete and at work on assembly and testing of the converted V2's. The first launching from White Sands, New Mexico was on 16 April 1946 and the launchings of the captured V2 rockets continued for six

years during which time the U.S. Army continued to move forward from the V2 technology under von Braun's guidance.

In 1947 von Braun was given permission to return to Bavaria to marry his 18 year old second cousin Maria Louise von Quistorp, and there are two daughters and a son of the marriage.

In October 1949 the Secretary of the Army gave formal approval for the transfer of the rocket group to the Redstone Arsenal in Huntsville, Alabama. The move in 1950 involved 500 military personnel, several hundred U.S. civilians and the technical core of von Braun and 130 members of his original Peenemünde staff. Hamill was in charge, with von Braun as director of the Guided Missile Development Division.

These were critical years for the US missile developments—spurred by the needs of the Korean War the Redstone missile was developed. First fired from Cape Canaveral in August 1953 it was the first missile to be in operational use in the US Army and was soon to play an unforeseen and dramatic part in the space research.

At that time intelligence sources found evidence that the USSR was in an advanced stage of development of an intercontinental ballistic missile (ICBM). All three US armed services were thereby spurred to vigorous development of an ICBM. The creation of the Army Ballistic Missile Agency in Huntsville under General Medaris was centered around von Braun as technical director. The Redstone rocket was upgraded and two upper stages were added to form the vehicle known as Jupiter C. The first stage



Wellcome ToxHA test kit

**new, simple
and
rapid**

Few people can resist the temptation to stroke or fondle a kitten or cat. Unfortunately, the complex life cycle of the causal organism in toxoplasmosis, *Toxoplasma gondii*, centres on the cat family in which all stages of this coccidian occur including the highly resistant and infective oocyst.

The Wellcome ToxHA test Kit is a standardised, indirect microtitration system for the rapid detection of antibodies to *T. gondii*. Because the test detects mainly the somatic but also the cuticular antibody, it can indicate the stage of infection when used in conjunction with the Wellcome Toxoplasma fluorescent antibody test.

The Wellcome ToxHA test Kit, Code TA21

- * Utilises extracts from purified trophozoites of *T. gondii* RH strain to sensitised aldehyde-treated tanned turkey red blood cells and avoids the use of living toxoplasmas.
- * Is suitable for manual or semi-automated use.
- * The agglutination patterns can be read, in V-well plates, within 90 minutes.
- * The test is simple to perform, requires no complex equipment and, because it requires only micro-volumes of reagents, it is quite inexpensive.
- * The test cells have a consistent titre, meeting the WHO International Standard for anti-toxoplasma sera.
- * 2 x 50 test kit, lyophilised, which will remain stable at 4°C for at least one year from date of manufacture:

Test Cells/Control Cells/Positive and Negative Control Sera/
Diluent Buffer.



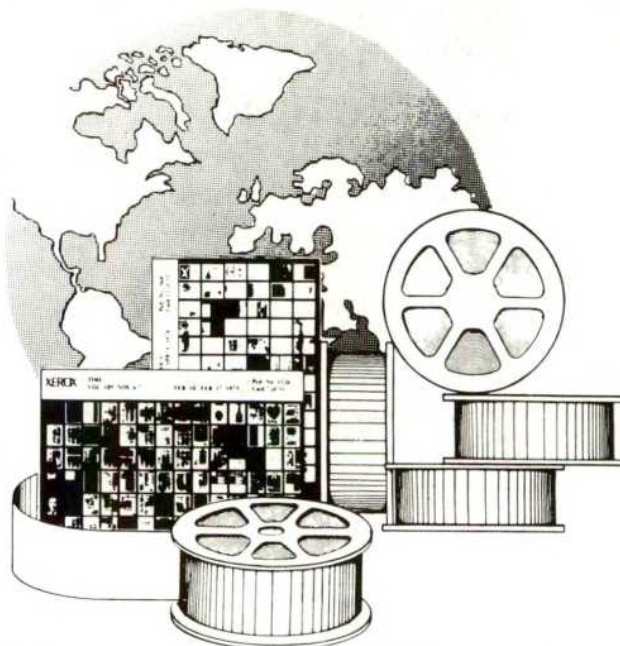
Wellcome Reagents Limited

303 Hither Green Lane
Hither Green London SE13 6TL

Circle No. 16 on Reader Enquiry Card.

This Publication is Available in MICROFORM

FOR INFORMATION,
CUSTOMERS OUTSIDE
THE UK AND
EUROPE SHOULD
WRITE:



University Microfilms International

300 North Zeeb Road
Ann Arbor, Mich. 48106
U.S.A.

18 Bedford Row
London, WC1R 4EJ
England

OTHER CUSTOMERS (UK AND EUROPE) SHOULD CONTACT:
Newspaper Archive Developments Limited, 16 Westgate Road, Reading RG2 2DE, England

Enzymes from T4 infected E. Coli cells for molecular biology:

cloning, labelling, ligation, sequencing, synthesis.

Free of contaminating enzymes,
high activity, and available from stock.

NEW! from BRL

BRL is proud to offer these new products. They have been subjected to rigid quality controls and meet the highest standards of excellence.

BRL Cat. No.	Product
5224	T4 DNA Ligase
5225	T4 RNA Ligase
5226	T4 DNA Kinase
5227	T4 DNA Polymerase

For further information or to place an order write or telephone collect (301) 340-0448.

We're first so you can be.

BRL

Bethesda Research Laboratories, Inc.

411 North Stonestreet Avenue, Rockville. MD 20850. (301) 340-0448.

Circle No. 07 on Reader Enquiry Card.

A GUIDE TO RADIATION PROTECTION

J. Craig Robertson

A guide for the layman faced with the problems of dealing with radioactive materials during the course of his work. Assuming no previous scientific knowledge, the book discusses, amongst other things, different types of radioactive sources and how to shield the various radiations they emit.

October 1976 £4.95

For further details please write to Anne Calcott (N), The Macmillan Press, Little Essex Street, London WC2R 3LF

M MACMILLAN
PRESS

The Science Dictionaries

Three useful and up-to-date works of reference intended not only for students, teachers and researchers but also for science journalists and the general reader.

Each book is designed for easy use – entries are arranged alphabetically and defined under the most common form of term, with synonyms in brackets. S.I. units are used throughout and all entries are thoroughly cross-referenced.

A Dictionary of Life Sciences

E. A. Martin

The recent trend to treat the biological/life sciences as a whole rather than divided into separate units is reflected in this concise dictionary which brings together in one volume all the main branches of zoology and botany. Selected terms in related fields of biochemistry, endocrinology, animal behaviour and palaeontology are also included. All major plant and animal classes are fully described as well as the more important orders, and common names of animals and plants are cross-referenced to their appropriate taxonomic groups.

1977 £5.95 392pp

A Dictionary of Earth Sciences

Stella E. Stiegeler

The subjects covered here include geophysics, structural geology, geomorphology, oceanography, stratigraphy, palaeontology, sedimentology, climatology, meteorology, soil science, cartography, mineralogy, petrology and surveying. The terms defined reflect the recent modification of traditional concepts and the resulting development of terminology in the earth sciences. In soil science, for example, a new system of classification is now in use, with a completely new terminology for the soil orders.

1977 £5.95 320pp

A Dictionary of Physical Sciences

John Daintith

This book covers the main branches of physics, chemistry and astronomy – from Abbe condenser to zoom lens and zwitterion. A selection of relevant terms from the related fields of electronics, computer science and mathematics is included. Full accounts are given of the properties and uses of the chemical elements and many of their compounds. All the chemical terminology used is that recommended by the International Union of Pure and Applied Chemistry.

1977 £5.95 352pp

To order a copy of any of these books, please send £5.95 plus 50p carriage to Technical Book Services, P.O. Box 79, Maidenhead, Berkshire SL6 2EG.

M MACMILLAN
PRESS

was a Redstone missile lengthened to hold more propellant and the engine was modified to burn a new fuel—hydrazine, a mixture of unsymmetrical dimethylhydrazine and diethylene triamine. The second stage was a cluster of eleven solid-propellant rockets and the third stage fitting inside the ring of the second stage comprised three solid fuel rockets. Von Braun stabilised the system by spinning it rapidly during flight and on its first flight from Cape Canaveral in September 1956 this remarkable vehicle reached an altitude of 682 miles and travelled 3,400 miles.

Ironically and almost immediately the Army was ordered to concentrate on short range missiles while the control of the ICBM programme passed to the Air Force. External events, however, once more determined a different destiny for von Braun.

In 1954 he showed that it would be possible to launch an earth satellite using the Redstone rocket assemblies as in the Jupiter missile. His scheme, under the code name Project Orbitor, was considered by an advisory group under Assistant Secretary of Defence Donald A. Quarles who outvoted the idea by 7 to 2 at a meeting on 9 September 1955, in favour of Project Vanguard, an alternative proposal from the Naval Research Laboratory. The official record states that the decision was based on technical recommendations of the advisory group. More probably the phrase covers the persuasion of President Eisenhower that the military association and the constraints of the secrecy classification on the Jupiter C rocket would be harmful to the peaceful intent of the satellite project. In the event Vanguard, based on sounding rocket technology and aside from the main stream development of the U.S. military ballistic rocket, inadequately financed and with no appropriate priority, was an almost total disaster.

In the turmoil created by the success of Sputnik One on 4 October 1957 von Braun again pressed his case from Huntsville that he should be allowed to use the army's Jupiter missile to launch a satellite. Three weeks after the launching of Sputnik he was given three and a half million dollars with a target date of 30 January 1958. Once more he triumphed—on 31 January the Jupiter rocket placed Explorer 1, the first American satellite in Earth orbit which within a few days discovered the zones of trapped particles around the Earth (the van Allen zones). The decade of intense technological rivalry between the US and USSR following these events would have borne a radically different aspect if von Braun's advice had been followed in 1955.

After these events the Eisenhower administration took immediate action to create a unified US space effort. Following the advice of a committee under James R. Killian steps were taken to create a strong civilian-orientated agency to direct the manned and unmanned exploration of space. The National Aeronautics and Space Act became law on 29 July 1958. Successively this National Aeronautics and Space Administration absorbed the 8,000-strong National Advisory Committee for Aeronautics, the Vanguard and NRL groups, the 2,800 staff of the Jet Propulsion Laboratory of CalTech and then in January 1960, President Eisenhower decreed that the vital army group, with 4,600 personnel under von Braun should be transferred from army to NASA control. On 1 July 1960 the transfer was effected when the President personally visited and dedicated the facility as the George C. Marshall Space Flight Center with von Braun as director.

Four years earlier von Braun had begun studies of rockets which would be far more powerful than the Redstone combinations. He knew that the rockets required for intercontinental missiles would never provide the thrust required to place man into space. In August 1958 he already had approval to develop a multiple Redstone/Jupiter combination with a thrust of 1.5 million pounds, and by the time of his absorption into NASA he had a priority rating for the development of vehicle known as Saturn 1. In October 1961 this 162 ft long carrier rocket weighing a million pounds had its flawless test launching from Cape Canaveral. By January 1964 a 3,700 pound test payload was placed in orbit. Thereafter von Braun progressed majestically and incredibly to the targets of the Apollo programme and to the fulfilment of his vision.

These early Saturn vehicles were mere stages towards the mammoth Saturn 5, the development of which was approved by NASA in January 1962. Standing 364 ft high, Saturn 5 weighed over six million pounds and could send 47 tons of payload to the Moon or place 140 tons in Earth orbit. The landing of Armstrong and Aldrin on the Moon in July 1969 was an operation of extraordinary complexity; an immense collaborative enterprise which von Braun and the Huntsville team made possible by designing this multi-million pound thrust rocket. From assembly to the count-down the monitoring system alone needed 10 miles of tape storing over 2.5 million words.

Von Braun witnessed the fulfilment of a youthful dream in his fifty-eighth year. No longer did it seem technical fiction that he wanted to proceed even

further along this path. He had designs to get man even further into space. First he would have strapped on solid rocket motors to the first stage of Saturn 5, then to get the men to Mars he planned to replace the existing liquid hydrogen-oxygen third stage by a nuclear engine. But this remained a dream; NASA was forced to cut the space programme and within a few years of the climax of Apollo 11, von Braun had the chagrin of witnessing the dismantling of the facilities and the dispersal of the men who had made Saturn a reality.

In 1970 von Braun was made Deputy Associate Administrator for Plans in NASA, an office which he held until his retirement at the age of 60 two years later. He then joined Fairchild Industries as Vice President for Engineering and Development but became ill with cancer and retired finally a few months before his death.

Von Braun was an extraordinary symbol of the age. His vision was of a nature which the establishment looked upon as science fiction but by single-minded persistence he astonished the world by succeeding through the labyrinth of war and peace, first as a German and then as an American citizen. Although von Braun was granted American citizenship in 1955 and rescued the Americans from their despair in the face of the Soviet Sputnik, there were those who could not forget that he laboured for the Nazis. But for von Braun the transformation was complete. He built his rockets to get men away from the earth—his imprisonment during the Peenemünde era resulted from an unwise remark that the V2 would help towards space travel—and he succeeded.

Bernard Lovell

D. W. Holder

At the time of his death on 18 April 1977, Professor Holder had been Head of the Department of Engineering Science at Oxford for close on sixteen years. In that time the laboratory had expanded at both the undergraduate and postgraduate level and much of the planning for this growth stemmed from his own initiatives.

He was educated at Imperial College in the Department of Civil Engineering between 1941 and 1943 and subsequently in the Aeronautics Department under Sir Leonard Bairstow. After graduating he immediately joined the Ministry of Aircraft Production at the Aircraft and Armament Experimental Establishment at Boscombe Down but soon transferred to the Aero-

dynamics Division at the National Physical Laboratory where he remained until 1961. His promotion from the junior grades to Deputy Chief Scientific Officer in 1957 was rapid and his career at the NPL spanned some of the most dramatic changes seen in aeronautics.

At the end of World War II, little was known about the behaviour of aircraft at transonic and supersonic speeds but the next decade was to change all that and Douglas Holder played an important role from the outset. The first problem was to design and build the wind tunnels which would enable the aerodynamic phenomena to be investigated. The Aerodynamics Division chose to use induced flow tunnels and a series of these ranging from the original pilot model 9 inches by 3 inches in cross section to 36 inches by 14 inches was constructed.

Before 1949 research on flow at Mach numbers close to unity had been impossible because of the reflection of the normal shock from the tunnel wall. Extensive studies of slotted walls were conducted in the UK and the USA and Holder's group at NPL made substantial contributions to this new technique which could eliminate tunnel interference effects. In addition to this work on tunnel design he made, with Gadd and Pearcey, important advances in shock wave boundary layer interaction and aerofoil design so that the division as a whole established an enviable international reputation.

Following closely on this pioneering research on transonic and supersonic aerodynamics Holder next led the development of a series of shock and wind tunnels for the study of flight at hypersonic speeds, i.e. above about Mach 7. Completely new techniques were needed to drive such facilities and Holder, now head of all high speed aerodynamics at NPL, took a major role in planning and designing these devices. The flow durations in shock tubes and shock tunnels range from tens of microseconds to a few milliseconds but Holder was equally at home in this new field. He had very early demonstrated a highly developed skill in wind tunnel design and had the ability, which may be the mark of the true engineer, to make rapid estimates of all the important design parameters for a new facility. Throughout this period he published, as he had always done, widely, and some of his papers are classics of their kind.

In 1961 he was invited to take the Chair of Engineering Science at Oxford to succeed Thom who was then retiring, and became at 37 the fourth holder of that post. The early sixties saw in Oxford, as in the UK as a whole, a rapid expansion of university engineer-

ing departments and under Thom a major development had been initiated. Holder took over this work and guided it through to completion. Although he continued to take a keen interest in fluid dynamics he also saw the importance of maintaining and extending a broad base of knowledge and there are now two new chairs in structures and electricity, largely due to his foresight and persistence.

His long association with aeronautics at the NPL made him an obvious choice as a consultant for industry and government and he was also much in demand in educational establishments. He was elected FRS in 1962 and served on many of its committees as well as its Council between 1969 and 1971. His wide range of activities outside Oxford constantly suggested new lines of research and development and together with Professor R. B. Duthie he established in Oxford a centre for orthopaedic engineering and encouraged work in physiological fluid dynamics.

Outside of Oxford his government advisory work was at the highest level and must remain obscured by confidentiality although he will be remembered for his chairmanship of the enquiry into Precision Approach Radar following the Gatwick disaster in 1969 and later, in 1971, his involvement at ministerial level in the technical decisions on the future of the RB-211 engine.

By nature he was reserved and the extent of his influence in both aeronautics research and engineering education is a testimony more to his patient and persuasive skills than to any justifiable forcefulness in argument. He was an exceptionally able administrator and dealt with what would appear a crippling work load with quiet efficiency. He was always ready to listen to his staff and research students and to encourage them to pursue new lines of investigation and he was generous to a fault to those who were in difficulties, although few around him would be aware of his very personal interventions. In this respect he was particularly effective on the University Staff Committee where he will long be remembered for his patient and skilful administration.

In his views of the part that universities should play in the industrial life of the country he was ahead of his contemporaries and with such wide experience and extensive contacts he often put his finger on promising lines of new work long before they were apparent to others. There are, alas, only a few of his calibre and he will be sadly missed in both university and industrial bodies.

D. L. Schultz

Yves Guitton

PROFESSOR YVES GUITTON died on 6 July 1977 in Perpignan (France) at the age of 46. He leaves a wife and two sons. Head of the Laboratory of Plant Physiology, he was also chairman of the University Group for the Biology of Development and Parasitology and Vice-president of the University of Perpignan.

With a wide-ranging scientific background, involving organic chemistry, nuclear energy research and microbiology, he entered plant physiology and biochemistry because he felt the development of this field to be essential for the future. His doctoral thesis was devoted to the metabolism of arginine during the germination of *Pinus*, and his contribution to the study of amino acids and nitrogenous substances was invaluable to French biochemistry.

In 1966 he was appointed to the newly-created University of Perpignan, addressing himself to a problem which intrigued him: the role of nucleic acids in developmental processes. Arriving alone in his new laboratory, geographically isolated from the scientific community, Professor Guitton nevertheless created within ten years a laboratory equipped for all techniques of molecular biology. The research team which he built up is a leading group in French physiology, collaborating with others both at home and abroad.

His recent contributions concern the metabolism of mRNA during the first hours of seed germination, mRNA stability and development-related changes in chromatin structure and composition. With his collaborators he was the author of around fifty publications in French and international journals.

In addition to his brilliant scientific career he involved himself with equal enthusiasm in his outside interests. An excellent footballer in his youth, he turned his attention to tennis after a serious accident, rapidly reaching a high standard. Many will remember his arrival at scientific conferences complete with tennis racquet, looking for partners among his American and British colleagues. In politics he was a highly active member of the Socialist party, deeply involved in the problems of his city.

For those who knew him Yves Guitton will be remembered not only for his exceptional achievements, but perhaps even more for his enthusiastic approach to all which concerned him and his warmth and friendliness in any company. His premature loss will be deeply regretted by his many friends in the scientific community and in his city. Michel Delseny

nature

20 October 1977

Protection for the non-academic student

MOST people's image of the educational system is of a pyramid structure, with a broad base up to the age of about 16 and then a progressively narrowing superstructure as more and more students peel off into employment, until the giddy and narrow heights of the doctorate are reached. In a recent report the Organisation for Economic Cooperation and Development (OECD) asks some profound questions about the suitability of this structure for the present day (*Selection and Certification in Education and Employment*, OECD, 2 rue André-Pascal, Paris). One of the most interesting questions concerns the so called terminal/transfer dilemma, and has a particular relevance in a period when secondary schools are being required to accept children of a very wide range of abilities into so-called comprehensive education.

The OECD points out that such schools have to combine two functions previously fulfilled on separate tracks: that of preparing students to leave the educational system for employment, and that of training and most often selecting students for transfer to the next higher level. In practically all cases, claims the OECD, the result of this uneasy merger is that curricula and assessment practices are geared primarily to meet the needs of those who will move up, even if this number is actually a minority. The higher levels of the pyramid exert a strong influence on those beneath. It might be added that in many universities the same pattern exists for the sciences: the need to select and

train the postgraduate unduly influences the undergraduate curriculum.

At the secondary level of education the move towards comprehensive schools has clearly been seen by its proponents as a move towards greater equality of opportunity and freedom to find one's own level. If however, comprehensive schools develop in the way that the OECD describes, they will only tend to emphasise failure. Those for whom such a school was never other than their last base before getting a job will find themselves poor relations alongside those with obvious university aspirations who are catered for by examinations and school curricula with the word 'university' written all over them.

If the so-called holistic structure of an educational system acts to the disadvantage of those with 'mere' manual and technical skills, does the alternative 'aggregative' model, with tertiary education pursued in a much more diverse way, work any better? Certainly it could help to break a narrow academic grip on schools, but maybe at the high cost of fragmented tertiary courses, much poorer staff-student relationships and a general lack of direction in many students' development.

Is there a middle path, that maintains the best of academic traditions without casting out the non-academic student into outer darkness? We must find an answer in the next few years; the OECD report could be a valuable basis for discussion. □

Read all about something like it

"MANY killed in earthquake . . . where's the pun in that?" a sub-editor on The Guardian is supposed, in newspaper mythology, to have said whilst appraising headlines. 'Buy now while stocks last' ran the headline to a leader in The Guardian last Saturday; true to form the content of the leader was not about declining resources or exceptional bargains—it was another little poke at the quaint language that specialists use. Only a few weeks ago The Guardian was pointing out the intelligibility of New Society as opposed to the opacity of New Scientist and Nature. Now it returns to the fray to renounce its faith even in the accessibility of the social sciences on the basis of an advertisement in New Society for a book *An Eclectic Approach to Primal Integration*, and it snips a highly technical sentence on adenylate cyclase out of last week's New Scientist to reassure readers that we scientists are still at it.

No doubt our colleagues on the New Scientist know how to defend themselves against these outrages, but for our part we simply say that at least our customers know what they are getting. Anyone who picks up an article

entitled 'Analgesic activity of lipotropic C fragment depends on carboxyl terminal tetrapeptide' can be under no illusions about what they are in for, whereas readers of The Guardian may have to search for a serious article on Indian corruption under a heading such as 'Currying Favour'. Nor is ambiguity restricted to The Guardian. Every evening as we walk wearily from office to station we are assailed by placards for the evening newspapers proclaiming 'London-bound train crashes', only to discover that a freight train from Glasgow has run off the rails outside Preston.

Our enthusiasm for this theme of misrepresentation having been raised, we were just about to go on in the same vein to wonder how many horticulturalists had bought John Betjeman's *A Few Late Chrysanthemums* by mistake, how many glaciologists Margaret Drabble's *The Ice Age* and so on when an uneasy feeling crept over us. After all in the office we do get a steady stream of offers of wildlife pictures. And we get the occasional phone call asking if we have the address of the World Nudist Federation. □

Of time and the energy wars

In another of an occasional series, Alvin M. Weinberg, Director of the Institute for Energy Analysis at Oak Ridge, Tennessee, comments on a difficult trade-off

QUANTUM mechanics tells us that energy and time are conjugate variables. I am therefore much bemused these days by an observation pointed out to me by a young Swiss physicist, Daniel Spreng, that in a certain social sense energy and time are conjugates. What Dr Spreng means by this is simply that man expends energy in order to save time; moreover, when given the choice, he usually chooses to save time over saving energy. (To be more accurate, it is availability rather than energy that is usually meant in these contexts).

This surely was the case during man's early civilisation. Domestic animals did the job quicker, and slaves were acquired because they were a source of intelligent energy. The slave-owner therefore had more freedom of choice in using time than did the peasant who had to work his field himself. And each technological achievement (at least until the advent of information technology) represented such a trade-off: the industrial revolution, the transportation revolution, the agricultural revolution—always it was man in a hurry ready to spend more energy in order to save time.

Actually this exchange of energy for time has a basis in thermodynamics, despite the well-known fact that thermodynamics never treats time explicitly. In thermodynamics we learn that the minimum free energy is expended when a process, like desalting, is performed reversibly. A reversible process, however, is usually considered to require an infinite amount of time; if we wish to save time, we must expend more energy since the process will then be performed irreversibly. Thus the minimum amount of work required to desalt 1,000 gallons of seawater is 3 kWh; the actual amount used in a practical desalting device is ten or more times higher than this.

Heat transfer processes can be performed *reversibly* in a finite time if the apparatus, particularly the heat transfer surface, is sufficiently large. In that case the temperature drops approach zero, and reversibility is achieved. But we must remember that the total energy required to conduct a process is actually the sum of two energies: the usual energy which is transferred from one body to another; and the energy that went into constructing the apparatus. Ordinarily we ignore the latter. But if the apparatus becomes very large we ought not to ignore this since the energy that went into making the apparatus—the mining and refining of the metal, its fabrication, its transport—becomes very large. Thus the total energy goes through a minimum as the degree of irreversibility diminishes. To perform a task in a finite time requires more energy than to perform it in a longer time, either because we do it irreversibly or because to do it reversibly requires a very large apparatus that itself embodies a great deal of energy.

Though I am bemused by the fact that time and energy can be regarded as conjugates, I do not put this forth as a magical talisman for guiding our course in the great energy debate, but simply as an antidote for the view that thermodynamics can guide energy policy better than can the evolving values of our society, particularly as embodied in economics. This view seems to be promulgated, especially in the United States, by Barry Commoner and Amory Lovins, to mention the most notable. They assert, with truth, that in principle we can save energy if we do a better job of matching the quality of the heat source with the quality of the heat required to achieve a given end. If water is wanted at 70 °C, why use a flame with a temperature of 1,000 °C as the heat source? And in two widely read



Camera Press

Time savers, or energy wasters?

articles, Commoner's "The Poverty of Power", which appeared in the *New Yorker* and then as a book, and Lovins' "Energy Strategy: The Road Not Taken?" which appeared in, of all places, *Foreign Affairs*, much was made of maximising this 'second law efficiency'. Indeed, the lesson thermodynamics teaches us for energy policy, according to Commoner and to Lovins, is mainly that we must maximise second law efficiency: in so doing we will save energy and create the good, that is, decentralised society, not to say eliminate nuclear energy.

But all this is so one dimensional. To be sure energy is a finite good, and we suffer from a shortage of it. But there are other goods that are finite, and of these I would consider time to be the most fundamental. This, I suppose, is one of the prices we pay for our knowledge of our mortality: we value time because we know it is limited, and we will do much to save it and to expand our choices in using it. If the underlying trade-off is between energy and time, then we mislead if we try to use thermodynamics as a primary guide to energy policy since thermodynamics gives no value to time: it is prepared to speak of processes that are infinitely slow, that ignore man's mortality.

There are those who argue that we have passed the point where energy can be exchanged for time. For example, S. Linder in *The Harried Leisure Classes* points out that our energy-intensive technologies themselves rob us of time: to maintain our gadgets, to commute, to listen to the semantic drivel that our new technologies impose upon us. And I cannot deny the merit of Linder's thesis: the Leisure Class is harried.

Yet who is to judge whether the trade-off between energy and time is to favour energy or time—the energy revolutionaries such as Commoner and Lovins, or the rest of us? We are short of energy, but we are also short of other things, like time. A free society allows each of us to make the choice, to allocate as we each decide. Economics, in the broadest sense, integrates all these trade-offs and judgments—it allows us to weigh time against energy, or for that matter, any other good against energy. I would hope the energy revolutionaries could be persuaded to be a little less revolutionary and not reject these homely truisms of engineering economics for a world they would impose on us with their emphasis on thermodynamic analysis. □

Planning Antarctica's future

The thirteen signatories to the Antarctic Treaty met in London earlier this month. **Paul Cheeseright** reports

IN THE face of increasing international economic pressures, the Antarctic Treaty is on the verge of substantial change, which could dilute, at least to some extent, the environmental stringencies practised to date by the signatory powers. The essential problem is how to control an area without having the recognised forms of sovereign jurisdiction.

For three weeks in London the thirteen consultative powers, as the signatories like to call themselves, tried to come to terms with the problem of finding regimes for the control of marine and mineral resources, while at the same time preserving the non-political approach which has hitherto dominated their scientific work. Inevitably their success has been only partial.

This inevitability is the direct result of the framework in which the Treaty powers had to work. There are thirteen of them: Argentina, Australia, Belgium, Chile, France, Japan, New Zealand, Norway, Poland, South Africa, Britain, the USA and the USSR. They make an oddly variegated bunch of states, having either geographical, historical, political or scientific links with Antarctica.

The Treaty which binds them was signed in 1958 and came into effect in 1961. Its effect has been to make the most inhospitable continent in the world a gigantic laboratory, where the work of one was subject to the inspection of all in a demilitarised nuclear-free zone reserved for peaceful purposes. "The question of man's impact on the Antarctic environment" is, the Treaty powers say, "a subject which subsumes all the main preoccupations of the Antarctic Treaty powers with regard to the area".

The early years of the Treaty's existence provoked no problems. The area was out of the way. The scientific work was expensive and only the powers involved were interested in making provision for it. But in 1969 questions were being asked in the New Zealand parliament about the mineral resources of the region. The seeds of difficulties were being sown.

There were two reasons for this. The first was that the Treaty itself made no mention of resources in the area and what to do about them. The second was that the Treaty effectively froze all territorial claims to the area for thirty years. It was at least partly in order to lay the claims to rest for a generation that the Treaty was signed

in the first place.

Seven Treaty signatories have claims: Argentina, Australia, Chile, France, New Zealand, Norway and Britain. But these claims are not recognised by the other signatories and are not accepted even among the claimant powers, because the claims of Argentina, Chile and Britain overlap. In addition some 15% of the Antarctic land mass is unclaimed. The danger of the mineral resources problem was that it could bring out into the open the territorial claims, because in the event of mineral exploration or exploitation there would need to be some jurisdiction over the activity.

Rights questioned

In recent years however, the rights of the Antarctic Treaty powers to define even loose methods of control of the area have been questioned. The United Nations Law of the Sea Conference embraced the concept that the oceans were the heritage of mankind. There is no reason, some Third World powers have argued, why the same concept should not be applied to Antarctica.

In the event the problem of mineral resources has not had quite the same urgency as marine living resources. The latter involve primarily the krill, a crustacean seen by some scientists as a major untapped source of protein which could be harvested in sufficient quantities to double the world's annual fishing catch.

The depletion of the region's whale stocks has led to an increase in krill stocks, pointing to the role of the

creature in the ecosystem of the southern oceans. But detailed knowledge of the ecological interaction of the krill and other Antarctic marine species has not yet been accumulated. It is not even known whether the Antarctic contains one or numerous distinct krill populations.

Nevertheless the proliferation of the krill and its ready exploitation have already attracted the attention of West Germany, which has reported catches of 40 tonnes an hour, the USSR, Japan, Chile and Poland. It has also been the subject of survey by the Food and Agricultural Organisation (FAO).

The implications of all this for the Antarctic Treaty powers at the London meeting were serious. If environmental damage was to be prevented then it was necessary to find a regime of conservation, but the regime would have to be voluntary. Unless national territorial claims with 200 mile economic zones were to be recognised in defiance of the Treaty and of some of its signatories, there could be no policing. At the same time the lack of any internationally accepted system of authority meant that any nation could move its fishing vessels into the area.

The Treaty powers are planning a conservation regime, which they hope will be ready for signature at the end of next year. Such a regime will embrace several principles, they decided. In the first place it will include the "rational use" of resources, so harvesting is not prohibited. In the second place they are mainly concerned with the seas south of latitude 60°S.

The third principle is that the regime would exclude catch allocations. At the same time, however, it could involve a total catch figure. It is not clear how it is possible to have the latter without involving the former.

The fourth principle is extension of the freezing of territorial claims into the conservation regime. The effect of



Krill, caught on the wrong side of a whale

this is to ensure the rights of free access into the southern waters adjacent to the continental landmass. While this constitutes a leap over a major political hurdle and ensures a degree of order in the affairs of the continent, concomitantly it means looser control.

But the way has been opened for widening the international acceptance of the Treaty. The powers are considering inviting countries like West Germany to take part in the drawing up of a conservation regime, and international organisations like FAO could send observers.

In general the Treaty powers are anxious that any nation which is active in the area should accede, so that practical answers may be provided to practical questions. The Treaty is not closed, but the powers remain concerned about the possibilities of hegemony by an international organisation which might diffuse authority still further and act not as a spur to action but as a block.

Interim guidelines

In the meantime, pending a definitive conservation regime for marine living resources, interim guidelines have been established. But they do not go beyond commitments to cooperate in research, coordinate shipping programmes and the exchange of catch statistics. The

guidelines are accompanied by an acceptance of the need not to harvest the resources to the extent that there will be depletion.

In common with the principle adopted for marine living resources, control of the continent's mineral resources will not affect the position on territorial claims. But the Treaty powers have made their strongest commitment so far not to engage in mineral resource exploration until a regime of control has been established. This they intend to work towards. That said, however, there is no theoretical reason why a company should not start drilling in the area.

But this is not likely. Mining companies have already pointed to the distance of Antarctica from the world markets, the easier access available to resources in more hospitable climates and the expense of coming to terms with land covered by an ice sheet. The reservations of the companies are also held by experts from the Treaty powers. Their discussions and conclusions have taken a good deal of the pressure away from the need to reach quick solutions on control.

It is thought that it will take at least five years to amass the scientific and technical data necessary to provide a foundation for exploration and subsequent exploitation if conditions are to

be found which safeguard the continental environment. Exploratory drilling itself might take ten years. In short the possibility of exploiting mineral resources is at least 15 years away, and probably more like 25.

This assumes that the continent has hydrocarbon and other mineral resources economically worth exploiting. This is not proven. Geological history indicates the possibility, but so far only traces of mineralisation have been found on land, although unmetamorphosed tertiary sediments, often associated with oil and gas, have been discovered in areas offshore.

There is no technology available for oil production all the year round in Antarctica. The concepts for self-contained installations on the seabed exist but that is all. On land there is no technology available for drilling through the ice shelf.

Meanwhile the Treaty powers have the immediate problem of ensuring that the information they collect is made available to all who need it. The continent is vital within the international meteorological system, but the powers have discovered that only 25% of their observations ever reach the world system. Somewhere between the Antarctic research stations, the global telecommunications system and the world weather watch, the rest evaporates. □

Ninety days and more

As the Windscale inquiry draws to an end, Eben Wilson sums up progress

THE Windscale inquiry has become an institution—the British democratic tradition gone wild in the interest of a public debate about nuclear power. In its ninety days, independent and government scientists from throughout Britain and abroad have for the first time been disagreeing and airing their uncertainties in public, while wrestling with the thorny problem of the close links between nuclear power development as an energy option and its social and political consequences for the future.

The inquiry has taken over a civic hall of 1960s' architectural utility in Whitehaven, a small town perched almost inaccessibly on the Irish Sea coast to the West of England's Lake District, eleven miles from Windscale. Inside one large room, the inquiry inspector, Mr Justice Parker, a high court judge, and his two assessors, Sir Frank Warner and Sir Edward Pochin, sit trapped at a green baize table among a pile of papers. They look out

on five rows of lawyers, scientists and environmentalists who hide behind their green baize tables loaded with documents, articles and studies on nuclear power.

Out of that sea of paper a pile of daily transcripts now four feet high has appeared. Many say these will become an historic document, the quintessential reference work for the international nuclear debate.

Although the inquiry is specifically trying to decide on a planning application to build an oxide fuel reprocessing plant (THORP) alongside Britain's present Magnox reprocessing facilities at Windscale, it has become the platform for discussions on Britain's future energy options. Ranged against British Nuclear Fuels (BNFL), who own the present plant, is an array of pressure groups and individuals, from the established Friends of the Earth (FOE) to local housewives worried about their children.

The Friends of the Earth have waged

war on the economic case for reprocessing and have convinced the inquiry that the British taxpayer will have to pay somewhere between £300 million and £500 million to recycle spent oxide fuel from Britain's advanced gas cooled reactors (AGR). The exact figure depends on where the price of uranium lies between \$30 per pound and \$100 per pound, and a notional price for plutonium above £90,000 per tonne. BNFL estimate an ex-works price for recycled fuel of £260,000 per tonne or above depending on whether permission is given for a 1,200 tonne per year plant with capacity for foreign reprocessing contracts, or a 600 tonne a year plant only for British fuel.

The FOE alternative consists of importing uranium for fuel, storing fuel elements on a long term basis and abandoning reprocessing. They have asked for a ten-year delay to THORP while long term storage is researched. If this fails, they say, the delay would at least give time to try to evolve safeguards against plutonium proliferation.

British Nuclear Fuels have an unhappy history of Magnox fuel elements deteriorating in their cooling ponds at Windscale and have revealed that AGR fuel, still less than ten years old, is already showing signs of corrosion.

They view reprocessing as good waste management and point out that against the cost of reprocessing has to be put the cost of fuel storage, which nobody knows, and that storage itself does not guarantee that fuel elements will not eventually have to be dealt with either by glassification (at £450,000 per tonne), or even reprocessing to prepare fuel for glassification.

Scientific struggles

The real scientific struggle has been over the level of discharges from Windscale into the Irish Sea. These contain long-lived α -emitters, ^{239}Pu and ^{241}Am and shorter lived isotopes of ^{137}Cs .

Hours of evidence and reams of paper have shown disturbing inadequacies in the research done by the Ministry of Agriculture, Fisheries and Food (MAFF) and the Fisheries Radiological Laboratory (FRL) into transuranic marine pathways back to man's environment. The Isle of Man government produced Dr Vaughan Bowen, an expert in marine pollution from America, who castigated the FRL for its methods of measuring silt movements and biological pathways. Dr Brian Wyne for the Network for Nuclear Concern and a research lecturer at Lancaster University, cross-examined both MAFF and FRL at length on the critical group method of evaluating derived working limits for the consumption of contaminated organisms. Cod, muscles, porphyra (an edible seaweed), silt and more have been metaphorically and literally dissected to examine possible synergistic effects of low level contamination or the possibility of biological systems concentrating transuranics.

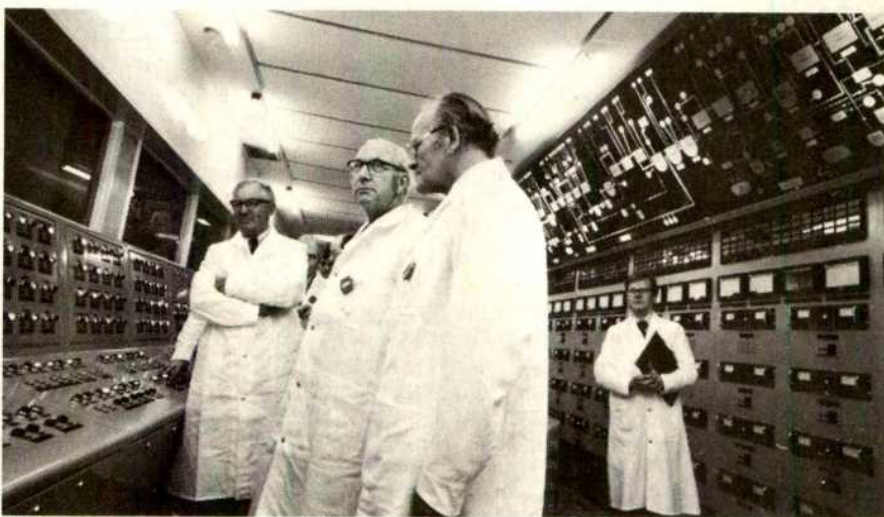
The result of the public analysis has been a mixture of scientific humility and confusion, and human arrogance and anger, each coming from both objectors and government scientists. Researchers using the transcripts after the inquiry will find long lists of documents showing the present confusion over the effects of low level radiation and the concept of a threshold for radiation below which humans are not affected.

Much evidence has been presented attacking the present International Commission on Radiological Protection (ICRP) limits. Dr Sadao Ichikawa from the Laboratory of Genetics at Kyoto University, Japan, in particular, presented disturbing evidence on the long term genetic effects of low level radiation.

The weight of all the technical evidence threatened to swamp the inquiry in its sixteenth week in a shower of facts when Justice Parker admitted that he and his assessors were falling behind under a deluge of documents.



Awaiting assessment: the Windscale plant



Making assessment: Warner, Pochin, Parker

Equally, however, it has given the inquiry a certain authority, so that when Thomas B. Stoel from the National Resources Defense Council (NRDC) in Washington, DC, came to suggest that a full environmental impact study should be done for Windscale, Justice Parker was able to wonder aloud and a little wearily what he had been doing for the past 74 days.

The overall impression of the discharge arguments has not been that BNFL are pumping death every day into the sea, but rather that nobody yet really knows what happens to the radiopathogens once they get into the silt and marine food chains. Both Dr Wynne and Dr Bowen have pleaded that independent bodies be funded to allow a plurality of research into radiation hazards.

Justice Parker could be forgiven for sighing and wondering what all this has to do with building a reprocessing plant now. He is known to favour risk comparisons with other industries and a practical approach to hazards. He has acted swiftly on the more alarmist claims made at the inquiry. The Lake District's water has been tested for tritium, air sampling is being done at Ravenglass a few miles from Windscale, and twenty local volunteers are

undergoing whole-body monitoring tests at the plant. The scientific groups on both sides of this application have agreed that the tests will prove nothing—an ironic consensus, as witnesses have usually retreated behind the armour plating of “not enough information” when under pressure.

The Oxford Political Ecology Research Group (PERG) faced the problem of getting enough information when they attempted to calculate the consequences of an accident at Windscale. A computer programme was provided by the Safety and Reliability Directorate, but agreement could not be reached on a possible mechanism for an accident that would provide the inputs to the programme.

Stunning realism

Justice Parker has gained a reputation for realism. He stunned the objecting groups at the beginning of July by declaring that alternative energy strategies in place of nuclear power would have to be presented showing “how and when and to what extent, and at what cost the energy gap can be filled”.

Strangely perhaps, the presentation of alternative energy sources has been made by ex-motorway campaigner, John Tyme. Stephen Salter of Edin-

burgh University explained wave-power systems, prophesying that the first string of 50 "nodding ducks" will be in the sea off the north of Scotland by 1984. Dr Peter Musgrove of Reading University put four hundred windmills into the Wash in the 1990s to produce around a quarter of Britain's energy demand at a price not far above the cost of coal now. His figures are becoming firmer every year. Solar power, biomass, seabed organic sediment fluidisation, combined heat and power, fluidised bed combustion were all at different stages into the future with corresponding scarcity of operating costings. But more traditional benign systems like water power, tidal power, and of course conservation were available options today.

The problem is money, either for research or first generation operating plants. All the witnesses giving evidence on soft energy paths claimed Britain was being slow in investigating alternative options. Amory Lovins' book *Soft Energy Paths*, which appeared during the inquiry, rapidly became the 'bible' on the way and the will for a move towards these options.

Time-scale has been Justice Parker's concern. John Tyme's trump card has been Yorkshire miners' leader Arthur Scargill, presenting the case for bridging the gap before a soft energy future with a huge expansion of the coal industry. This almost worked, although BNFL did a pretty savage job in cross-examination on both the productivity record and safety record of the coal industry in comparison with the nuclear industry.

The energy establishment represented by the Central Electricity Generating Board (CEGB), the South of Scotland Electricity Board (SSEB) and the Department of Energy itself faced a barrage of criticism from objectors. Surplus generating capacity, the downturn in energy demand since the oil crisis, and a radical change in

energy coefficients of new innovations were cited as proof that present energy demand forecasts are far too high. In particular, Dr Peter Chapman from the Open University Energy Research Group tied up the present view of energy relying on supply needs, with the need to change to a soft energy scenario based on energy income.

The nuclear industry is of course cursed with one emotive argument above all—the Bomb. The inquiry has not denied that nuclear warfare is a terrifying prospect. But British Nuclear Fuels has been at pains to deny that it is a conceivable prospect for future generations living with large quantities of plutonium resulting from reprocessing. Britain will be custodian to fifty tons of plutonium by the end of the century if THORP goes ahead. Five tons would fuel the first commercial fast reactor CFR 1. Throughout the inquiry the shadow of safeguards and security has been cast over the proceedings.

The fall-out from this has gone in three directions—towards terrorism, international nuclear diplomacy and civil liberties. BNFL thought they had squashed terrorism by their insistence that "measures" could be taken to prevent theft of plutonium. "Spiking" (making it radioactive), only returning it on contract as refabricated fuel, and the sheer difficulty of making a device that explodes rather than produces a large criticality excursion, are enough of a deterrent to BNFL. They add that there are easier ways to achieve political ends without dabbling in radioactive materials.

But BNFL's confidence was rudely shaken by two American witnesses. Professor Arnold Wohlstetter from the University of Chicago has explained to the inquiry that a nuclear device has been exploded using reactor-grade plutonium in the USA. Tom Cochran, again from the NRDC in Washington, DC, presented evidence outlining the

numbers needed for an armed raid on a nuclear plant (around nine outside and three inside.)

Avoiding proliferation

FOE have maintained a consistent line on the international threat of plutonium proliferation. They see as inevitable the political pressures for reprocessing contracts that specify return of plutonium after a certain period. The motivating force of Walt Patterson, their scientific adviser, has been proliferation, with a specific image of a world where shipments of plutonium will become familiar, leaving contempt for safeguards to provide the inevitable disaster.

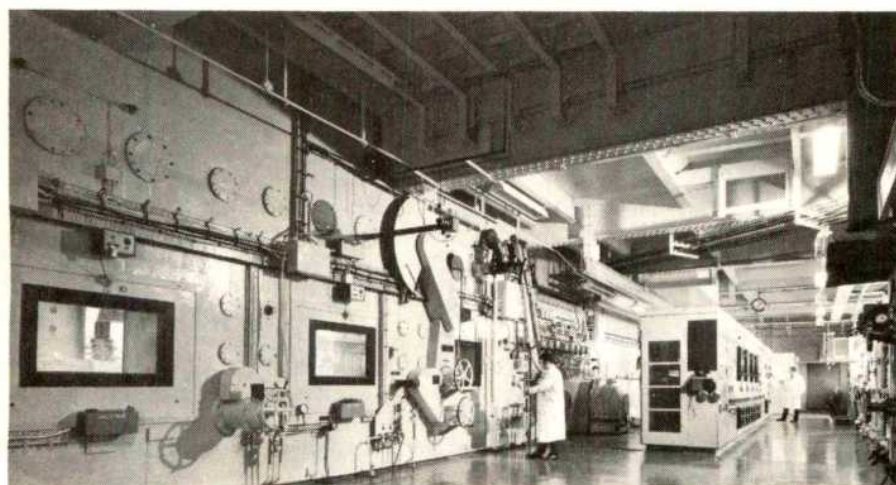
Positive suggestions to avoid proliferation have been made by Professor Joseph Rotblat from London University. He favours an international bank for buying in, storing, and allocating nuclear materials to establish the nuclear industry as a global enterprise. Research into storage options, reprocessing and the fast breeder would all be done under international licence. Reprocessing would be abandoned until proved essential.

Whatever the solution to proliferation, the attempt is to avoid the Orwellian 'plutonium economy'. Both the National Council for Civil Liberties and Justice made pleas that future generations would not be committed to a life of surveillance, power parks guarded by automatic weapon carrying police, and hunts for subversives.

By holding a mental map of all this in his head, Justice Parker has gained immense respect for the intellectual feat he is attempting. The weight of evidence is with the objectors, there being more of them, but he will be typically concerned with common sense applied to reprocessing now in Britain.

In retrospect Whitehaven has been an essential part of the spectacle. Inquiry faithfuls staying in enclaves throughout the town have been indulging in increasingly frenetic energy outlets as time goes on. Beach barbecues, pony-trekking, and a treasure hunt, where a vital clue was missing leaving ten stranded inquirers tearing around the Cumbrian countryside in complete confusion, have added colour to the interminable days of evidence and cross-examination.

Foreign visitors comment on the lack of armed police at the doors and the politesse of the participants. Perhaps it's one of the problems of the inquiry that the British will never see something as *that* important. The feeling now as summing up begins is "we wish the end of term party would come". But although the work is ending for some, for Justice Parker it is just beginning. □



Windscale's head-end plant: no longer working, it has reprocessed oxide fuel

PROFILE

Chris Sherwell on Professor Geoffrey Allen

SRC's new head, old hand

GEOFFREY ALLEN heads a 400-strong organisation that wields a budget of over £125 million and influences the fate of the great majority of Britain's basic researchers. He is not new to its workings. But he is new to his post. He took over from Sir Sam Edwards as chairman of the Science Research Council at the beginning of the month.

The job demands practical experience of research and of administration and, if possible, of the 'real world' outside both. It also demands more than a little political nous. An erratic economy makes consistent support for Britain's research effort hard to achieve. To fight for money on behalf of scientists at large, and then dispense it on behalf of the community at large, is just as difficult.

So how did Professor Allen come to it? Under the British system which chooses to recruit through the grapevine, he wasn't the first man approached. But he is realistic about this. He accepts the system and says he has used it himself. Equally, he'd be happy if jobs like his were advertised—provided the minister concerned (at Education and Science) was not constrained by the list of applicants. He is also sure that if the job had been advertised, he wouldn't have applied for it.

He is admirably qualified, of course: a polymer scientist of 48 elected last year to the Royal Society, he has SRC and industry (especially ICI) connections going back many years. The problem was that he had recently taken up the chair of chemical technology at Imperial College, and was starting a research group there; he couldn't easily ask for a 4-year leave of absence. When the approach came, however, the decision wasn't difficult for him personally; rather, it was simply a matter of working out the possibilities and reaching agreement with Sir Brian Flowers at Imperial and with the Department of Education and Science.

What Allen calls his pragmatic view of life and work comes, he says, from his background. He was born in the Derby mining town of Clay Cross, the son of an engine driver. A Methodist Sunday School teacher taught him self-discipline. And a donnish ex-Cambridge maths teacher at grammar school inspired his love for science. He sees nothing significant in the fact that he has since

'made good'. "The good thing about science", he says, "is that it doesn't signify where you come from. It's what you do that matters".

Having graduated in chemistry at Leeds and completed a doctorate there on the thermodynamics of solutions, he took the advice of Fred Dainton, now head of the University Grants Committee (UGC), and went to Canada in 1952 to learn about spectroscopy. He wanted an academic job when he returned two years later, but the pickings were thin. Geoffrey Gee, who had just left the Rubber Producers' Research Association to take up a chair in chemistry at Manchester, offered him a job "provided you work in my field and bring the techniques you've learned to bear on my problems". Allen stayed for twenty years.

Gee taught him three things: that it was possible to do good physical science on polymers; that good science is always the basis of good technology ("That coloured the whole of my career . . . I never had the hang-up some of my contemporaries had that there was something nasty about industry"); and that "scientists have to think more widely than just being a scientist"—a view reinforced by contact in Manchester with Brian Flowers and Sam Edwards, his SRC predecessors.

Allen acquired his experience of administration and industry during the 1960s, sitting on government and SRC committees and consulting for companies. Then, in 1969, in a development he sees as a crossroads, Allen helped set up the ICI/Manchester Unit Joint Laboratory he directed at Manchester. He insisted from the start that the laboratory be closed after five years, on the grounds that any worthwhile work could at least be run down steadily, any dynamic work would be picked up by ICI or the university, and any decision to re-start it could take account of experience. A little later he went half-time to the ICI corporate laboratory, also for five years.

Before Allen came to the SRC he declared all his consultancies, but served notice that he might wish to take up two while in office; this was agreed. "It would be nice to do some consulting as a working scientist", he says, "and I could do this without compromising my position". For the curious, he says his income is likely



Professor Geoffrey Allen

to stay roughly the same at the SRC, with a "chance there could be a drop".

As for the coming four years, he knows the problems awaiting him. Support for high energy physics and space, for example, can't go any lower. "They're at the margin", he says. After a declining curve in the overall budget, he now wants a flat one; an inclining one, he acknowledges, would be too much to expect. But it will probably have to come out of a larger Science Budget, that is, economic growth. Among the arguments at his disposal for a greater share of that budget, however, is one that must appeal to the scientists who dispose of it: that applications for money clearly deserving support are being turned down.

Allen here distinguishes a lack of money from a surfeit of ideas. But both are problems, even if the former is sad and the latter a sign of health. He also says that the 'dual support' system for university research is not working, save in specific cases—as when the UGC had some money for new projects and could be persuaded to put it into the Interactive Computing project for British universities.

Allen also knows the standards he needs to follow. He says he'll be running "an open office", like Flowers and Edwards before him. He is conscious of a need for proper and responsible dealings with pressure groups, though he doesn't name names. He likes tackling problems, "provided they're amenable to logic", and, he says, "I can be persuaded to change my mind. Administrators must be able to do that".

He hopes to leave his successor with an operation looking as good as the one Edwards has left him. And like Edwards, he hopes to return to science, his first love. He still works at it, after all. Last year he got a grant from the SRC.

BRITAIN

ICRF appointment

The Imperial Cancer Research Fund has announced a new director for its research laboratories. Chris Sherwell reports

THE Imperial Cancer Research Fund (ICRF) last week announced the appointment of Walter Bodmer, Professor of Genetics at Oxford, to succeed the present director of its research laboratories, Dr Michael Stoker. Stoker, a virologist, leaves the post he has held since 1968 a year early, in September 1979, when he is 61; he returns to academic life and research in Cambridge. Bodmer, 41, a population geneticist involved more recently in immunology and tissue typing, has held his Oxford chair since 1970.

Bodmer said this week that nothing significant should be read into the fact that a geneticist was replacing a virologist. In welcoming the appointment, however, Stoker described the appointment of a geneticist as "very timely", paralleling in its recognition of contemporary developments his own appointment as a virologist years earlier. Over the next couple of decades, he said, the cancer problem would be dissected at the molecular genetics level, specifically in the realm of mutagenesis. Bodmer was "one of the leading younger geneticists in the country", with an all-round ability as well as a specialisation in the human leucocyte antigen (HLA) system.

Though Bodmer will almost certainly be receiving a higher salary than he currently enjoys, the real attractions are the opportunities, facilities and support ICRF offers. He recognises that he will be giving up one sort of administration for another, but hopes not to be too tied down by such matters. There will be no teaching, no university committees, a curtailed administrative load outside but—most importantly—an allotment of space at the ICRF's sophisticated laboratories in Lincoln's Inn Fields, central London. This he can fill with his own appointments—perhaps up to a dozen—who will continue the research he has led at Oxford, though perhaps with a change of emphasis. He says he will establish an HLA typing lab there.

In spite of recent allegations about the way ICRF uses its money, which drew a sharp response from ICRF and subsequently a libel suit for the paper that made them, Bodmer sees the job as no more controversial than any similar one in universities and other

research institutes which have to justify their particular paths in research. That the job is important he has no doubt—"otherwise I wouldn't be moving". It combines, he says, "the best opportunities for biochemical research in the country". But if the post hadn't come along, he would not be leaving the country, though he admits the alternatives beyond Oxford are limited.

Both Bodmer and Stoker believe in the virtues of limited tenure as a means of maintaining high quality in research. The same purpose is also served, both men believe, by the presence of foreign visitors, another distinguishing feature of ICRF research. Stoker is a firm advocate of another controversial view relating to research—that of early retirement from positions of power. This belief, along with his appointment as Foreign Secretary at the Royal Society, which has dragged him from his research, has encouraged his own departure. He adds that two years is the sort of notice that has to be given for a job like this.

Thus the far-reaching changes of which the ICRF Council chairman, Sir Eric Scowen, spoke in his annual report (published at the end of March) are now coming to pass. The announcement of a new man to head the biggest single private research effort in the country is the outcome of a search conducted by a special ICRF committee over the past few months. Stoker's intention to relinquish his post was confirmed in the annual report; and Stoker's deputy, Dr Renato Dulbecco, retired last month. The post of deputy will remain vacant until Bodmer takes up his appointment and makes his own choice. In the meantime the deputy's role is handled by Dr John Cairns, who heads ICRF's lab at Mill Hill. □



Walter Bodmer

USSR

Space retrieval

THE cancellation of the *Soyuz 25/Salyut 6* link-up coincided most unfortunately with the celebration meeting held on October 11 to mark the 20th anniversary of *Sputnik 1*. This anniversary had been invested with considerable importance by the Soviet authorities.

In his anniversary address, Academician Boris N. Petrov, head of the *Interkosmos* programme, did his best to retrieve the situation. Reviewing the events of the last twenty years, he stressed especially the successes of the automatic space probes and the *lunokhody* and Venus descent craft in particular. Even the *Salyut* space stations, he noted, were designed to operate in both manned and unmanned modes, and in either could "solve important problems of science and technology". Since the (manned) *Soyuz* programme already had two major setbacks in the past three years (*Soyuz 15* in 1974 and *Soyuz 23* in 1976 both failing to achieve a link-up with the *Salyut* stations), Petrov naturally concentrated on the more successful unmanned ventures.

Ironically, however, it was not the crew but the automatic part of the *Soyuz 25* mission which was responsible for its failure. The main part of the link-up programme is effected automatically by the *Soyuz* on-board computer with possibly some orbital adjustments of the *Salyut* by ground control. This system operates to within 120 m of link-up, after which the cosmonauts take over the control for final approach. What appears to have happened in recent aborted missions is that the automatic system has over-shot such that the *Soyuz* has not had sufficient power reserves to attempt a second, manually-controlled pass.

Although some *Soyuz* craft have carried solar panels to recharge their batteries, it seems likely that on the *Soyuz 25* these were sacrificed to permit additional weight to be allotted to stores and/or experimental equipment. This would tie in with speculations that the crew were to attempt to break the current Soviet record of 63 days or the US record of 84 days in space.

It is not impossible, however, that a further attempt to link up with *Salyut 7* will be made before the 60th anniversary of the Revolution next month.

Vera Rich

IN BRIEF

Call for dementia research

IN its report *Senile and presenile dementias: a report of the MRC sub-committee*, the Medical Research Council recommends that senile dementia and the rare presenile dementias be designated areas of high research priority, although it does not recommend setting up a special MRC Unit yet. The main message of the report is clear however; an estimated 5–10% of elderly people in the UK suffer from senile dementia about which virtually nothing is known. The MRC should encourage clinical units prepared to undertake the intensive studies needed and support special workers within institutions caring for demented patients, who would organise research and liaise with laboratory workers

carrying out parallel biochemical, pharmacological and histological studies.

One of the most urgent needs is a clarification of the various types of dementia and their comparison with the normal ageing process. Little is known about the early stages of senile dementia and the report suggests that community studies of the elderly might be grafted onto surveys already in progress for other purposes.

German nuclear funds

IN the Federal German government's budget proposals for 1978, DM1.45 thousand million is ear-marked for nuclear research and technology. Allowing for a 4% inflation rate, this means an increase of 8.5% over the

1977 figure of DM1.3 thousand million. As before, financing the government nuclear research centres will be a main consideration and DM700 million is being made available for their maintenance.

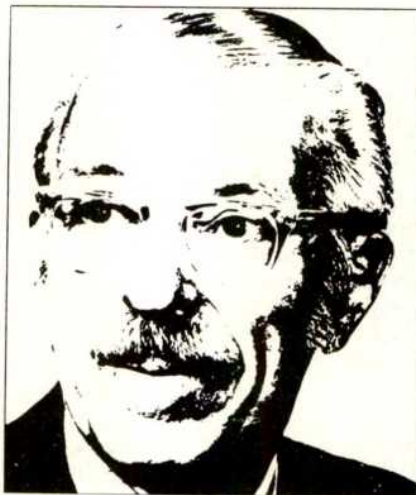
The prototype 300MW fast breeder reactor under construction at Kalkar is costing DM2.86 thousand million at present rates and 90% is being borne by the state, with the Belgian and Dutch governments contributing DM719 million. The cost of this reactor has greatly increased year by year—DM200 million from 1977 to 1978 alone—and its completion is expected in 1983. The costs of the 300MW high temperature reactor being built at Schmehausen are running at DM1.75 thousand million.

AGRICULTURAL technology in the USA has evolved with a rapidity that is seldom perceived except by participants in its use. The consequences of migration from rural to urban areas are evident to most people, but few city-dwellers know much about what happens on the farm these days.

The change from muscle-power to petroleum has proceeded almost to completion, and is to all intent irreversible. The Council for Agricultural Science and Technology recently published an energy-use report prepared by 22 agricultural scientists. They point out that to produce today's US crops by 1918 technology would require 61 million horses and mules that would need almost half of the cropland now in cultivation to supply their food. Performing the necessary additional hand labour thus involved would call for the relocation of almost one-third of the total working population in the United States. The average cost of their toil, at \$26.50 per day, would be about nine hundred times as much as the cost of electrical energy to carry out the same amount of physical work. Very few people have first-hand memories of how crops were raised and harvested 50 years ago, and not many can comprehend what life would be like if horses were, once again, the main source of transportation.

Agricultural production uses only 3% of the energy consumed in the USA. Jet aircraft use more than this. More food is being produced than ever before, and with less human labour. In 1976, the US gained \$23 billion in foreign exchange from exporting agricultural products, as compared with \$34 billion spent on imports of energy for all purposes.

One-third of the energy used in agriculture is employed for producing fertilisers, and only 5% for production of pesticides, principally herbicides, insecticides and fungicides. One estimate is that 41% of food and

Energy for crops**THOMAS H. JUKES**

fibre production in 1960 would have been lost if pesticides had not been used. Biological control as a replacement for chemicals is 'in the news', but, as the energy-use report says, "Biological control of plant pests is the ideal in theory, but progress in practical implementation is slow and difficult."

On 27 September the Secretary of Agriculture criticised the use of pesticides as being wasteful of petroleum. He did not mention that their production and application consume less than 0.2% of the country's energy total. I wonder how he allocates

priorities: certainly he doesn't seem to be 'thinking agriculture'. There is much dissipation of fossil fuel for purposes of convenience and pleasure as contrasted with its use for food production. Tourism and family reunions must account for a substantial proportion of jet plane travel. The highways are clogged with passenger automobiles and recreational vehicles, and the waterways resound to the hum of motorboats. Give us the petroleum-powered luxuries of life, and we will dispense with the necessities. Maybe! If food really becomes scarce, there will be plenty of trouble.

Statistics are dry and boring compared with the actual rural scene. Huge tractors till the vast wheat country, clean of officious fence or hedge, in the Great Plains. The big fields of hybrid maize in the corn belt grow apace in the hot sun, nourished by synthetic ammonia, and protected by chemical weed-killers. Further west, green discs of the new centre-pivot irrigation systems dot the landscape. These are yet another replacement of manpower by energy. There has been recent criticism of the University of California for developing new farm machinery that does the work of human beings. This is like protesting against the tractor, the combine, the plough and other inventions that lifted the burden of hand labour.

Only 5% of the energy used on farms is derived from electricity. Optimistically speaking, solar energy could perhaps supply another 25% by the year 2,000, says the report. When it comes to agricultural production, no complete or near-complete replacement seems to be in sight for the dwindling supplies of petroleum.

correspondence

Melatonin in serum

SIR,—We have recently completed a preliminary collaborative study of radioimmunoassay methods in the determination of melatonin in serum in which the different techniques used in seven laboratories were compared. To each laboratory we sent the same material derived from calf serum with a melatonin level of less than 10 ng per l. To this serum melatonin was added in a final concentration of 200 ng per l or 0.86 nmol per l. Three ml aliquots of the calf serum from the pool were pipetted into glass ampoules. The calf serum in each ampoule was lyophilised and the ampoules were tightly sealed. The serum was dissolved in 3.00 ml of water and melatonin was assayed in 35 samples from 8 ampoules and was found to be 0.87 nmol per l \pm 12.9% r.s.d. by our own radioimmunoassay.

Twenty ampoules were sent in December 1976 to each laboratory. They were requested to add 3.00 ml of water to each ampoule and to perform assays using their own method, reporting back the results in ng melatonin per l redissolved calf serum. The results of the different assays are given in Table 1, and are presented as ng per l and are also transformed to nmol per l according to the SI-system. It should be pointed out that the extraction techniques in the methods differed considerably between the different laboratories and that each laboratory used its own antiserum except for numbers 4 and 5 who used the same one.

The results indicate that melatonin can be measured accurately using different radioimmunoassay techniques. We now intend to repeat and extend the study to include human reference serum with different concentrations of melatonin. This will allow an evaluation of the linearity and parallelism of the standard curves, as well as give

further data on the sensitivity and specificity of the different assays. All laboratories wishing to participate in the extensive cross-validation study planned at the beginning of 1978 are welcome to submit their names to us.

LENNART WETTERBERG

Karolinska Institute,
St Goran's Hospital,
Box 12500, S-11281,
Stockholm,
Sweden

Technics rather than technik

SIR,—I thoroughly agree with what Fores and Rey wrote (1 September, page 2) about the need for making a clear distinction between science and what they propose to call *Technik*. However, I should like to point out that a well-established, but now little used, word already exists in the English language for the concept of the useful arts. This is *technics*, and I would urge the revival of this old English word, rather than the importation of a foreign-sounding one, to describe this area of our culture.

Lewis Mumford's use of it throughout his great book *Technics and Civilization* (1934, Routledge) bears out what Fores and Rey say about the need for a word to express this concept; for Mumford, unlike Bronowski and Mazlish, does not make the mistake of confusing science and technics. As he says, "science and technics form two independent yet related worlds: sometimes converging, sometimes drawing apart".

As Fores and Rey indicate, this distinction is as important in current discussions and controversies concerning the place of science and technics in contemporary culture and society as it is in historical studies. Much current discussion of the financing, control and effects of 'science' would be far less

woolly-minded if a clearer distinction were made between science and technics. However, the lack of a familiar word to express the idea of technics is I think only part of the problem. Equally important is the (perhaps only partly conscious) desire by many scientists and others deliberately to blur the distinction, and to claim for 'science' and scientists the credit for all the benefits resulting from technics.

This shortsighted attitude may have boosted funding and respect for science in the short run but in the longer run, as people become more critical of technology, it is in part responsible for the increasing disillusion with science and the growth of antisience movements. The anti-science critics are even more at fault for failing not only to make a distinction between science and technics, but also between these and the socio-political decisions which have led to particular technical developments that they dislike, and for which we are all responsible as citizens, whether scientists, technicians (surely a better word than *Techniker*, if its status can be raised) or layman. For a healthy society and a healthy science I believe we should support those, like Sir Andrew Huxley (29 September, page 366), who insist on making these important, but often unpopular, distinctions.

T. CAVALIER-SMITH

University of London King's College,
UK

The Messinian crisis

SIR,—Just a thought or two on "The Messinian salinity crisis . . . etc" by Adams, Benson, Kidd, Ryan and Wright (29 September, page 383).

A Crisis—a word often seen in the news. Is a term which most journalists

frequently use
With reference to sterling or else to a ban
On the import of Datsuns from far off Japan.

But vaporisation of Miocene seas
Is a natural process and nothing like these;
Evaporite bodies will oft mark the coast
Of an ancestral ocean which gave up the ghost.

Applying that hackneyed and trite little word
To a normal event is quite clearly absurd.
Else what might we see if left up to the Press—

The Messinian Horror or Shocker or Mess?

ANON

Comparison of different radioimmunoassays for melatonin

Laboratory Number	Melatonin concentration of reference calf serum containing 200 ng per l or 0.86 n mol per l.	
	pg per ml	nmol per l
1	239 \pm 35	1.03 \pm 0.15
2	214 \pm 11	0.92 \pm 0.04
3	210 \pm 41	0.90 \pm 0.18
4	190 \pm 28	0.82 \pm 0.12
5	188 \pm 10	0.81 \pm 0.04
6	135	0.58
7	128 \pm 15	0.55 \pm 0.06
Mean s.d. of all 7	186 \pm 41	0.80 \pm 0.17

All values are mean \pm s.d. Different laboratories have assayed different numbers of samples.

news and views

Observation of photon antibunching

from Peter Knight

VERY few measurable optical effects require field quantisation and the idea of a photon for their explanation. One notable example of this rare class is photon antibunching in intensity correlation of resonance fluorescence (*Nature* **265**, 683; 1977), predicted by Carmichael and Walls (*J. Phys.* **B9**, L43; 1976; *J. Phys.* **B9**, 1199; 1976) and now observed by Kimble, Dagenais and Mandel (*Phys. Rev. Lett.* **39**, 691; 1977). Whereas most optical coherence and correlation experiments have an adequate semiclassical interpretation, antibunching requires quantisation of the emitted fluorescence and the idea of a 'quantum jump'.

The radiation emitted from the resonantly-excited atoms is collected, split into two, and imaged onto two photomultiplier tubes whose output is electronically correlated. The intensity correlation of interest is

$$g^{(2)}(\tau) = \frac{\langle \hat{I}(t+\tau) \hat{I}(t) \rangle}{\langle \hat{I}(t+\tau) \rangle \langle \hat{I}(t) \rangle}$$

where $g^{(2)}(0) = 2$ if the radiation was chaotic (this positive correlation, or bunching is the famous Hanbury Brown Twiss result) and $g^{(2)}(0) = 1$ for coherent light. Fully quantised and semiclassical approaches are here in agreement. However there are quantum states of the electromagnetic field which do not have a classical description, but the experimental realisation of such states has proved difficult. States exhibiting negative photoelectric correlation ($g^{(2)}(0) - 1 < 0$) or 'antibunching' are much-discussed examples of an effect requiring field quantisation: it is hard to visualise a function $I(t)$ representing the classical intensity whose mean square is less than the square of its mean. Carmichael and Walls showed that the resonance fluorescence radiated by a single two-level system driven by a coherent field possesses the required statistical properties to exhibit antibunching. This can be demonstrated easily since $g^{(2)}$ may be expressed in terms

of the two-level Pauli operators representing the atomic source fields as

$$g^{(2)}(\tau) \sim \langle \sigma_+(t) \sigma_+(t+\tau) \sigma_-(t+\tau) \sigma_-(t) \rangle$$

so that $g^{(2)}(0) \sim 0$. This behaviour at $\tau \sim 0$ is a strictly quantum feature: the atom emits a photon at time t and is unable to radiate again immediately after having made a quantum jump down to the lower state.

The measurement of $g^{(2)}$ consists of two parts: detection of the first photon (ensuring the de-excitation of the source atom) and a delay while the atom is re-excited by the resonant driving field for the subsequent re-emission of a second photon. So

$$g^{(2)}(\tau) \propto p(t, \tau)$$

where $p(t, \tau)$ is the probability of finding an atom, initially in its lower state at t , re-excited at τ . Cohen-Tannoudji (*Frontiers in laser spectroscopy*; Les Houches 27; North Holland, 1977) has stressed that the antibunching effect in resonance fluorescence is a striking example of wavepacket reduction in action. We expect first a lack of coincidence counts at short times τ , and further expect the Rabi nutational oscillation of $p(t, \tau)$ to be reflected in $g^{(2)}(\tau)$ which

should exhibit periodic bunching and antibunching as the driven atom undergoes Rabi oscillations. For a sufficiently intense resonant driving field for which the Rabi frequency Ω is much greater than the spontaneous decay rate β ,

$$g^{(2)}(\tau) = 1 - \exp(-3\beta \tau/2) \cos \Omega \tau$$

If there is more than one atom in the observation region the interpretation is a little harder and since fluorescence could then be produced by two or more atoms this would increase $g^{(2)}$ and obscure the antibunching.

The experimental arrangement of Mandel and coworkers resembles that used by earlier groups to study resonance fluorescence spectra. Sodium atoms in a well-collimated atomic beam are prepared by optical pumping to ensure a two-level transition and are driven by an intensity and frequency-stabilised CW dye laser at the $3^2S_{1/2} F=2, M_F=2$ to $3^2P_{3/2} F=3, M_F=3$ transition frequency. The fluorescence emitted at right angles to both atomic and laser beams is collected, divided by a beam splitter, imaged on to two photomultiplier tubes, and the correlation evaluated under computer control. The atomic beam current is designed to allow only one or two atoms to contribute to the collected fluorescence at the same time. The experiment shows direct evidence for antibunching of photoelectric pulses. After various corrections have been made for accidental pair correlations produced by background laser scatter, their results are shown in Fig. 1, where the broken curve shows the theoretical single atom prediction normalised to the same peak for $\Omega/\beta = 4$.

The observed $g^{(2)}(0)$ is approximately 0.4 rather than the zero predicted theoretically for a single atom. Mandel *et al.* estimate $g^{(2)}(0)$ to be 0.5 if the field were produced by two radiating atoms located at random positions. At large τ the atomic flight time through the photomultiplier field of view decreases $g^{(2)}(\tau)$. The overall result is as expected and plainly exhibits both bunching, and more importantly, for the first time antibunching. \square

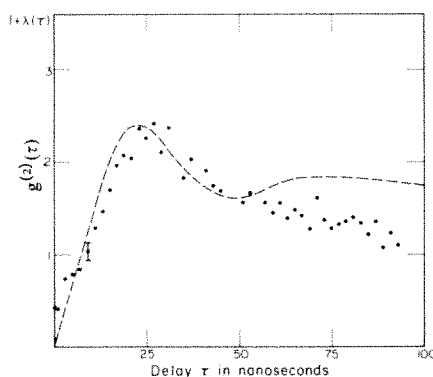


Fig. 1 Values of $g^{(2)}(\tau)$ derived from the data, demonstrating photon antibunching. The broken curve is the theoretical prediction, suitably normalised to the same peak.

Immunoglobulin genes and the immune response

by Miranda Robertson

The Tenth Harden Conference on the Molecular Genetics of the Immune Response was organised by Professor A. R. Williamson and took place at Wye College on 12–16 September, 1977.

IN a meeting which was characterised throughout by lively and uninhibited discussion of almost every controversial issue in modern immunology, the outstanding topic was without question the unexpected character of immunoglobulin light chain chromosomal DNA. The surprise lies at the junction between the constant and the variable region sequences. S. Tonegawa and his associates have shown (Hozumi & Tonegawa *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628; 1976) that whereas in embryonic cells the chromosomal DNA coding for the V region is on a different restriction fragment from that coding for the C region, in antibody-secreting myeloma cells they are both on the same restriction fragment. This has been widely interpreted to mean that V and C sequences are separated in the germ line, but become joined in committed B cells. But evidence from four sources, including Tonegawa's own laboratory, now shows that the V and the C sequences of myeloma chromosomal immunoglobulin DNA are separated by an untranslated 'spacer' sequence.

Discontinuous Ig gene

This explains the otherwise puzzling results of S-I nuclease digestion of hybrids between chromosomal DNA and cDNA made on a mRNA template. S. Longacre, as reported by B. Mach (Geneva University), hybridised separate V and C region cDNAs with restriction fragments of embryonic and myeloma chromosomal DNA, expecting to find more single-stranded tails on the embryonic than the myeloma DNA. But when she compared the hybrids retrieved by hydroxyapatite extraction with those retrieved from S-I nuclease digestion, which removes single-stranded stretches, she found that the proportions were the same for embryonic and myeloma DNA.

In a different experiment on similar principles, T. Rabbitts (MRC Cambridge) has compared the susceptibility of liver chromosomal DNA and myeloma chromosomal DNA hybridised with complete kappa chain cDNA to digestion with S-I nuclease. Liver

DNA is assumed to be equivalent to embryonic DNA where immunoglobulin genes are concerned, so that if separate V and C sequences are joined in plasma cells the entire V-C sequence should be protected by the cDNA probe in myeloma DNA, but only separate V and C fragments in liver DNA. In fact, gel electrophoresis of the digests reveals only the C region fragments regardless of the source of the chromosomal DNA. The fate of the V region is unknown—it may have undergone partial digestion or be hidden in the C region peak—but at the very least the data imply a gap between the V and the C regions in which a short stretch of single-stranded DNA is exposed to the nuclease. Similar nuclease digestion evidence for the separation of V and C regions of active immunoglobulin genes was presented by G. Mathysens (Basel Institute for Immunology), who, however, found both V and C region fragments on electrophoresis.

The results of the nuclease digestion studies are moreover fully borne out by electron micrograph evidence from Tonegawa's own laboratory, presented at the conference by O. Bernard (Basel Institute for Immunology). Hybrids of cloned myeloma chromosomal DNA with the complementary lambda chain mRNA showed clearly in the electron microscope a single-stranded stretch of DNA which appeared as a loop between the V and C regions. Approaching the question another way, Tonegawa and his colleagues have annealed separate V and C region mRNAs with double-stranded chromosomal DNA. Because the DNA hybridises preferentially with the mRNA, this results in openings in the double strand where the RNA has hybridised with one strand of the DNA. In the case of lambda chain mRNA, two such loops (known as R loops) are separated by a roughly 1,000-nucleotide 'spacer' sequence.

It seems likely that the 'spacer' DNA has some regulatory function and that function is not peculiar to immunoglobulin genes. There are strong indications, hammered home by Mach, that spacer sequences may be a general feature of eukaryotic genes. There have recently been reports in three other systems of discontinuous genes on chromosomal DNA—in adenovirus (see *News and Views* **268**, 101; 1977), in *Drosophila* tyrosinase tRNA (Goodman *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press) and mouse globin (Leder *et al. CSH Symposium*, 1977). Although the spacer sequences are not

present in mRNA, that is not necessarily to say that they are not transcribed, and the obvious speculation is that the transcript is none other than so-called heterogeneous nuclear RNA. Many have remained unconvinced that hnRNA is anything more than an artefactual aggregate of mRNA molecules, but the evidence has recently become more convincing, at least for globin (see *News and Views* **269**, 9; 1977), and indeed according to Mach, Leder finds that the size of the globin spacer corresponds to the size of the extra sequence on globin precursor mRNA. Some first steps in investigating immunoglobulin precursor mRNA were reported at the conference by R. Wall (University of California, Los Angeles) who has identified 40S and 24S precursors of kappa light chain mRNA. He has yet to find out where the V and C regions map on these precursors and whether they will hybridise with chromosomal DNA.

The answers might have been available sooner had not the early stages of the work been held up by indecision on the part of NIH about the safety of the plasmids used by Wall to clone cDNA probes for the precursor mRNAs. Delays of the same sort are affecting Rabbitts and his colleagues who are waiting for permission to clone myeloma chromosomal DNA in an *E. coli*-λ phage EKII system. Now that it is clear that cDNA is by no means necessarily a copy of chromosomal DNA and that spacer sequences may hold the answers to important questions of genetic regulation, delays of this kind are likely to lead to acute frustration, particularly where, as in the case of Rabbitts's experiments, permission has already been granted elsewhere (in Switzerland and the USA). It may not matter very much in the light of eternity whether fundamental discoveries in biology are made now or in six months' time, in Britain or in the USA—but it matters very much to biologists.

Sequence diversity in frameworks

There are no sequence data yet on the myeloma gene, but Bernard did present the first nucleotide sequence of an immunoglobulin gene: that of a cloned V region fragment of embryonic chromosomal DNA, the fruit of a collaboration between Tonegawa's group and W. Gilbert's group at Harvard. This has revealed another untranslated region, 54 nucleotides long, in the precursor sequence at the 3' end. Otherwise, the sequence corresponds largely to the amino acid sequence of

the light chain V region of myeloma MOPC 315 which produces lambda chains of subgroup ii. A curious feature of the sequence is that at three of four of the positions where it disagrees with the MOPC 315 sequence it corresponds to that of MOPC 104R, which produces lambda i light chains; in the fourth position it agrees with neither. C. Milstein (MRC Cambridge) gave voice to a general feeling that this pattern of substitutions is unlikely to be random. More sequence data should clarify the issue, but in the meantime the data are reminiscent of the amino acid sequences of mouse kappa V regions, reported by M. Weigert (Institute for Cancer Research, Philadelphia). Some residues in these chains seem to switch between closely related subgroups.

Whether more amino acid sequence data will help to clarify the question of the generation of antibody diversity is controversial. Amino acid sequences were presented at the meeting in support of an insertion theory (D. Capra, University of Texas) and of a somatic mutation theory (Weigert) but neither set of data could strictly eliminate alternative theories. It is now generally conceded that antibody diversity arises by a somatic mechanism acting on a limited number of germ-line genes (probably two or three copies for lambda chains and perhaps two or three copies for each of an unknown number of kappa chain subgroups) (Rabbitts & Milstein *Contemporary Topics in Molecular Immunology* 6, 117; 1977; Tonegawa *et al. Immun. Rev.* 36, 29; 1977) and there is a school of thought that the solution to the generation of diversity will be found in cellular mechanisms rather than sequences. In the meantime, the rational position on GOD seems to be agnostic.

Idiotypes and immunoregulation

Sequence data may, on the other hand, help to clarify the structural and functional significance of idiotypes, which if Jerne is right may be the basis for intercellular recognition in a regulatory network. At what levels might Jerneian immunoregulation take place? Capra presented unequivocal evidence that the so-called 'anti-Ars' idiotypic on mouse anti-arsonate antibodies is determined by specific residues in the hypervariable regions which also determine antigen-binding specificity. However, there is ample serological evidence that idiotypic is not always associated with the antigen-binding site, and T. Feizi for instance (MRC Clinical Research Centre, Harrow) has found that cross-reacting idiotypes on human antibodies against erythrocyte Ii antigens can also be found on antibodies which lack that binding specificity. In competitive bind-

Unification confrontation

MOST physicists hope that the electromagnetic and weak interactions are unified and this hope has been strengthened over the past few years as the Weinberg-Salam unified model of the weak interactions has notched up success upon success. But a small cloud appeared on the horizon last year; and it has not gone away.

Take the Weinberg-Salam model, add some atomic physics calculations, and the result is a prediction of small, but measurable, parity-violating effects in atoms. Preliminary results from experiments aimed at detecting these effects were reported last year (*Nature* 264, 528; *News and Views* 264, 505; 1976) and were disturbing—the predicted effects were not seen.

These experimenters now have their final results (*Phys. Rev. Lett.* 39, 795; 39, 798; 1977) and have reached a sensitivity an order of magnitude better than the prediction. No parity-violating effects are seen and the threat to the unified theories must now be regarded as serious.

There is only one foreseeable escape route—perhaps the atomic physics calculations for the bismuth atoms used in these experiments could be in error. Experiments planned with hydrogen will resolve this final uncertainty.

ing assays, the idiotypes can be identified as a heavy chain framework determinant that seems to identify a sub-subgroup.

Subgroups of subgroups were a particular feature of Weigert's partial sequence data on 22 mouse kappa light chains, and raised the question of how variable a framework residue has to be before it becomes part of a hypervariable region. (Is GOD leaky? asked Milstein). Indeed Weigert and his colleagues have now found in mouse light chains a fourth distinct hypervariable region, like that reported earlier by Capra *et al.* in heavy chains. The fourth hypervariable region (like the second, at least in those antibodies whose crystal structure is known) is remote from the antigen-binding site.

F. Melchers (Basel Institute for Immunology) was enormously interested in these results, on the grounds that the fourth hypervariable region might provide the basis for recognition in Jerne's immune network. He has indirect evidence for idiotypic regulation of mouse antibodies to streptococcus A

carbohydrate, which carry the A5A idiotypic. Primary immunisation with either the streptococcal antigen or antibodies against the A5A idiotypic stimulates the production of idiotypic-positive antibodies 40% of which bind the antigen and 60% of which do not, regardless of the priming stimulus. Melchers interprets these results—which strictly apply only to lipopolysaccharide (LPS)-sensitive cells (one third of B cells) since LPS was used to expand the number of cells *in vitro* for counting—as evidence for lymphocyte triggering by idiotypic rather than by antigen-binding site in these cells.

The structural significance of idiotypic arose again in connection with the T cell receptor—another crucial element in any immune network. The recent discovery that T and B cells can carry the same heavy chain idiotypic and that anti-idiotypic antibodies can trigger T cell help *in vitro* (see *Cold Spring Harbor Symp. quant. Biol.* 61, 1976 and *News and Views* 263, 10; 1976) has been interpreted to imply a T cell antigen receptor coded partly by genes in the pool coding for the V region of the heavy chain. However, K. Rajewsky's group, as reported by T. Cramer and T. Imanishi (Cologne University), now finds that in SJL mice T cells but not B cells carry an idiotypic associated with the heteroclitic binding of the hapten nitrophenol (NP). Genetic experiments suggest that this heavy chain idiotypic is expressed only in cells which produce lambda light chains and its absence in SJL B cells is due to the very low production of lambda chains by that strain. This leaves a puzzle: what produces the conformational change that gives the idiotypic on SJL T cells? So far, the evidence has been against any light chain gene contribution to the 'T cell receptor.'

The proper characterisation of the T cell receptor will really be possible only when one can be isolated in large quantities. This may be soon. G. Fathman and H. Hengartner (Basel Institute of Immunology) reported a technique for producing 3-month cultures of T cells with specific reactivity in a mixed lymphocyte culture—a potential source of receptors for histocompatibility antigens.

'Hybridomas'

The final session of the meeting amounted to a progress report on the developing range of hybridomas. Since its beginning with immortal clones producing antibodies against sheep red blood cells, the hybridoma army has recruited clones producing antibodies against haptens TNP, NIP and NP; soluble proteins peroxidase, lysozyme, and chick gamma-globulin; and influenza virus surface antigens, rat

MHC antigens, T, G (A - - L) and streptococcus A carbohydrate.

To this Milstein added a line producing antibodies against an allotypic determinant on mouse molecules with the characteristics of IgD (Pearson *et al. Eur. J. Immun.*, in the press). and mouse hybrids producing antibodies against three rat bone marrow differentiation antigens (Williams *et al. Cell*, in the press). With some overlap, these antibodies seem to define the lymphoid, myeloid and erythroid populations of the bone marrow.

G. Hämmerling (Cologne University) reported the production of three hybrids secreting monoclonal antibodies against mouse H-2 antigens; these three are the survivors of five isolated from 1,000 hybridisations.

The recent extension of hybridisation techniques to T cells produced two exciting reports. Hengartner and Fathman have evidence for sensitivity to specific MHC haplotypes in two hybrids made from lymphoma cells and T cells sensitised to H-2 haplotypes in mixed lymphocyte reactions. And L. Herzenberg (Stanford

University), and colleagues has a hybrid expressing antigens coded by genes in the I-J region which is associated with T suppressor functions. The hybrid is derived from T cells in a mouse suppressed for the Ig1b allotype but evidence for a suppressor factor is so far inconclusive. Supernatants from the cultured cells have shown no reliable suppressor activity against the allotype, and although sonicates have given more positive results they too are inconsistent.

Research reported at the conference represented the entire immunological continuum between what Fathman characterised as immunochemistry on the one hand and immunomagic on the other. But although structures and sequences may be cleaner than cells and sonicates, there remain problems that at this stage are not amenable to the immunochemical approach. Herzenberg was prepared to defend immunomagic. Knowing the structure of the immunoglobulin molecule, he pointed out to participants who included R. R. Porter, has not told us how B cells work. □

arranged around the inner surface of the neck near the entrance hole or ostiole. Fertile female fig wasps, *Blastophaga psenes*, enter these winter figs and lay their eggs in the neuter flowers. On completion of their development, the male offspring emerge first and bore into the flowers occupied by females and fertilise them before emergence. The males then die. Females subsequently emerge from their pupae and leave the fig through the ostiole, but in doing so become coated by pollen from the dehiscent anthers of the male flowers.

Pollen-bearing female wasps are then believed to enter figs containing a mixture of female and neuter flowers. The former have longer styles than the latter, and this prevents oviposition by the fertile females. They do succeed, however, in ovipositing within the neuter flowers, where the next generation of wasps develop, and they also inadvertently scatter their load of pollen within the fig, resulting in the pollination and subsequent fertilisation of the female flowers.

Emergence and fertilisation of the wasps takes place as before, and the fertile females now escape and invade a third type of fig which contains only neuter flowers. At this stage, obviously, there is no pollination process, but a third generation of wasps can be established within the neuter figs. Fertile females of this generation emerge in winter and lay their eggs in the neuter flowers of the winter fig (which also contains male flowers) thus completing the annual cycle.

Of most immediate interest is the provision by the plant of certain flowers, and indeed an entire inflorescence generation, which are of no

tors. There are on record some striking examples of complex symbiotic relationships between plants and pollinators, but few more remarkable than that of the figs (*Ficus* spp.) and the fig wasps.

The false fruits of the figs are hollow, swollen receptacles which bear on their inner surface small, unisexual flowers. In the wild progenitor of the edible fig (*Ficus carica*) a specialised winter fig is formed which contains mainly neuter flowers, with some male flowers

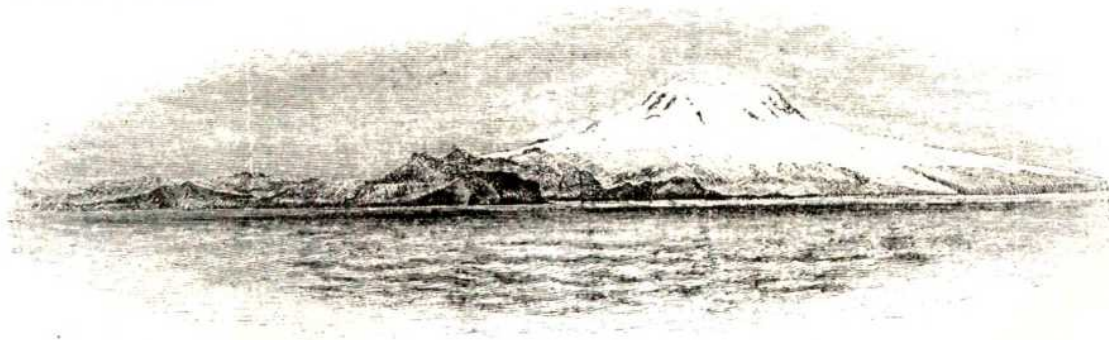
Figs for wasps

from Peter D. Moore

THE phenomenal success of the flowering plants may well be due, at least in part, to the way in which their floral evolutionary development has been related to the structural and behavioural modifications of insect pollina-

Peter D. Moore is a Senior Lecturer in the Department of Plant Sciences, King's College, London.

A hundred years ago



THE NORWEGIAN DEEP-SEA EXPEDITION The next day was wonderfully calm, but the fog covered the higher parts of Jan Mayen. We went on shore the sea being so calm that we could step on short without any inconvenience. The shore consisted of volcanic ash and sand, quite black, and was, higher up, covered by driftwood thickly strewn on a level surface. To the left

was a steep cliff wonderfully rich in colour, the abode of thousands of seabirds, whose inner slope, consisting of ash and scoriae, showed it to be a part of a former crater. The scientific party spread in different directions and made the best of the time in surveying, collecting specimens of plants and rocks, and drawing; one polar fox was killed. The plants found belong to very

few species, the vivid green we had seen from the ship being only a cover of moss. The flowers had just come out and all the lower part of the island up to about 2500 feet was generally free from snow, the snow lying only in the lower regions where a larger mass was gathered in ravines.

From *Nature* 16, 18 October, 526; 1877.

direct value in terms of seed production. The energy expenditure must be worthwhile in terms of maintaining a pollination vector population for the time when they are needed.

The wasps themselves have also attracted some attention recently, for the process of pollen transfer has proved more complex than was originally anticipated. It is difficult to account for pollination by a simple accidental carriage of pollen by emergent females, for as the wasp enters the ostiole of the receptor inflorescence, it is subjected to considerable mechanical stress as it forces its way between tight ostiolar scales. Indeed the entry slits are so narrow that wings and antennal flagellae are often lost. Thus the likelihood of pollen being carried into the fig inflorescence by superficial adhesion is very low. Galil and Eisikovitch (*Tijdschr. Ent.* **112**, 1; 1969) discovered specialised pollen pouches on the ventral side of the thorax in the fig wasp *Ceratosolen arabicus*, which is the pollinator of the African *Ficus sycamorus*. In the same year Ramirez (*Science* **163**, 580; 1969) described similar pouches in New World species of fig wasps. Subsequently Galil and Snitzer-Pasternak (*New Phytol.* **69**, 775; 1970) described the behaviour of the fig wasp *Blastophaga quadraticeps* as it loads and unloads pollen from its pollen pockets during pollen transfer in *Ficus religiosa*. Cleaning movements of the legs transfer pollen to the thorax and the pouches open at the touch of the legs to receive pollen. So, in many fig wasps complex morphological and behavioural adaptations aid the process of pollen transfer, which in turn ensures the fertilisation of fig ovules.

But this does not seem to work in the case of *Blastophaga psenes* and the common fig *Ficus carica*. Galil and Neeman (*New Phytol.* **79**, 163; 1977) have re-examined this relationship and have found no evidence of the complex pouches which had been noted in other fig wasps. Yet the superficial carriage of pollen was most unlikely, for not only does the wasp experience physical scouring when passing between ostiolar scales, it also cleans itself very thoroughly after emergence from the *Ficus* inflorescence and removes all external pollen.

Serial sections of wasps were taken immediately after this cleaning and the only pollen to be found was that lodged in temporary folds within the intersegmental membranes. Galil and Neeman also found that these invaginations are produced as a result of water loss from the insect after emergence from the pupa which causes infolding of the membranes. About 20–30% shrinkage of the abdomen was found to occur within the fig inflores-

cence after emergence from the pupa. They experimentally raised the relative humidity of some figs to 100% and found that wasps failed to pick up pollen.

Unloading in the receptor fig probably occurs in the reverse manner. On entering a higher humidity atmosphere the wasp's body would swell and the pollen trapped in folds would be liberated. Thus the transfer of pollen in *Ficus carica* is more complex than originally thought, but it entails neither the sophisticated anatomical modification nor the purposive behavioural mechanisms on the part of the vectors which are found in other fig/fig wasp relationships. Galil and Neeman point out that the common fig has about 800 stamens in a fig, compared with only about 15 in *Ficus religiosa*, the vector of which has pollen pockets. The relationship in *Ficus carica* can thus be regarded as evolutionarily primitive and energetically expensive and wasteful as far as the plant is concerned, for most of the pollen produced and disseminated is cleaned off by the unwitting vector. □

Origin of the Rio Grande rise

from Peter J. Smith

THE Rio Grande rise is a large, well known, aseismic structure stretching through at least 10° of both latitude and longitude to the west of the mid-Atlantic ridge in the South Atlantic Ocean. To say that it is well known, however, is to imply little more than that it is a conspicuous topographic feature which has received a certain amount of bathymetric attention and frequent mention in the geophysical literature. It is true that Thiede is about to publish a report (*Am. Assoc. Petr. Geol. Bull.*) in which he concludes from sedimentary and stratigraphic data that the rise was once a large island which lay 2–3 km above sea level some 75–85 Myr ago. But the origin of this sediment-covered and supposedly sunken island remains uncertain, largely because of lack of information on the nature of the basement.

Unfortunately the recent attempts by the Deep Sea Drilling Project to sample the igneous basement beneath the Rio Grande rise were unsuccessful (Leg 39, site 357). On the other hand, as Fodor *et al.* point out (*Earth planet. Sci. Lett.* **35**, 225; 1977), a large quantity of igneous material was dredged from the rise by the research

vessel *R. D. Conrad* in 1973. Dredge samples are obviously not as satisfactory as cores recovered from *in situ* rock; nor in the present case have the samples been dated. Nevertheless, from microscope, electron microprobe and bulk chemical studies on a selection of the 1973 dredge material, Fodor and his colleagues have been able to come to some general conclusions about the origin of the Rio Grande rise.

The material in question comprises basaltic rocks, some of which contain mafic nodules and megacrysts, and volcanic breccias composed largely of basaltic fragments. The bulk compositions enable the rocks to be classified as alkalic basalts, trachybasalts and trachyandesites. Moreover, the compositions of the pyroxene, plagioclase and interstitial alkali feldspar within the material are typical of volcanic rocks having alkalic affinities. The dredged samples are thus clearly characteristic of alkali basalt suites and bear no resemblance to oceanic ridge tholeiites. The compositional range (basalt to trachyandesite) also shows that the volcanic material of the Rio Grande rise has undergone magmatic differentiation.

Both alkalic composition and differentiation seem to be typical features of aseismic ridges. Indeed, the Rio Grande samples are very similar in many respects to those from the islands of Gough and Tristan de Cunha and, more significantly, to those from the Walvis ridge, the linear structure whose position almost mirrors that of the Rio Grande rise across the mid-Atlantic ridge. On the other hand, there are some distinct differences between the Rio Grande and Walvis rocks, which fact draws attention to the disadvantages of having to use dredge samples. It is reasonable to assume (although it cannot be proved) that the material dredged from the Rio Grande rise comes from the crystalline basement. But how typical is it of the basement? Does it or does it not represent all the main rock types present? There is no way of knowing. Thus the observed differences between the Walvis and Rio Grande rocks may (but also may not) simply reflect inadequate sampling. Moreover, the impossibility of knowing whether or not the sampling is representative makes it impossible to say that the Rio Grande basement does not also include tholeiitic material.

But these reservations apart, the best information available suggests that the Rio Grande rise consists of alkalic basaltic rocks covered with later sediment. And as alkalic basaltic rocks in oceanic provinces are usually associated with volcanic islands or seamounts, it

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

seems reasonable to propose, as do Fodor and his colleagues, that the rise originated as a series of volcanic islands and/or seamounts which underwent magmatic differentiation and subsided as the South Atlantic opened. The evidence for subsidence is that the Rio Grande sediment contains shallow-water fossils incompatible with its present depth. Those same sediments also contain little or no volcanic material, suggesting that the igneous activity represented by the dredge samples must have occurred early in the South Atlantic's history.

However, the question of how the volcanic islands formed is still unsolved. They appear to be consistent with generation at a hotspot, although volcanic activity along fracture zones cannot be ruled out. The origin of the Walvis ridge is similarly uncertain.

European tree-trunks reveal post-glacial changes

from J. M. Fletcher

A symposium on Dendrochronology was held on 19 August, 1977, as part of the X INQUA Congress at Birmingham.

DENDROCHRONOLOGY, the dating of wood remains by the number and pattern of tree-rings has been extensively used to date wooden artefacts and timber structures from prehistoric to relatively modern times (see *News and Views* 268, 402; 1977). Some of the research reported at the symposium demonstrated that the type of tree-ring analysis involved is also revealing and dating major changes in palaeogeography over the past 10,000 years and calibrating radiocarbon ages with greater accuracy than hitherto.

In Europe, Bruno Huber (1899–1969) established German preeminence in this subject and this position is being maintained by the work of his pupils, in particular by that of B. Becker (University of Hohenheim, Stuttgart). From his comprehensive study of the subfossil tree trunks that lie at various depths in the sediments occupying former river channels in central Europe Becker has been able to trace and date with precision changes in fluvial activity and in climate from 9,600 BP (in

Preboreal time) to 780 AD, after which few trunks were deposited in the drainage systems studied.

The information on climatic changes has been derived from the changes in species present in the immediate post-glacial period and from the dating by dendrochronology of 2,000 oak tree-trunks in association with the variations in their average ring-widths (that is growth rates) at different times in the Holocene.

Periods at which depositions ceased simultaneously occurred in several river systems, for example the Danube, Rhine, Main and Werra. At other periods, for example 6,700 to 4,500 BC, the ring-widths and associated remains from peat indicate a slow rate of growth due to wet sites with a high ground water level.

The dates of major changes in the drainage system are pin-pointed by these results. For example, in late Atlantic times there was renewal of trunk accumulation due to high fluvial activity from 4,300 to 3,100 BC. Becker interprets his results for the Late Holocene as providing evidence not only for the activity of the rivers, but, from changes in the widths of the annual rings, for the clearance of forests by man with the production of deep alluvial soils.

A paper of particular interest to those concerned with the calibration of radiocarbon dates dealt with results at Queen's University, Belfast on large samples (175–200 g), each covering 20 years growth, of the sub-fossil oaks already dated in the same department by tree-ring analysis. After a lengthy period of critical examination of the human and other errors that can arise in radiocarbon analysis, G. Pearson has achieved an accuracy in which the uncertainty on each of many results is as little as ± 25 yr. The importance of this accuracy, which is comparable to that (about ± 20 yr), now achieved at Seattle by M. Stuiver on comparable samples of recent wood, lies in the reliability of the information for the first period to be examined systematically, namely from 3,600 to 4,600 BP. Whereas previous results from US laboratories a decade or so ago were interpreted as showing distinct 'wiggles' in that part of the calibration curve, the Belfast results virtually conclusively demonstrate their absence.

Two papers in the session dealt with conifers at the timberline in the Alps. Françoise Serre (Laboratory for Palaeobotany and Palynology, Marseilles) has discovered what may well be some of Europe's longest-lived trees. Most of the 38 larches sampled at an altitude of 2,100 m in the Maritime Alps (France) are over 1,000 years old. About 20% of their ring-width sequen-

ces have been cross dated and the 'signature' years compared with climatic variations. The research extends to about 500 km, the distance in the Alps over which ring-width features for this species are common to particular years.

For the eastern Alps, the High Tavern of Austria, H. W. Posamentier (Department of Geosciences, Rider College, New Jersey) described how narrow and wide rings of stone pine and larch at the timberline are related to records of glacier advance or retreat. For stone-pine, the correlation coefficient (r) is -0.69 for the years 1891–1969. The tree-ring record implies 10 or 11 periods of glacier advance since 1585: these include the years 1627–28 and 1815–22 when ring-widths were at their lowest values. □

Snow geese, lizards and sunbirds

from John Krebs

The 15th International Ethological Conference was held at Bielefeld, FRG on 25–31 August, 1977.

THERE are two genetically distinct colour phases of the lesser snow goose, *Anser caerulescens* breeding in Northern Canada. At the Eastern end of Hudson's Bay the large La Pérouse Bay colony contains 25–30% 'blue' and 70–75% 'white' morphs. At the conference, F. Cooke (Queens University, Ontario) described a remarkable long term study of the mating preferences of the two colour morphs. At La Pérouse Bay, most birds choose a mate of their own colour: only 16% of all pairs are mixed, compared with an expected 40% if birds had been choosing mates at random. In a series of experiments with captive geese, Cooke was able to show that the bird's preference for a mate of its own colour comes about through imprinting (learning during early life). The field observations and experiments established the following facts. Birds of mixed parentage choose mates at random with respect to colour, so that own colour has no direct effect on mate choice. In order for a clear preference to be established, a young bird has to grow up with parents and siblings of the same colour. Birds reared with parents and siblings of different colours showed no clear mating colour preferences, although they did avoid mating within

J. M. Fletcher holds a Leverhulme Trust Fellowship granted through the University of Oxford to the Research Laboratory for Archaeology and History of Art.

John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology at the University of Oxford.

their own foster group (an interesting parallel with learned 'incest' avoidance in human children). How do mixed pairs arise in nature? Cooke constructed a model based on the observed facts, and was able to show that about 40% of the offspring from pure broods must be mating at random in order to account for the observed level of mixed pairs. An appreciable proportion of birds become imprinted on the 'wrong' colour as a result of nest parasitism, for example a blue pair laying an egg in the nest of a white pair, but this alone is not enough to account for the high level of 'mistakes'. Cooke suggests two possible additional hypotheses: there is genetic variation in imprintability, or birds take a mate of the wrong colour when there is no alternative around. Cooke's study is the clearest demonstration of sexual imprinting in the wild, but the selective forces acting on the snow goose polymorphism are still a mystery. White, blue, and mixed pairs all have similar reproductive success, yet the frequency of morphs varies from one colony to another. Whether this reflects an historical accident, or the action of unidentified selection pressures remains to be discovered.

The phenomenon of sexual imprinting itself is hard to explain in functional terms. Why should birds have to learn their mating preferences rather than having a purely inborn response to their own species? P. P. G. Bateson (University of Cambridge) proposed the intriguing idea that sexual imprinting is primarily involved with recognition of kin. Bateson argued that animals should avoid mating with their own siblings (because of the adverse effects of inbreeding), but they should also prefer to mate with partners from the same population, whose genes will be adapted to the same environmental conditions. The simplest way to achieve this optimal level of outbreeding would be to learn the phenotypic characteristics of close kin, and choose a mate which is similar but not too similar to the parents and siblings. This prediction could well be tested experimentally.

Although the main theme of the conference was behavioural ontogeny, there were several outstanding contributions dealing with more ecological problems. J. A. Stamps (University of California at Davis) described how young lizards (*Anolis aeneus*) defend feeding territories, and in addition that the level of aggression shown to tethered conspecific intruders depends on the size ratio of resident to intruder. The lizards are most aggressive to individuals of the same size, which have the biggest overlap in feeding niche, and are hence the most severe competitors. L. L. Wolf (University of

Syracuse, New York) and F. B. Gill (University of Pennsylvania, Philadelphia) also reported on a study of feeding territories, and described how sunbirds (Nectariniidae) utilise their defended feeding area in a way which results in an individual avoiding recently depleted flowers so that they always harvest from those flowers which have been accumulating nectar for a long time.

Finally, T. H. Clutton-Brock (University of Cambridge) showed in a most elegant study how red deer stags settle disputes over the ownership of females by roaring contests, in which there is a gradual escalation of roaring rate until the loser gives up, presumably exhausted. There remains the nagging question of why a weak stag does not lurk in the background while two stronger males roar each other to exhaustion, and then step in to claim the hinds. □

Origin of cosmic rays

from a Correspondent

The 15th Biennial International Cosmic Ray Conference was held at Plovdiv, Bulgaria on 13-26 August, 1977.

SOMEWHERE in the Universe cosmic ray nuclei are produced and accelerated up to energies as high as 10^{20} eV. The bulk of cosmic ray nuclei, falling at the rate of $10^4 \text{ m}^{-2} \text{ min}^{-1}$ on the Earth's atmosphere, are known to be single protons and to have energies $\sim 10^{10}$ eV. The flux of such particles falls off steeply with energy ($\approx E^{-2}$) so that at 10^{20} eV the rate is very low indeed, 1 per 10 km^2 per 10 years. The propagation paths of the cosmic ray particles are stirred up by interstellar magnetic fields to such an extent that nearly all sense of source direction is lost. Thus the origin of cosmic ray particles remains a continuing topic for debate, and pervaded the conference. No definite solution was to emerge but some progress was achieved.

Experimentally the main attacks on the problem are concerned with the determination of the mass composition, the energy spectrum and the arrival directions of the cosmic ray particles. At lower energies ($\sim 10^{10}$ eV) interest still focuses on the relatively recent discovery of ultraheavy cosmic ray nuclei. New results were presented by the combined University of Bristol/

Dublin Institute for Advanced Studies group on the analysis of large balloon-borne lexan plastic and nuclear emulsion sandwich stacks flown from Sioux Falls, USA. A total exposure of 120 m^2 days was achieved at $\sim 3.8 \text{ g cm}^{-2}$ atmospheric depth. A total of 274 tracks of nuclei with charge number $Z > 36$ were found, of which 96 had $Z > 65$. The charge distribution featured a peak in the neighbourhood of platinum and a high uranium group flux. The conclusion appears to be that these particles are produced by rapid neutron capture (r-process) presumably in supernovae or similar outbursts.

At higher energies up to 10^{17} eV and beyond the evidence with regard to the overall mass composition is very indirectly obtained and with controversy existing as to interpretation it is probably best (but not necessarily safe) to conclude that the mass composition is much the same as that found at lower energies, that is, 90% protons, 9% helium nuclei, 1% rest, at a fixed energy per nucleon.

For many years now it has been well established that the energy spectrum steepens significantly beyond 10^{15} eV although the explanation of the steepening remains in doubt. New results presented at the conference from the large Extensive Air Shower Arrays at Haverah Park (England), Yakutsk, (USSR), and Volcano Ranch (USA) consistently indicate a flattening in the spectrum again at close to 10^{19} eV. This flattening may perhaps represent a transition from dominantly galactic rays to extragalactic sources.

Any weight given to this latter conclusion must be strongly influenced by the almost complete isotropy in arrival directions of cosmic ray particles up to the highest energies. Harmonic analysis in right ascension of the data at these highest energies ($> 10^{18}$ eV) does suggest a measurable anisotropy creeping in, but the phase seems to change with energy and no obvious source direction is indicated. Some of the highest energy events clearly come from high galactic latitudes, strongly suggesting extragalactic origin.

The outstanding problem for protagonists of the extragalactic origin rests with the lack of cut-off above 10^{19} eV expected from the interaction of the high energy particles with the universal 2.7 K photons. This suggests that the particles observed at these energies come from distances within 10^8 light years.

In the opening invited talk at this conference V. I. Ginzburg (Institute for Nuclear Research, Moscow) argued forcefully that as the galactic halo had now been well established from radio astronomy, there really was

no problem in the bulk of the cosmic rays being of galactic origin. Later in a more detailed analysis of the situation V. S. Berezinsky (Moscow) maintained the Soviet line by suggesting a theory that could fit the total experimental data. According to his 'naïve theory', at energies below 10^{19} eV cosmic rays are mostly protons of galactic origin. The phase of anisotropy varies with energy due to a focusing effect of the galactic magnetic fields. At higher energies an origin transition occurs with the cosmic rays reaching the Solar System largely coming from the local supercluster of galaxies with a maximum from the Virgo direction. For the moment this theory of the origin of cosmic ray particles seems to be the best compromise. □

Do populations regulate themselves?

by Mary Lindley

A symposium on Population Control by Social Behaviour was held at the Institute of Biology in London on 20–21 September, 1977. It was organised by D. M. Stoddart (King's College, London) and F. J. Ebling (University of Sheffield), and the proceedings will be published by the Institute of Biology.

TWENTY years ago V. C. Wynne-Edwards propounded a hypothesis to explain why populations of animals do not expand unchecked until stopped by the exhaustion of food resources. Many and perhaps most animals, he suggested, must regulate their population densities before this stage is reached, and they do that through social competition. His work on the red grouse (*Lagopus lagopus scoticus*), which was carried out at the University of Aberdeen and which he recalled at the symposium, proved him correct as far as that species was concerned. Each autumn young males take up territories in a contest with established males, and the many unsuccessful birds are cast out and usually die within six months. Thus population density is regulated.

One of the purposes of the symposium was to examine how much more evidence has emerged in favour of the hypothesis. Some of the eleven speakers addressed that theme more directly than others.

Reviewing 30 years of field work

with great tits (*Parus major*) in Oxford and Holland, J. R. Krebs (University of Oxford) said that the birds' behaviour seems to affect population size during three stages of the annual cycle. First, during the spring breeding season the number of eggs laid by each bird can fluctuate considerably, with fewer produced when the population density is high than when it is low. This fluctuation does not seem to be a direct consequence of changes in the amount of food available. The immediate influence is more likely to be a behavioural mechanism. Second, the survival of young birds in the summer and early autumn is an important influence on the size of the population, and aggressive interactions are at least in part the cause, with heavier birds most likely to survive. The third period when behaviour seems to be regulating population is early spring, when birds compete for territories, and exclude those that are unsuccessful. But Krebs stressed that little is known about the environmental resources to which the regulating behaviour is geared.

Indications of similar regulatory influences on populations of the woodmouse (*Apodemus sylvaticus*) have emerged from equally long term studies carried out in the United Kingdom and elsewhere in Europe. J. R. Flowerdew (University of Cambridge) described how from winter to winter the number of woodmice within a population remains relatively stable. From summer to summer, however, there is considerable variation and the population is smaller in summer than in winter. The size of the winter population seems to be regulated by a density-dependent process initiated in summer. Social behaviour seems to be a part of that process, involving, for example, aggression towards immigrants and dispersal of juveniles. But more information is needed about the nature of the behaviour and about the influence of other factors, especially genetics, predation and starvation.

In primatology, however, positive conclusions seem even further away. Although primates might seem to be ideal mammals for the study of social behaviour and population structure, most of them live too long. A. Jolly (University of Sussex) pointed out that there has been pathetically little long-term fieldwork with primates. Hope is offered, however, by the lemurs of Madagascar which have a relatively short life cycle. Since 1963 sixteen studies have been carried out in the favourable circumstances of the Barenty Reserve, and although the objectives have been different in each case, the data can be combined to

represent a long-term study of the sifaka (*Propithecus verreauxi*) and *Lemur catta*. A picture is emerging of stable populations, with *P. verreauxi* maintaining the same territories throughout the year and from year to year, while *L. catta* fluctuates in its use of the environment. This seems to be an ideal situation for critical study of social behaviour and population regulation. And that was as far as Jolly was prepared to go.

Her fellow primatologist J. Deag (University of Edinburgh) seemed to offer even less hope. He launched an attack on primatologists, including himself, for the way they have generally discussed the adaptive significance of social behaviour. He criticised the approach of inductive reasoning, whereby hypotheses are developed to explain behaviour on the assumption that it is adaptive. This had led to much speculation, with in many cases alternative hypotheses which are difficult or impossible to test. Deag called for a more critical approach based on established facts and involving more rigorous testing of the predictions of hypotheses.

There is growing evidence that odours are involved in many inter-relationships between behaviour and population processes in mammals. Reviewing what is known so far, D. M. Stoddart (King's College, London), explained that data on the role of odours in reproduction are hard to interpret because all were obtained in the laboratory, which is very different from the field. But three aspects of population ecology provide a function for odour. First, social dominance can be marked by odour in several species, including rabbits and marmosets. The emerging picture is of a rise in social dominance associated with increased production of scent, increased marking behaviour and a change in the composition of the odorous secretions. Second, odour can be used to mark out and maintain territory. It chiefly deters intruders and serves as a landmark by which the occupier recognises its own territory. Third, odour seems to be involved in the transmission of danger and warning signals, although so far the evidence is not strong. As Stoddart pointed out, the role of odour in population regulation is another underdeveloped area of investigation.

By the end of the symposium the only conclusion to have emerged was the familiar plea that more work needs to be done. Wynne-Edwards professed himself completely content that discussion about his hypothesis is still alive. As he said, you cannot expect answers of the sort given at meetings of the British Association in Victorian times. □

review article

Recent excitement in the DNA replication problem

Bruce Alberts* & Rolf Sternglanz†

It is now possible to reproduce most of the reactions involved in DNA replication using prokaryotic enzymes in vitro. Such systems have revealed that DNA replication is a complex process depending on a relatively large number of proteins, and that nucleoside triphosphate hydrolysis energy is used at several discrete steps. Much of the complexity of DNA replication may arise from the need for extreme copying fidelity.

DNA REPLICATION represents a central biological problem whose general outlines were solved "in theory" by Watson and Crick. For the past 24 years, geneticists, biochemists and molecular biologists have been hard at work attempting to translate into chemical reality the prediction that "each chain acts as a template for the formation on to itself of a new companion chain"¹. Progress has been slower than many had expected, as both the mechanism and the enzymology have turned out to be surprisingly elaborate. The following general conclusions concerning events at a functioning replication fork now seem reasonable, and will be used as section headings in this brief review of the present status of the field. Note that we have not attempted to deal with the important problem of how replication forks are initiated, but only with how, once formed, they progress along the parental helix to duplicate the DNA.

DNA is replicated by a multienzyme complex

DNA is replicated by means of a multienzyme complex, sometimes called a "replication apparatus". This complex, because it is held together by relatively weak protein-protein interactions, has been isolated only as smaller individual protein components. Much of the challenge in this field is a consequence of the difficulty of recognising these components in cell extracts, and then purifying them and characterising their activities in larger reconstituted systems.

The first components to be identified were of course the DNA polymerases, the intricate and elegant enzymes which catalyse the actual polymerisation process, while following the dictates of the base sequence of a DNA template strand². These large enzymes (molecular weight about 10^5) have so far failed to receive the attention they deserve from enzymologists, chemists and X-ray crystallographers. As a result, we know rather less

about their detailed chemistry than we should, despite some intriguing initial studies³.

Because it is smaller and simpler than the DNA polymerase, considerable attention has been paid in recent years to defining the chemistry and structure of a second component of the replication apparatus, henceforth designated the "helix-destabilising protein" (abbreviated as HD-protein)[§]. The prototype for proteins in this class is the T4 HD-protein (T4 gene 32 protein), originally designated a "DNA-unwinding protein"⁵, and in more recent years called by some a "DNA-melting"⁶ or "DNA-binding"⁷ protein. Quantitative data reveal that by binding tightly, co-operatively and specifically to single strands, the T4 HD-protein and its analogues in other organisms greatly reduce the thermodynamic cost of DNA helix melting within the cell^{5,6,8,9}. However, as discussed below, additional energy seems to be needed to open the parental DNA helix ahead of the fork. Further, HD-proteins interact in specific ways with many other proteins which function on DNA, and can thereby play a deciding part in determining their mode of action^{7,10-13}.

The other proteins which interact with the DNA polymerase and helix-destabilising protein to complete a replication apparatus are less well characterised. Here geneticists have contributed much to recent advances. Their work forecast the complexity of *in vitro* systems now in use, revealing as early as 1963 a requirement for numerous gene products in a large replication apparatus^{14,15}. Moreover, the many mutants they isolated made it possible for biochemists to develop "*in vitro* complementation assays". Such assays, which quantify the activity of mutationally defined proteins, have allowed numerous essential proteins of previously unknown function to be identified and purified to near homogeneity from *E. coli*^{10,11}, bacteriophage T4^{16,17} and bacteriophage T7¹⁸⁻²⁰ replication systems. The development of an *E. coli in vitro* DNA replication system using only soluble components²¹ was another major advance. So far, the number of putative replication fork proteins identified by genetics and/or biochemistry is at least 13 from *E. coli*^{10,11}, 6 from the T4 system^{17,22} and 4 from the T7 system^{18-20,23}. Possibly some of the extra proteins in the *E. coli* system are essential because the assembly of the replication complex needs to be much more carefully controlled in a bacterium than in a bacteriophage.

An interesting and so far unexplained feature of these multienzyme complexes is the large number of different replication proteins which hydrolyse nucleoside triphosphates to nucleoside diphosphates and inorganic phosphate. These include *dna* B protein²⁴, copolymerase III*²⁵, replication

*Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143.

†Department of Biochemistry, State University of New York, Stony Brook, New York 11794.

§ The new term "helix-destabilising protein", and the HD-protein abbreviation, are suggested here after consultation with other workers in this field. They are designed to avoid confusion with other types of proteins which seem to use ATP hydrolysis energy to aid in DNA-helix unwinding (see text). As an example of the convention proposed, the extensively characterised *E. coli* (3) and calf (4) analogues of the T4 HD-protein (gene 32-protein) are to be designated henceforth as the *Eco* HD-protein I and the calf HD-protein I, respectively. This nomenclature is designed to be descriptive enough to emphasise the striking homologies which exist between HD-proteins from different organisms, without focusing on any one of the possible consequences of thermodynamic DNA double-helix destabilisation (e.g., double-helix unwinding, acceleration of DNA renaturation, and single-strand extension; see ref. 5).

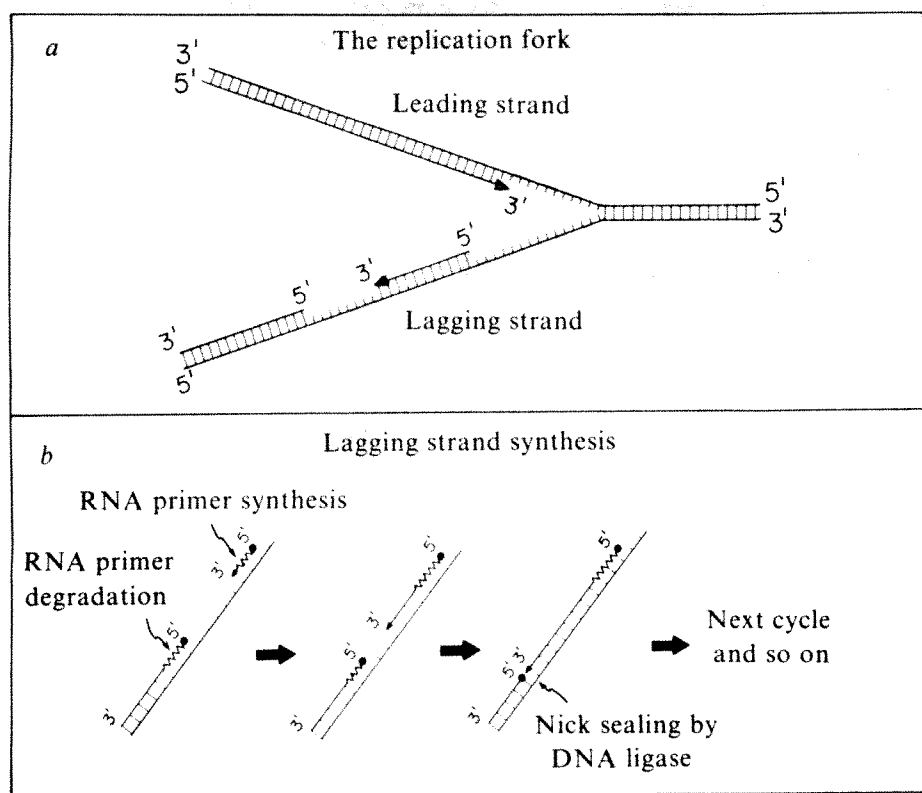


Fig. 1 *a*, Schematic representation of a replication fork, in which synthesis on the leading and lagging strand differs, but is carried out by the same type of DNA polymerase. *b*, Lagging strand synthesis in more detail: a cycle of synthesis and degradation of the RNA primer believed to start Okazaki fragments (for details, see ref. 38).

factor Y²⁶, *rep* protein²⁷, and presumably DNA gyrase²⁸ from *E. coli*; gene 44/62 protein and gene 41 protein from T4^{22,29}; and gene 4 protein from T7³⁰.

Fidelity constraints on the mechanism probably generate the observed asymmetrical replication fork

Most of those working on the biochemistry of DNA replication in the early 1960s tended to view replication as a relatively simple process. Cairns had shown by autoradiography that the two parental polynucleotide chains become separated from each other, and at about the same time become paired with newly made complementary chains, at a structure termed a "replication fork"³¹. The simplest mechanism to draw was one in which DNA polymerases continuously elongate the DNA chains on the two sides of this fork. Because of the antiparallel orientation of strands in the DNA double helix, only one of these DNA polymerases could proceed by chain elongation in the 5' to 3' chain direction (by attack of a 3'-OH of a polymer chain end on the activated 5' end of an incoming 5'-deoxyribonucleoside triphosphate monomer). A second type of DNA polymerase had to be postulated for which the growing chain end carried the 5' triphosphate activation, and the incoming 5'-triphosphate monomer was "backed in" to present its 3'-OH for the polymerisation reaction. Efforts to find such a second DNA polymerase were unsuccessful. Moreover, pulse-labelling with ³H-thymidine revealed 1,000–2,000 nucleotide-long "Okazaki fragments" of DNA at the growing fork³², apparently synthesised in the 5' to 3' chain direction only³³, and subsequently joined together by DNA ligase^{34–36}. These observations suggested that at least one of the two daughter DNA chains grows discontinuously at the replication fork, and in conjunction with detailed electron microscopic analysis of replicating molecules³⁷, led most workers in the field to switch to favour the type of model for replication schematically illustrated in Fig. 1*a*. Here, because of the asymmetry of synthesis on the leading and lagging sides of the fork, only a DNA polymerase which elongates chains in the 5' to 3' direction is required. However, fresh complications arise from the (at first sight perverse) inability of known DNA polymerases to start DNA chains *de novo*^{2,39,40}. Such new chain starts are

required at frequent intervals on the lagging side of the fork (Fig. 1*a*). Partly for this reason, a new primer-generating polymerase was sought, and eventually found in the *E. coli* replication system⁴¹. Its properties, combined with data from another system^{42,43}, made it seem likely that Okazaki fragments are initiated on short RNA primers which are elongated by DNA polymerase and then erased (and replaced by DNA) before DNA ligase-catalysed sealing of the lagging-strand DNA fragments. One such hypothetical cycle of primer synthesis and degradation is schematically illustrated in Fig. 1*b*.

The type of mechanism outlined in Fig. 1 seems at first sight much more complex and unwieldy than necessary. Is it possible that nature has been foolish and wasteful in engineering these crucial processes? In attempting to answer this question, it is important to recognise that DNA replication must be extremely accurate. Indeed, the observed templating fidelity is such that only one mistake is made per 10⁹ to 10¹⁰ base pairs replicated in *E. coli*⁴⁴.

The initial finding that DNA polymerases require a pre-existing primer chain to begin synthesis^{39,40}, began to be understood in the context of fidelity when it was found that for polymerisation, both T4 and *E. coli* DNA polymerases demand a Watson-Crick base-paired residue at the 3'-OH terminus of the primer to which nucleotides are being added. When confronted with a template-primer with a terminal mismatch, these polymerases use their built-in 3'→5' exonuclease activity to clip off unpaired primer residues by hydrolysis; this continues until enough nucleotides are removed to regenerate a base-paired terminus and create an active template-primer⁴⁵. As a result, these polymerases will efficiently remove their own polymerisation errors^{46–49}. As demonstrated by studies with mutant DNA polymerases^{48,49}, this self-correcting feature allows the enzyme to select for the proper template base-pairing of each added nucleoside triphosphate in a separate backward reaction, in addition to its strong selection for base-pairing of nucleoside triphosphates during the initial polymerisation. Theoretically, the intrinsic mistake frequency at this first step due to base mispairings can be reduced by the same factor in the proofreading step. It has been argued from chemical considerations⁵⁰ that the intrinsic mistake frequency is unlikely to be less than 10⁻⁵. In this case, at least one proof-

reading step would be absolutely required to approach a tolerable mutational load ($10^{-5} \times 10^{-5} = 10^{-10}$).

Accepting this view, it becomes clear why no DNA polymerase is able to start a new polynucleotide chain *de novo*, whereas DNA-dependent RNA polymerases can do so. We postulate that the need to be self-correcting gives DNA polymerase a stringent requirement for a perfectly base-paired primer terminus; to ask this enzyme to start synthesis in the complete absence of primer, without losing any of its discrimination between base-paired and unpaired primer 3'-OH termini, seems contradictory. In contrast, the RNA polymerases need not be self-correcting, inasmuch as relatively high error rates can be tolerated during transcription.

This line of reasoning likewise suggests a possible explanation for the failure to find a second DNA polymerase which adds deoxyribonucleoside 5' triphosphates in such a way as to cause chains to grow in the 3'→5' direction, despite the relatively simple type of replication fork mechanism that this would allow. With such a 3'→5' polymerase the growing 5' chain end (rather than the incoming mononucleotide) carries the activated triphosphate. Thus, mistakes in polymerisation cannot be hydrolysed away without a special enzymatic system for reactivating the bare 5' chain end thus created.

Finally, this view suggests why an erasable ribonucleotide-containing primer might be preferred for priming, rather than a non-erased DNA primer which might otherwise seem more economical. The argument that a self-correcting polymerase cannot start chains *de novo* also implies its converse: that an enzyme which starts chains *de novo* cannot do a good job of self correcting. Thus, any enzyme which primes discontinuous synthesis will of necessity make a relatively inaccurate copy (say, more than one error in 10^5). Even if the amount of this copy which is retained in the final product constitutes only 1% of the total genome (say, 10 primer nucleotides per 1,000 nucleotide Okazaki fragment), the resulting increase in overall mutation rate could be substantial. Thus, it seems reasonable to suggest that the use of ribonucleotides in synthesis of primer was of great evolutionary advantage, since it automatically marked these sequences as "bad copy" to be removed.

In conclusion, it seems likely that the type of mechanism for DNA replication sketched in Fig. 1 was specially selected to ensure that the entire DNA sequence is very accurately copied (that is, copied by a self-correcting type of DNA polymerase). Additional complexities in the mechanism, including the poorly understood requirements for protein cofactors which hydrolyse nucleoside triphosphates to nucleoside diphosphates (see above), may be similarly designed to help generate fidelity in as yet unexplained ways (see, for example, refs 51 and 52).

ATP energy can be used to aid DNA helix-unwinding at the replication fork

The early suspicion that cellular energy sources might be directly used to aid in the mechanics of DNA helix-unwinding^{53,54} has been dramatically confirmed by recent data. The first direct hint that this might be the case was the discovery of *E. coli* "DNA-unwinding enzyme I"^{55,56}. This enzyme, whose function *in vivo* is still unknown, can denature DNA only when it hydrolyses ATP, like the ATP-dependent nucleases discovered earlier^{57,58}. Later, a different *E. coli* DNA unwinding enzyme was identified, and found to correspond to the *rep* gene product²⁷. Like the above ATP-dependent enzymes, the *rep* protein can be assayed as a DNA-dependent ATPase, with ADP and P_i as products. In a recent elegant series of experiments, the *rep* protein has been shown to be necessary for DNA polymerase action on a double-helical template. Specifically, ϕ X174 double-stranded DNA circles have been used for efficient synthesis of single strands of viral DNA by the combined action of: a four-protein complex called *E. coli* "DNA polymerase III holoenzyme", *rep* protein, *Eco* HD-protein, and the ϕ X-coded *cis* A protein^{27,59}. In this reaction, the *cis* A protein nicks the (+) strand of the circular ϕ X DNA at a unique site, and attaches covalently to the 5' end of the

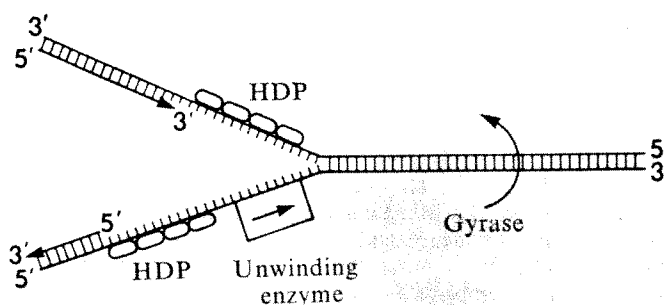


Fig. 2 Possible energetic contributions to helix unwinding. HDP stands for helix-destabilising protein (see footnotes). For a description of HD-protein, unwinding enzyme and DNA gyrase, see text. The arrows show the expected directionality of the motion generated by unwinding enzyme and by DNA gyrase, respectively, due to conformational changes occurring when bound ATP is hydrolysed by these proteins^{27,28}. The assignment of unwinding enzyme to the lagging strand side of the fork is rather arbitrary, and is based upon the fact that stoichiometric quantities of *E. coli* DNA-unwinding enzyme I seem to invade a DNA double helix by "walking" along a single strand from its 5' toward its 3' end⁶³.

DNA. The adjacent 3'-OH end created serves as a primer for the DNA polymerase, which in conjunction with HD-protein and *rep* protein synthesises the (+) strand in a modified "rolling-circle"⁶⁰ mode of replication. Approximately two molecules of ATP are hydrolysed per deoxyribonucleotide polymerised in this system.

The hydrolysis of one ATP molecule releases about 14 kcal of free energy at intracellular concentrations of ATP, ADP and P_i ⁶¹. The energy cost of melting a base pair on the template should range from about 1.2 kcal mol⁻¹ ($\frac{A}{T}$ next to $\frac{A}{T}$) to 5.0 kcal mol⁻¹ ($\frac{C}{G}$ next to $\frac{C}{G}$), depending on the particular base pair being melted and its nearest neighbour. (These numbers were derived for RNA helices at 25°, but values for DNA helices should be similar⁶².) Thus, coupled ATP hydrolysis can in principle make a significant contribution to parental DNA helix unwinding.

How an unwinding enzyme such as the *rep* protein may function in the replication of double-stranded DNA is schematically illustrated in Fig. 2. This scheme is designed to take into account the following facts: (1) only catalytic amounts of the *rep* protein are required for DNA synthesis²⁷; (2) the *rep* protein can hydrolyse ATP and cause DNA strand separation with *Eco* HD-protein even in the absence of DNA polymerase^{27,59}, and (3) unidirectional mechanical motion can result from coupling the hydrolysis of a bound nucleotide to allosteric changes in protein conformation (as in muscle)^{64,66}. From a recent report, the T7 gene 4 protein seems to perform a function similar to that of *rep* in the T7 DNA replication system³⁰.

Nevertheless, additional data make it clear that ATP-driven unwinding enzymes are only one of several kinds of components contributing to helix unwinding. First of all, while T7 gene 4 mutants are conditional-lethal and fail to replicate T7 DNA, all known *E. coli* *rep* mutants are viable (the rate of *E. coli* replication fork movement is slowed by only half when the *rep* gene is mutated; ref. 66). Second, an exciting new *E. coli* enzyme, DNA gyrase, has been discovered which uses ATP energy to pump negative twists into the DNA double helix²⁸, as schematically illustrated in Fig. 2. Such negative super-twisting will make the DNA helix easier to open by an estimated 1 kcal mol⁻¹ base pairs (M. Gellert, personal communication; see also ref. 67). DNA gyrase is inhibited by the drugs novobiocin⁶⁸ and nalidixic acid (M. Gellert, personal communication; N. Cozzarelli, personal communication). Since these drugs stop DNA replication immediately in *E. coli* (and many other prokaryotic systems), the contribution of DNA gyrase to helix unwinding in these system may be essential.

Finally, an important contribution to helix unwinding is

likely to be made by the helix-destabilising proteins. Apparently homologous HD-proteins have so far been found in a wide variety of organisms, including T4 and T7 bacteriophages^{5,69} *E. coli*³, *Ustilago maydis*⁷⁰, and calf⁴. As described above, *Eco* HD-protein is required for the catalytic *rep* protein-mediated DNA melting observed with ϕ X174 DNA in the presence of the ϕ X *cis A* protein^{27,59}, although its energetic contribution to melting is not yet known. Similarly, while the T4 DNA polymerase cannot displace strands on double-helical templates by itself, the addition of intracellular concentrations of T4 HD-protein to the complete T4 *in vitro* replication system allows this enzyme to proceed through double-stranded DNA at close to the rate *in vivo* of 800 nucleotides per second¹⁷. ATP hydrolysis by the T4 44/62 protein is required for this synthesis, but the stoichiometry of coupled hydrolysis is apparently less than 1 ATP hydrolysed per 5 deoxyribonucleotides polymerised (J. Barry and B. M. Alberts, unpublished results).

Since no DNA gyrase seems to be used in the T4 *in vitro* system (E. Belikoff and R. Sternglanz, unpublished results), a great deal of the 12→50 kcal mol⁻¹ required to melt one helical turn (10 base pairs) of DNA in this system may have to come from T4 HD-protein binding to the single-strands produced, as schematically illustrated in Fig. 2. Recent studies carried out on the T4 HD-protein⁶ would suggest that inside the cell, not more than 10 to 20 kcal mol⁻¹ helical turn melted should accompany its binding (assuming a concentration *in vivo* of about 10⁻⁵ M). However, stronger binding is seen when the C-terminal 8,000-dalton segment of T4 HD-protein is removed proteolytically, and the interesting suggestion has been made that the known interaction of T4 HD-protein with DNA polymerase⁷¹ and other T4 replication proteins²² similarly increases this energy of DNA binding at the replication fork⁷².

To summarise the situation as it seems to be now, a large part of the energy required for DNA helix unwinding ahead of the DNA replication fork can, in principle, be derived from the sum of the several forces illustrated in Fig. 2. No one of these proteins need be sufficient to accomplish the task alone. Surprisingly, different replication systems seem to use a different balance of the mechanisms illustrated, in some cases all three. Finally, the fact that DNA polymerase I of *E. coli* can use double-helical DNA templates in the absence of any of the proteins in Fig. 2 (ref. 2) means that considerable energy for helix unwinding can also come from the deoxyribonucleoside triphosphate hydrolysis which accompanies polymerisation. We can assume that the replicative DNA polymerases also derive energetic contributions to helix unwinding from this source.

Okazaki fragments need not all arise from *de novo* priming events

In this section we discuss the current status of work on the nascent DNA chains known as Okazaki fragments. Referring to the replication fork shown in Fig. 1a, it is clear that the lagging strand must be synthesised discontinuously with *de novo* priming events. Electron microscopic evidence demonstrates that such priming events occur at least every few kilobases³⁷. In principle, the leading strand can be synthesised continuously, with no *de novo* priming events (except possibly at the origin of replication). However, early work on *E. coli* and T4³² showed that after a short pulse of ³H-thymidine, all the labelled DNA is found as slowly sedimenting chains in alkaline sucrose gradients (10S, corresponding to 1,000–2,000 nucleotides). Thus, the data suggested that both strands are synthesised discontinuously. Experiments with DNAl igase^{73,74} and DNA polymerase I mutants⁷⁵ reinforced this idea, since such mutants accumulate 10S fragments even after relatively long labelling periods, with little or no label found in large DNA. Demonstrations that *E. coli*⁷⁶ and phage P2⁷⁷ accumulate more fragments on the lagging strand than on the leading strand have been reconciled with the above observations by noting that the fragments on the leading strand are joined more rapidly than those on the lagging strand^{78,79}.

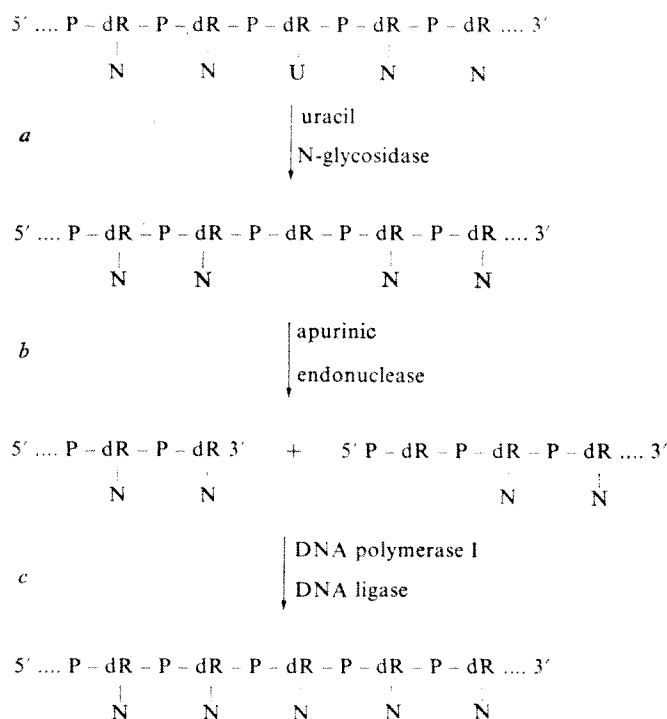


Fig. 3 A possible scheme for the excision-repair of uracil from DNA⁸². The phosphodiester backbone of the DNA is represented by ...P-dR-P-dR..., N represents any of the 4 normal bases (A, G, C, T) and U represents uracil. The repair process takes place on double-stranded DNA, but the complementary chain has been omitted for simplicity. *a*, Uracil *N*-glycosidase⁸³ removes uracil, leaving an intact phosphodiester backbone with a missing base. *b*, Apurinic endonuclease cleaves the DNA chain at the missing base⁸⁴. Recent data indicate that an alternative pathway, in which endonuclease V⁸⁵ recognises uracil and cleaves the DNA chain directly, is less active. *c*, DNA polymerase I and its associated 5'→3' exonuclease nick-translates⁸⁶, removing the damaged region and filling in the gap. Finally, DNA ligase joins the nick⁸⁴ to complete the process.

However, recent work reveals that not all DNA fragments labelled during a short pulse need arise from *de novo* priming events. In an *E. coli* mutant originally called *sof* (an acronym for small Okazaki fragments), pulse-labelling yields nascent DNA chains 5–10 times smaller than normal⁸⁰. It has now been demonstrated that *sof* mutants are identical in genotype and phenotype to *dut* mutants, deficient in dUTPase⁸¹. Such mutants have a higher than normal ratio of dUTP to TTP, and some dUTP is therefore incorporated into the DNA. The uracil is then rapidly excised by an excision-repair system which leaves temporary breaks in the DNA. Cells have a repair system to remove uracil residues from DNA, probably because of the frequent spontaneous deamination of cytosine in DNA to yield uracil, which is a potentially mutagenic event. A hypothetical scheme⁸² for the uracil excision-repair process is presented in Fig. 3.

In view of the above results with *dut* mutants, the question arises whether some Okazaki fragments in wild-type (*dut*⁺) strains are due to uracil incorporation and subsequent excision, rather than to true discontinuous synthesis. Even with a very active dUTPase there should be a finite level of dUTP available for polymerisation in the cell. In several *in vitro* DNA replication systems, low concentrations of dUTP strongly affect the size of the fragments synthesised (ref. 87; I. R. Lehman, personal communication). Conceivably then, all of the fragments on the leading strand (and some on the lagging strand) could be due to uracil misincorporation and subsequent excision-repair. If this were so, one might expect only 50% of a ³H-thymidine pulse-label to appear in Okazaki fragments in a uracil *N*-glycosidase mutant (see Fig. 3a). But pulse-labelling experiments have shown no difference between this mutant

(*ung*; ref. 88) and wild type (I. R. Lehman, personal communication; R. Sternglanz, unpublished results). Although results in the expected direction have been observed with the *ung* mutant in a cellophane disk *in vitro* system⁸⁷, the relevance of this result to the *in vivo* situation is unclear.

It should also be pointed out that other repair processes may similarly act on the nascent DNA to give rise to Okazaki fragments⁸⁹. For example, *E. coli* *dam* mutants (deficient in DNA adenine methylase) exhibit abnormally high recombination frequencies⁹⁰ (as do the *dut* mutants⁹⁰), presumably because of nicks and gaps in the DNA. Thus, the delayed methylation of nascent DNA could reduce its size. Because of such possibilities, the important question of what fraction of Okazaki fragments arise from *de novo* priming events remains unanswered, as does the question of whether the leading strand is synthesised continuously or discontinuously.

The priming mechanism for Okazaki fragments is complex

As discussed above, at least some of the Okazaki fragments on the lagging strand (and possibly on the leading strand) must be due to *de novo* priming events catalysed by an enzyme other than DNA polymerase. There are several important questions about such priming. Is the primer RNA and does it have a unique length and sequence? Are there unique start and stop signals for the primer on the template strand? Finally, what proteins are required for synthesis of the primer?

Definitive work on the priming of Okazaki fragments comes from a study of polyoma DNA fragments synthesised in an *in vitro* nuclear system. These fragments have been shown to have a 10-nucleotide-long stretch of RNA at their 5' ends, terminating in a 5'-triphosphate. The RNA is homogeneous in length but the sequence is not unique, not even at the RNA-DNA junction^{42,91}. Comparable results have been reported recently for human lymphocyte DNA fragments synthesised in an *in vitro* replication system⁹². However, it is still possible that an Okazaki fragment from a unique region (for example, isolated by hybridisation to a specific small polyoma DNA fragment) has a unique RNA primer sequence.

It has proved more difficult to demonstrate the presence of an RNA primer on *E. coli* Okazaki fragments. The most direct evidence comes from studies on fragments synthesised *in vitro* in toluenised cells; in that case a RNA-DNA junction has been chemically demonstrated⁹³. However, the evidence for RNA on *E. coli* DNA fragments synthesised *in vivo* is much more indirect. The initial experiments involving density shifts in Cs_2SO_4 equilibrium gradients⁹⁴ are now known to have been unreliable^{91,95}. The main evidence comes from experiments in which the nascent chains are treated with alkali, which hydrolyses the presumed RNA primer leaving a DNA chain with a 5'-OH terminus. The presence of the 5'-OH is then demonstrated by digestion of the DNA with spleen phosphodiesterase⁹⁶ or by phosphorylation of the DNA chain with polynucleotide kinase^{93,97}. However, it should be noted that the production of a 5'-OH terminus after alkali treatment of an Okazaki fragment does not prove the existence of RNA on the chain. For example, the presumed intermediate in the excision of uracil from DNA (Fig. 3b) might yield a 5'-OH terminus after alkali treatment, due to hydrolysis of the terminal deoxyribose from the chain via a 3'-4' cyclic phosphate intermediate^{98,99}. Direct demonstration of ribonucleotides on Okazaki fragments⁹⁷ is complicated by the difficulty in purifying the DNA free from traces of contaminating RNA chains. No one has been able to find on the 5' ends of *E. coli* Okazaki fragments the triphosphates that are found on fragments from mammalian cells^{91,92}.

As mentioned previously, *E. coli* DNA polymerase I mutants accumulate Okazaki fragments. They presumably do so because of a defect in a reaction termed nick-translation; in this reaction, DNA polymerase I can use its 5' to 3' exonuclease to remove the RNA primer from the 5' end of an Okazaki fragment, while simultaneously filling in the resulting gap with DNA⁸⁶. Those

mutants with the most severe defects in nick translation are actually conditional-lethal¹⁰⁰. Thus, one would predict that at least some of these mutants would accumulate the RNA primer. This prediction has been borne out in two laboratories using the aforementioned criterion of a 5'-OH terminus on the DNA chain after alkali treatment (ref. 97 and D. Denhardt, personal communication). However, two other laboratories have been unable to detect an RNA primer using the same or similar mutants (ref. 101; and B. Olivera, personal communication).

It is surprising that it has been so difficult to demonstrate directly the existence of RNA primers at the 5' end of Okazaki fragments synthesised *in vivo*. Some workers remain sceptical about the *in vivo* existence of RNA priming for this reason. On the other hand, it is not unreasonable to postulate that the primer is normally very rapidly removed *in vivo*, being preserved only in *in vitro* replication systems where the primer removal mechanism has been altered.

The enzymology of priming has been worked out in some detail for the *in vitro* replication of 3 different single-stranded circular bacteriophage DNA molecules; their conversion to the double-stranded form is carried out by the host *E. coli* replication proteins. Since these small DNA molecules do not have ends, DNA synthesis is absolutely dependent on the synthesis of a primer. Historically, RNA priming was first discovered in one of these systems¹⁰². Even though the priming reactions are carried out on specialised single-stranded DNA templates, they serve as possible models for the priming of Okazaki fragments at a replication fork.

The protein requirements for priming depend on the particular single-stranded bacteriophage DNA template used. For DNA from the filamentous bacteriophages, M13 and fd, the *E. coli* RNA polymerase responsible for most cellular RNA synthesis produces an RNA primer, initiating synthesis at a unique place on the single-stranded, circular DNA template^{102,103,104}. For bacteriophage G4 DNA, the primer is produced instead by the *E. coli* *dnaG* protein, an enzyme which, surprisingly, can polymerise either ribo- or deoxyribonucleoside triphosphates *in vitro* with nearly equal efficiency¹⁰⁵. This primer also begins at or near a unique site on the G4 DNA. It has recently been reported that the *dnaG* protein requires ADP in addition to the nucleoside triphosphates (ribo- or deoxy-) in order to form a primer on G4 DNA¹⁰⁶. For both M13 and G4 DNA, primer formation also requires enough *Eco* HD-protein I to completely cover the single-stranded DNA¹⁰³⁻¹⁰⁶.

Priming on bacteriophage ϕ X-174 DNA is much more complex. The *dnaG* protein synthesises the primer in this system as well, but at least 6 additional proteins are required: *Eco* HD-protein, *dnaB* and *dnaC* proteins, and additional DNA replication factors termed either X, Y and Z^{10,26} or i and n¹⁰⁷. These proteins, in an ATP-dependent reaction, are thought to form sites on ϕ X-174 DNA that can be recognised by the *dnaG* protein, which in turn synthesises the primer at these sites^{107,108}.

Since genetic evidence indicates that *dnaB*, *dnaC*, and *dnaG* proteins are all required for *E. coli* DNA replication, it seems likely that the mechanism of primer synthesis for *E. coli* Okazaki fragments is similar to that found for ϕ X-174 DNA. In fact, the *dnaG* protein has been indirectly implicated in the initiation of *E. coli* Okazaki fragments¹⁰⁹. Questions which remain to be answered for both ϕ X and *E. coli* primer formation include: (1) the exact role of the proteins whose action precedes the actual *dnaG* protein-mediated primer synthesis, and (2) the composition and sequence of the primer (RNA or mixed RNA-DNA copolymer).

The analogous RNA priming reactions detected in the bacteriophage T4 and T7 replication systems are as yet less well characterised; for discussions see refs 17, 20, 22, 29 and 30.

Prospects and conclusions

In spite of the reservations expressed above, the basic outlines

of DNA replication fork enzymology are now clear. DNA replication is a complex process, in which the asymmetric replication fork shown schematically in Fig. 1a moves along the parental DNA helix at rates of up to 1,000 nucleotides per second (in prokaryotes). In this process, multiple protein components, including DNA polymerases, helix-destabilising protein, DNA unwinding enzymes, DNA ligase, and RNA primer synthesising and erasing enzymes all have an important role. Several purified systems have been developed from prokaryotes which come close to reproducing this basic reaction *in vitro*.

DNA synthesis on the lagging side of the fork is clearly discontinuous, and the present evidence suggests that synthesis on the leading side of the fork may often be discontinuous too. One possibility is that lagging and leading strand syntheses are coupled, perhaps by a protein-induced folding of the DNA at the fork into an unique tertiary structure.

An unforeseen aspect of the replication process is the widespread use of ribonucleoside triphosphate hydrolysis energy. Presumably, each hydrolysis event drives an ordered allosteric change in protein conformation⁶⁴. Some of these changes generate force to aid in parental DNA helix unwinding^{27,28,56}, while others may exist only to facilitate protein-protein assembly reactions^{29,110} and/or to enhance replication fidelity^{51,52}.

In this connection, fidelity is a crucial aspect of the *in vivo* replication process which has not yet been adequately examined in any *in vitro* system. We have proposed above that the enormous replication fidelity which must be maintained *in vivo* (one error in 10⁹ to 10¹⁰ bases copied) has placed severe restraints on possible replication mechanisms. In fact, much of the apparent complexity of the process may arise for this reason. Yet, present *in vitro* systems are routinely assessed as "faithful" by criteria (infectivity or restriction mapping) which would probably detect errors only if they exceed one part in 10⁴. Thus, until a more sensitive assay for fidelity is used, these *in vitro* systems cannot be said to be truly "biological." They may even be missing central protein components which have little or no effect on synthesis rates, but which have major effects on fidelity.

In eukaryotes, replication forks must progress over bound nucleosomal histones, which may explain in part both the slower rate (100 nucleotides per s) and the smaller size of Okazaki fragments (a few hundred nucleotides) compared with prokaryotes¹¹¹. Replication over nucleosomes demands major conformational changes in the chromatin¹¹², which probably cannot be achieved *in vitro* without eukaryotic replication proteins. If only for this reason, the enzymology of a eukaryotic replication system needs to be worked out in detail, and a purified multi-component *in vitro* replication system developed, analogous to those discussed above from prokaryotes. Also to be learned from such a system is the source of the high fidelity in eukaryotic replication, especially if the eukaryotic DNA polymerases turn out to lack the 3' to 5' proofreading exonuclease of the prokaryotic enzymes^{111,113,114}.

We thank all those investigators who sent us preprints before publication. This work was supported by grant number GM 24020 from the National Institutes of Health, while R.S. was on sabbatical at the University of California.

- Watson, J. D. & Crick, F. H. C. *Nature* **171**, 964-967 (1953).
- Kornberg, T. & Kornberg, A. in *The Enzymes* (ed. Boyer, P. D.) 3rd edn, **10**, 119-144 (Academic, New York, 1974).
- Sigal, N., Delius, H., Kornberg, T., Gefter, M. L. & Alberts, B. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3537-3541 (1972).
- Herrick, G. & Alberts, B. M. *J. biol. Chem.* **251**, 2124-2132 (1976).
- Alberts, B. M. & Frey, L. *Nature* **227**, 1313-1318 (1970).
- Jensen, D. E., Kelly, R. C. & von Hippel, P. H. *J. biol. Chem.* **251**, 7215-7228 (1976).
- Molineux, I. J. & Gefter, M. L. *J. molec. Biol.* **98**, 811-825 (1975).
- Kelly, R. C. & von Hippel, P. H. *J. biol. Chem.* **251**, 7229-7239 (1976).
- Kelly, R. C., Jensen, D. E. & von Hippel, P. H. *J. biol. Chem.* **251**, 7240-7250 (1976).
- Wickner, S. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4120-4124 (1974).
- Schekman, R., Weiner, J. H., Weiner, A. & Kornberg, A. *J. biol. Chem.* **250**, 5859-5865 (1975).
- Mosig, G. & Breschkin, A. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1226-1230 (1975).
- Mosig, G. & Bock, S. J. *Virology* **17**, 756-761 (1976).
- Epstein, R. H. *et al. Cold Spring Harb. Symp. quant. Biol.* **28**, 375-392 (1963).
- Wechsler, J. A. & Gross, J. D. *Molec. gen. Genet.* **113**, 273-284 (1971).
- Barry, J. & Alberts, B. M. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2717-2721 (1972).
- Morris, C. F., Sinha, N. K. & Alberts, B. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4800-4804 (1975).
- Modrich, P. & Richardson, C. C. *J. biol. Chem.* **250**, 5508-5514 (1975).
- Hinkle, D. C. & Richardson, C. C. *J. biol. Chem.* **250**, 5523-5529 (1975).
- Scherzinger, E., Lanka, E., Morelli, G., Seiffert, D. & Yuki, A. *Eur. J. Biochem.* **72**, 543-558 (1977).
- Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **69**, 965-969 (1972).
- Alberts, B. *et al.* in *Nucleic Acid-Protein Recognition* (ed. Vogel, H. J.) 31-63 (Academic, New York, 1977).
- Studier, F. W. *Science* **176**, 367-376 (1972).
- Wickner, S., Wright, M. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 783-787 (1974).
- Wickner, W. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3679-3683 (1973).
- Wickner, S. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3342-3346 (1975).
- Scott, J. F., Eisenberg, S., Bertsch, L. L. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 193-197 (1977).
- Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3872-3876 (1976).
- Alberts, B. M., Morris, C. F., Mace, D., Sinha, N., Bittner, M. & Moran, L. in *DNA Synthesis and Its Regulation, ICS-UCLA Symposium on Molecular and Cellular Biology* (eds Goulian, M., Hanawalt, P. & Fox, C. F.) **3**, 241-269 (W. H. Benjamin, Menlo Park, California, 1975).
- Kolodner, R. & Richardson, C. C. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1525-1529 (1977).
- Cairns, J. *J. molec. Biol.* **6**, 208-213 (1963).
- Okazaki, R. *et al. Cold Spring Harb. Symp. quant. Biol.* **33**, 129-143 (1968).
- Okazaki, T. & Okazaki, R. *Proc. natn. Acad. Sci. U.S.A.* **64**, 1242-1248 (1969).
- Gellert, M. *Proc. natn. Acad. Sci. U.S.A.* **57**, 148-155 (1967).
- Newman, J. & Hanawalt, P. *Cold Spring Harb. Symp. quant. Biol.* **33**, 145-150 (1968).
- Richardson, C. C., Masamune, Y., Live, T. R., Jacquemin-Sablon, A., Weiss, B. & Fareed, G. *Cold Spring Harb. Symp. quant. Biol.* **33**, 151-164 (1968).
- Inman, R. B. & Schnos, M. J. *J. molec. Biol.* **56**, 319-325 (1971).
- Kornberg, A. *DNA Synthesis* (W. H. Freeman, San Francisco, 1974).
- Bollum, F. J. *Science* **144**, 560 (1964).
- Bollum, F. J. in *Genetic Elements* (ed. Shugar, D.) 3-15 (Academic, New York, 1967).
- Bouché, J. P., Zechel, K. & Kornberg, A. *J. biol. Chem.* **250**, 5995-6001 (1975).
- Pigiet, V., Eliasson, R. & Reichard, P. *J. molec. Biol.* **84**, 197-216 (1974).
- Hunter, T. & Francke, B. *J. molec. Biol.* **83**, 123-130 (1974).
- Drake, J. W. *Nature* **221**, 1132 (1969).
- Brutlag, D. & Kornberg, A. *J. biol. Chem.* **247**, 241-248 (1972).
- England, P. T. *J. biol. Chem.* **246**, 5684-5687 (1971).
- Nossal, N. G. & Herschfield, M. S. *J. biol. Chem.* **246**, 5414-5426 (1971).
- Muzyczka, N., Poland, R. L. & Bessman, M. J. *J. biol. Chem.* **247**, 7116-7122 (1972).
- Lo, K.-Y. & Bessman, M. J. *J. biol. Chem.* **251**, 2475-2479 (1976).
- Topal, M. D. & Fresco, J. R. *Nature* **263**, 285-289 (1976).
- Hopfield, J. J. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4135-4139 (1974).
- Ninio, J. *Biochimie* **57**, 587-595 (1975).
- Watson, J. D. & Crick, F. H. C. *Cold Spring Harb. Symp. quant. Biol.* **18**, 123-131 (1953).
- Cairns, J. & Denhardt, D. T. *J. molec. Biol.* **36**, 335-342 (1968).
- Abdel-Monem, M. & Hoffmann-Berling, H. *Eur. J. Biochem.* **65**, 431-440 (1976).
- Abdel-Monem, M., Durwald, H. & Hoffmann-Berling, H. *Eur. J. Biochem.* **65**, 441-449 (1976).
- MacKay, V. & Linn, S. *J. biol. Chem.* **249**, 4286-4294 (1974).
- Wilcox, K. W. & Smith, H. O. *J. biol. Chem.* **251**, 6127-6134 (1976).
- Eisenberg, S., Griffith, J. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3198-3202 (1977).
- Gilbert, W. & Dressler, D. *Cold Spring Harb. Symp. quant. Biol.* **33**, 473-484 (1968).
- Simmons, R. M. & Hill, T. L. *Nature* **263**, 615-618 (1976).
- Tinoco, I. Jr *et al. Nature new Biol.* **246**, 40-41 (1973).
- Abdel-Monem, M., Lauppe, H.-F., Kartenbeck, J., Durwald, H. & Hoffmann-Berling, H. *J. molec. Biol.* **110**, 667-685 (1977).
- Hill, T. L. *Proc. natn. Acad. Sci. U.S.A.* **64**, 267-274 (1969).
- Boyer, P. D. in *Dynamics of Energy-Transducing Membranes* (eds Ernster, L., Estabrook, R. W. & Slater, E. C.), 289-301 (Elsevier, Amsterdam, 1974).
- Lane, H. E. D. & Denhardt, D. T. *J. molec. Biol.* **97**, 99-112 (1975).
- Wang, J. C. & Hsieh, T. *Biochemistry* **14**, 527-535 (1975).
- Gellert, M., O'Dea, M. H., Itoh, T. & Tomizawa, J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4474-4478 (1976).
- Reichen, R. C. & Gefter, M. L. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1846-1850 (1973).
- Banks, G. R. & Spanos, A. *J. molec. Biol.* **93**, 63-77 (1975).
- Huberman, J. A., Kornberg, A. & Alberts, B. M. *J. molec. Biol.* **62**, 39-52 (1971).
- Moise, H. & Hosoda, J. *Nature* **259**, 455-458 (1976).
- Konrad, E. B., Modrich, P. & Lehman, I. R. *J. molec. Biol.* **77**, 519-529 (1973).
- Gottesman, M. M., Hicks, M. L. & Gellert, M. *J. molec. Biol.* **77**, 531-547 (1973).
- Kuempel, P. L. & Veomett, G. E. *Biochem. biophys. Res. Commun.* **41**, 973-980 (1970).
- Louarn, J.-H. & Bird, R. E. *Proc. natn. Acad. Sci. U.S.A.* **71**, 329-333 (1974).
- Kainuma-Kuroda, R. & Okazaki, R. *J. molec. Biol.* **94**, 213-228 (1975).
- Kurosawa, Y. & Okazaki, R. *J. molec. Biol.* **94**, 229-241 (1975).
- Sternglanz, R., Wang, H. F. & Donegan, J. J. *Biochemistry* **15**, 1838-1843 (1976).
- Konrad, E. B. & Lehman, I. R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2150-2154 (1975).
- Tye, B., Nyman, P., Lehman, I. R., Hochhauser, S. & Weiss, B. *Proc. natn. Acad. Sci. U.S.A.* **74**, 154-157 (1977).
- Lindahl, T. *Nature* **259**, 64-66 (1976).
- Lindahl, T. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3649-3653 (1974).
- Verly, W. G. & Rassart, E. *J. biol. Chem.* **250**, 8214-8219 (1975).
- Gates, F. T. III & Linn, S. *J. biol. Chem.* **252**, 1647-1653 (1977).
- Kelly, R. B., Cozzarelli, N. R., Deutscher, M. P., Lehman, I. R. & Kornberg, A. *J. biol. Chem.* **245**, 39-45 (1970).
- Olivera, B. M., Tye, B. & Lehman, I. R. *Fedn Proc.* **36**, 654 (1977).
- Duncan, B. K., Rockstroh, P. A. & Warner, H. R. *Fedn Proc.* **35**, 1493 (1976).
- Werner, R. *Nature* **230**, 570-572 (1971).
- Konrad, E. B. *J. Bacteriol.* **130**, 167-172 (1977).
- Reichard, P., Eliasson, R. & Soderman, G. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4901-4905 (1974).
- Tseng, B. Y. & Goulian, M. *J. molec. Biol.* **99**, 339-346 (1975), and *Fedn Proc.* **36**, 654 (1977).
- Okazaki, R. *et al.* in *DNA Synthesis and Its Regulation* (eds Goulian, M., Hanawalt, P. & Fox, C. F.) 832-862 (W. A. Benjamin, Menlo Park, 1975).
- Sugino, A., Hirose, S. & Okazaki, R. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1863-1867 (1972).
- Probst, H., Gentner, P. R., Hofstatter, T. & Jenke, S. *Biochim. biophys. Acta* **340**, 361-373 (1974).

96. Kurosawa, Y., Ogawa, T., Hirose, S., Okazaki, T. & Okazaki, R. *J. molec. Biol.* **96**, 653-664 (1975).
97. Ogawa, T., Hirose, S., Okazaki, T. & Okazaki, R. *J. molec. Biol.* **112**, 121-140 (1977).
98. Tamm, C., Shapiro, H. S., Lipshitz, R. & Chargaff, E. *J. biol. Chem.* **203**, 673-688 (1953).
99. Seidel, H. *Biochim. biophys. Acta* **138**, 98-106 (1967).
100. Lehman, I. R. & Uyemura, D. G. *Science* **193**, 963-969 (1976).
101. Uyemura, D., Eichler, D. C. & Lehman, I. R. *J. biol. Chem.* **251**, 4085-4089 (1976).
102. Brutlag, D., Schekman, R. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2826-2829 (1971).
103. Geider, K. & Kornberg, A. *J. biol. Chem.* **249**, 3999-4005 (1974).
104. Wickner, R. B., Wright, M., Wickner, S. & Hurwitz, J. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3233-3237 (1972).
105. McMacken, R., Bouché, J.-P., Rowen, S. L., Weiner, J. H., Ueda, K., Thelander, L., McHenry, C. & Kornberg, A. in *Nucleic Acid-Protein Recognition* (ed. Vogel H.) 15-29 (Academic, New York, 1977).
106. Wickner, S., *Proc. natn. Acad. Sci. U.S.A.* **74**, 2815-2819 (1977).
107. Weiner, J. H., McMacken, R. & Kornberg, A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 752-756 (1976).
108. Wickner, S. & Hurwitz, J. in *DNA Synthesis and Its Regulation* (ed. Goulian, M., Hanawalt, P. and Fox C. F.) 227-238 (W. A. Benjamin, Menlo Park, 1975).
109. Lark, K. G. *Nature new Biol.* **240**, 237-240 (1972).
110. Wickner, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3511-3515 (1976).
111. Edenberg, H. J. & Huberman, J. A. *Annual Rev. Gen.* **9**, 245-284 (1975).
112. Weintraub, H., Worcel, A. & Alberts, B. M. *Cell* **9**, 409-417 (1976).
113. Chang, L. M. S. & Bollum, F. J. *J. biol. Chem.* **248**, 3398-3404 (1973).
114. Sedwick, W. D., Wang, T. S. & Korn, D. *J. biol. Chem.* **250**, 7045-7056 (1975).

articles

'Unmixing' of the deep-sea record and the deglacial meltwater spike

W. H. Berger, R. F. Johnson & J. S. Killingley

Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093

The Berger-Heath model of vertical mixing in deep-sea sediments provides a simple means of simulating an original input signal by 'unmixing' a signal found in deep-sea cores. When applied to an oxygen isotope record from the western equatorial Pacific, the possibility emerges that the entire ocean was covered by a low salinity water layer, during deglaciation.

DELETERIOUS effects of burrowing organisms on the resolution of the deep-sea record have been recognised for some time, and several mathematical models have been proposed to deal with signal distortions introduced by benthic mixing¹⁻⁵. The simplest one of these models² postulates a homogeneous layer at the sediment-water interface within which incoming particles are completely mixed with older sediment. In reality, of course, the intensity of mixing decreases with depth. In fact, mixing processes show a tiered structure, with intense mixing in an upper layer which is virtually homogeneous, and 'lumpy' mixing in a layer below. Sporadic displacement of sediment by large deeply burrowing organisms occurs to considerable depth. The upper two tiers of the entire mixed layer are of the order of 5 cm thick each, in the deep-sea carbonates studied, and the deep burrowing involves a layer at least 40 cm thick^{6,7}.

In spite of this complicated structure of the mixed layer, simple mathematical models can yield excellent results in simulating actual mixing processes. In particular, it can be shown that the distribution of particles within many ash layers found in deep-sea cores can be reconciled with a history of sudden emplacement of a thin homogeneous layer and subsequent simple exponential mixing⁸⁻¹⁰.

It seems worthwhile, therefore, to try to run the mixing model backwards, in order to reconstruct a stratigraphic signal as it might have appeared had there been no benthic mixing to distort it. Here we derive a simple unmixing equation and apply it to an oxygen isotope record of deep-sea carbonates from the western equatorial Pacific, spanning the past 20,000 yr.

Unmixing equation

The pertinent differential equation is derived as follows: c_0 is the concentration before mixing of some stable tracer, within the accumulating sediment; the concentration in the mixed layer is c . For discrete particles with any kind of label (such as forams and

their isotopic composition) $c = nr$, where n is the particle concentration and r is the average tracer value.

When a thin layer of sediment, dl , accumulates, it brings with it $c_0 dl$ units of tracer per unit area. At the same time, the mixed layer moves up a distance dl and so loses cdl units of tracer. The concentration of the tracer in the mixed layer then changes by an amount,

$$dc = (c_0 dl - cd)/m$$

dividing by dl , we get $c' = (c_0 - c)/m$; which means that the instantaneous gradient incorporated into the historical layer reflects the difference in the incoming signal (at the top of m) and the outgoing one (at its bottom), averaged over m .

Hence, to recover the original concentration c_0 from the record, we write

$$c_0 = c + c'm \quad (1)$$

This is the basic unmixing equation. In this model, the signal begins at the base of the mixed layer. When calculating sedimentation rates from a dated c_0 excursion, this shift has to be taken into account, that is, the distance to the sediment surface has to be reduced by m , before dividing by the age.

If sizeable changes occur in the proportion of signal-carrying particles, that is, in the proportion of the foraminifera whose isotopic composition is being studied, equation (1) has to be expanded. In the coarse sand fractions non-carbonate can be neglected, hence n , the particle concentration, is $s \times p$, where s is the grain size fraction and p the proportion of the species within that fraction.

The unmixing equation is applied first to n (that is, $[s \times p]$)

$$n_0 = n + n'm$$

and subsequently to $n \times r$

$$n_0 \times r_0 = n \times r + [n \times r]' \times m$$

and the solution sought is

$$\begin{aligned} r_0 &= n_0 \times r_0 / n_0 \\ &= (n \times r + [n \times r]' \times m) / (n + n'm) \end{aligned}$$

Table 1 Locations of box cores, depths from which they were raised, average carbonate content, and depth of the preservation spike at 14,000 yr b.p.¹²

Core	Latitude	Longitude	Depth (m)	CaCO ₃ (%)	Maximum preservation (cm)
ERDC 92 Bx	2° 13.5'S	156° 59.9'E	1598	83.6	26.5
ERDC 88 Bx	0° 02.9'S	155° 52.1'E	1924	88.2	21.5
ERDC 112 Bx	1° 37.5'S	159° 14.1'E	2169	84.3	23.5
ERDC 120 Bx	0° 01.0'S	158° 41.6'E	2247	85.0	26.0
ERDC 102 Bx	3° 36.3'S	161° 19.1'E	2306	87.1	17.5
ERDC 83 Bx	1° 24.1'N	157° 18.6'E	2342	83.0	23.5
ERDC 79 Bx	2° 47.1'N	156° 13.8'E	2767	84.4	19.5
ERDC 123 Bx	0° 01.3'S	160° 24.9'E	2948	83.1	26.5
ERDC 125 Bx	0° 00.2'S	160° 59.9'E	3368	82.8	21.5

Results from the first four and from the last four cores were pooled to derive the averaged signal in Fig. 1. Results from Core ERDC Bx 102 were rejected. Water depth is based on echo sounding, corrected for (assumed) regional differences in sound velocity.

where v_0 is the tracer signal, in our case oxygen isotope composition in ‰ PDB.

To test the efficacy of equation (1) (or its expansion) we can apply it to the ash data^{9,10}, where it will be seen that the saw-tooth-like frequency distributions of ash particles are readily converted to rectangular signal spikes, as expected. But, any noise in the record, that is, irregular excursions due to discrete burrowing, is immediately transformed into large erratic excursions. To avoid this it is necessary to collect several records of the same time interval, and to add them up in order to obtain a smooth average 'best' record.

Oxygen isotope record

Because of the considerable interest attaching to the climate and ocean dynamics of the past 20,000 yr, we have endeavoured to produce such an averaged oxygen isotope record for this period. Our cores are box cores from the western equatorial Pacific (see Table 1). The nine cores shown are well above the lysocline and are virtually free from dissolution effects, which distort the isotope record¹¹. The cores were sampled at closely spaced intervals, between 3 and 4 cm on the average. The isotopic composition of the planktonic foraminifer *Pulleniatina obliquiloculata* was determined by standard procedure, after establishing that the

isotopic signal of this species is similar to that of *Globigerinoides sacculifer*, in well-preserved sediment, except for an offset of 0.8‰^{11} . This offset reflects the fact that *P. obliquiloculata* grows its shell at somewhat greater depths than *G. sacculifer*, on the average.

Results of the determinations show a striking similarity of the oxygen isotope sequences in eight of the nine cores (see Fig. 1). Box core 102 does not follow the general pattern and is not considered further. Since all cores are from the same area, any idiosyncracies must be due to local disturbance.

We next average the eight acceptable sequences into one. Sedimentation rates are likely to differ somewhat, and isochrons must first be found, for proper addition. The exact dates of such isochrons are immaterial for this exercise.

We choose two such isochrons, besides the surface, for correlation. The first (13,000 yr b.p. according to ¹⁴C dating of Core 92 (ref. 11), is a point halfway between the late (isotopically light) segment of the high gradient transition from glacial to postglacial, and the segment of maximum preservation of the fine sand fraction (the preservation spike near 14,000 yr b.p.¹²). The second isochron (about 18,000 yr b.p.¹¹) is the point where the $\delta^{18}\text{O}$ values are presumed to be heaviest. The distance between the two isochrons in Core 92, which is ¹⁴C-dated¹¹ and in Core 112, where a very heavy isotopic value was obtained near 35 cm, was taken as a guide for determining where approximately this 18,000 yr b.p. level¹³ should be in the cores, in addition to the actual isotope determinations in each core.

The isochrons thus determined are shown in Table 2. A straightforward addition by core depth alone would yield a similar result: each core (except Bx 102) shows a small $\delta^{18}\text{O}$ minimum at the end of the steep transition segment, at almost identical depth, that is, near 20 cm. Our correlation procedure, therefore, besides being standard practice in deep-sea micropalaeontology, produces an acceptable match of the cores.

On the basis of this agreement as to depths in core (and not just sequence), we next construct two average isotope signals as a function of average depth with the $\delta^{18}\text{O}$ minimum centred on 20 cm (Fig. 1, two heavy lines). The first average signal is based on cores 92, 88, 112, and 120. The second is based on cores 83, 79, 123, and 125. Thus, the two average curves are derived completely independently. Their near-congruency is remarkable. We take this close agreement as further support for our correlation procedure. The median line between the two generalised signals (Fig. 1, fine line between two heavy lines) is our best estimate of a true oxygen isotope signal as would be recorded in an ideal core.

We consider this averaged signal to be more detailed and more reliable than any oxygen isotope record now available from deep-sea carbonates for this period. We realise also that this signal will have to experience some modifications as more data become available.

The result of applying our unmixing equation to this record is shown in Fig. 1. The reconstructed signal is represented by the shaded zone. The borders of the shaded zone show the possible amplitudes of the presumed original signal, for a mixed layer parameter m between 4 and 8 cm. This range for m has been established as reasonable for deep-sea sediment in general⁸ and also is indicated by unpublished results of closely spaced ¹⁴C

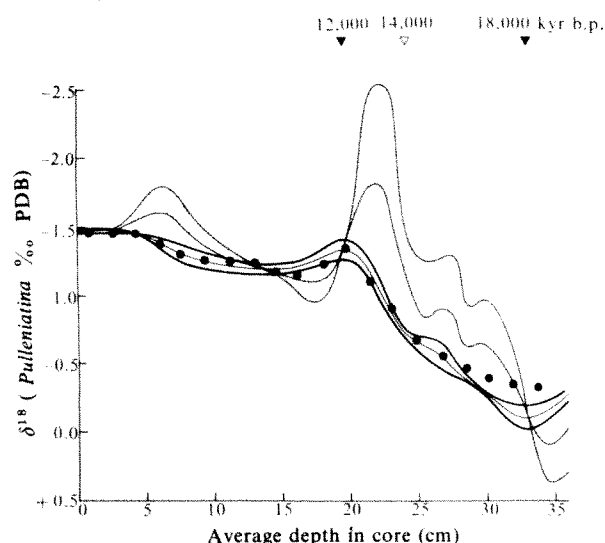


Fig. 1 Stacked and reconstructed oxygen isotope signal from eight box cores taken above the lysocline, on Ontong-Java Plateau. Heavy lines: results of two separate stackings, of four records each. Centre line between two heavy lines: overall average signal. Shaded area: approximate estimated shape of original signal, using unmixing equation $c_0 = c + c'm$. Outer bound (large amplitudes): $m = 8$ cm. Inner bound (small amplitudes): $m = 4$ cm. The age scale refers to the actual signal, not the reconstructed one. Fix points are 12,000 yr b.p. for the meltwater anomaly, 14,000 yr b.p. for maximum preservation, 18,000 yr b.p. for the glacial maximum, based on ¹⁴C dating of core ERDC 92^{11,12}. The ● represent an average isotope signal resulting from correlation by the preservation spike only (Table 1, last column) demonstrating the absence of bias in the combined isotope-preservation correlation procedure (see text).

Box core	Depth in core (cm)											
	0	5	10	15	20	25	30	35	40			
92	1.53	1.49	1.46	1.38	1.13	0.85	1.08	0.49	0.26	0.05		
88	1.68	1.61	1.53	1.44	1.50	1.69	0.88	0.91	0.78	0.69		
112	1.47	1.29	1.07	0.78	0.94	1.00	0.69	0.36	0.12	+0.19		
120	1.29	1.50	0.83	1.34	0.96	1.43	1.18	0.48	0.08	0.25		
102	1.39	1.45	0.95	1.00	+0.10?	0.50	0.54	.	0.58			
83	1.45	1.40	1.56	1.26	1.19	1.46	0.83	0.67	0.36	0.24	0.33	
79	1.47	1.38	1.32	1.46	1.17	1.25	1.29	0.56	0.40	0.34	0.43	
123	1.33	1.17	1.38	1.25	1.36	1.10	1.22	0.23	0.16	0.85?		
125	1.53	1.81	1.47	1.18	1.25	1.34	1.50	0.70	0.43	0.16	0.21	
	13 kyr b.p.										18 kyr b.p.	

Fig. 2 Oxygen isotope composition of *Pulleniatina obliquiloculata* in nine box cores from Ontong Java Plateau above the lysocline (~ 3,500 m). Decimal points indicate position of sampling midpoint. All values in $\delta^{18}\text{O}_{\text{‰}}$ PDB (negative, unless marked +). Stippled lines indicate estimated positions of two isochrons (~ 13,000 yr b.p. between (estimated) maximum anomaly of product [$e \times c$] and centre of maximum preservation; and ~ 18,000 yr b.p. at maximum value of $\delta^{18}\text{O}$).

dating of Core 92, by T. H. Peng. The larger m produces the larger amplitude, as seen from equation (1).

The resulting reconstructed signal, that is, the shaded field in Fig. 1, has some interesting features, the outstanding one being the pronounced $\delta^{18}\text{O}$ minimum (near 12,000 yr b.p.). One might argue that the amplitude of this minimum is partly due to our method of correlation, in which the isotope signal itself had one-half weight for determination of the 13,000 yr b.p. isochron. This objection, raised by N. J. Shackleton, in private discussion, is entirely justified. We have, therefore, re-done the correlation using only the centre of the preservation spike as a guide. The resulting average isotope signal is shown in Fig. 1, as a string of heavy dots. It is obvious that the outcome of an unmixing exercise on this signal would be quite similar to the reconstructed signal given.

In showing a pronounced $\delta^{18}\text{O}$ minimum near 12,000 yr b.p. and also in other details, our reconstruction resembles closely the raw oxygen isotope records found by Kennett and Shackleton¹⁴ and by Emiliani *et al.*¹⁵ in the Gulf of Mexico. Their signals are derived from cores with high sedimentation rates which are much less susceptible to distortion by benthic mixing than ours. Emiliani *et al.* date the prominent $\delta^{18}\text{O}$ minimum near 12,000 yr b.p., a date that agrees with ours and with the estimate by Kennett and Shackleton. Hence, we are reasonably confident that we are seeing a worldwide phenomenon, of which the Gulf of Mexico anomaly is a special regional manifestation. (Note that the age scale in Fig. 1

refers to the actual signal, not the reconstruction, so that the 12,000-yr date shown coincides with the original $\delta^{18}\text{O}$ minimum.)

A worldwide phenomenon

What is this phenomenon? The Gulf of Mexico results have been interpreted as showing an influx of meltwater from the Laurentide Ice Sheets^{14,15}. This influx would strongly decrease the $\delta^{18}\text{O}$ values of the upper water layer, and hence of the shells of the foraminifera living within it.

We propose that a similar effect may exist for the entire world ocean. Our interpretation supports the hypothesis of Worthington¹⁶, who postulated that the rapid influx of meltwater to the world ocean must have resulted in a low salinity upper water layer, forming a lid on the ocean. Below this lid, which prevented vertical mixing, CO_2 would have accumulated and this CO_2 enrichment would have led to increased dissolution of the shells of foraminifera on the sea floor¹⁶.

The increase of dissolution postulated by Worthington has been found, and occurs exactly at the point predicted¹². On renewed mixing, any deep-trapped CO_2 should have been released to the atmosphere. A rapid warming might be expected at this point. There is evidence that the warming at the beginning of the Holocene, which led to the Hypsithermal was very rapid^{17,18}. In our scenario, this warming event is the one recent geological analogue of the present large-scale release of industrial CO_2 to the atmosphere. Its course and its effects on the biosphere should hold great interest for future investigation.

This research was supported by the US NSF (grant OCE 75-04335) and by the Office of Naval Research (contract USN N00014-75-C-0152). The Stable Isotope Sediment Laboratory was set up with US NSF support (DES 75-04497, to W. H. Berger and M. Kastner) and with support from the Scripps Industrial Associates and from Chevron Oil, California. We thank E. Vincent for supervising sample preparation and for discussions. The box coring device was provided by R. R. Hessler, and G. Wilson assisted in taking the cores.

Received 23 May; accepted 25 July 1977.

1. Goldberg, E. D. & Koide, M. *Geochim. cosmochim. Acta* **26**, 417-450 (1962).
2. Berger, W. H. & Heath, G. R. *J. mar. Res.* **26**, 134-143 (1968).
3. Guinasso, N. L. & Schink, D. R. *J. geophys. Res.* **80**, 3032-3043 (1975).
4. Guinasso, N. L. *EOS* **57**, 150-151 (1976).
5. Goreau, T. J. *Nature* **265**, 525-526 (1977).
6. Berger, W. H. in *Benthic Processes and Geochemistry of Interstitial Waters of Marine Sediments* (eds Fanning, K. A. & Manheim, F. T.) (Joint Oceanographic Congress, Edinburgh, 1977).
7. Ekdale, A. A. & Berger, W. H. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* (in the press).
8. Berger, W. H. in *Treatise on Chemical Oceanography* (eds Riley, J. P. & Chester, R.) **5**, 265-388 (Academic, London, 1976).
9. Glass, B. P. *Earth planet. Sci. Lett.* **6**, 409-415 (1969).
10. Ruddiman, W. F. & Glover, L. K. *Bull. geol. Soc. Am.* **83**, 2817-2836 (1972).
11. Berger, W. H. & Killingley, J. S. *Science* **197**, 563-566 (1977).
12. Berger, W. H. *Nature* **269**, 301-304 (1977).
13. CLIMAP *Science* **191**, 1131-1137 (1976).
14. Kennett, J. P. & Shackleton, N. J. *Science* **188**, 147-150 (1975).
15. Emiliani, C. *et al. Science* **189**, 1083-1088 (1975).
16. Worthington, L. V. *Meteor. Monogr.* **8**, 63-67 (1968).
17. Morner, N.-A. *Geology* **3**, 109-110 (1975).
18. Webb, T. & Bryson, R. A. *Quat. Rev.* **2**, 70-115 (1975) (see Fig. 26).

Plate boundary within Tjörnes Fracture Zone on northern Iceland's insular margin

Robert L. McMaster & Jean-Guy E. Schilling

Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island, 02881

Paul R. Pinet

Department of Geology, University of Georgia, Athens, Georgia, 30602

The current North American-Eurasian plate boundary on Iceland's northern insular margin is defined. Overlapping rift zones and en echelon post-glacial volcanic eruptions oblique to the apparent transform direction of the Tjörnes Fracture Zone suggest that the fault is in a transient deformational stage.

ICELAND represents an anomalous segment of the Mid-Atlantic Ridge that remains unexplained by the simple elements of plate-tectonic theory. The abnormal build-up of the Iceland platform also coincides with the pronounced change of the ridge direction. Although the history of rifting over Iceland is complex, the zones of neovolcanic and tectonic activity now appear fairly well defined^{1,2}.

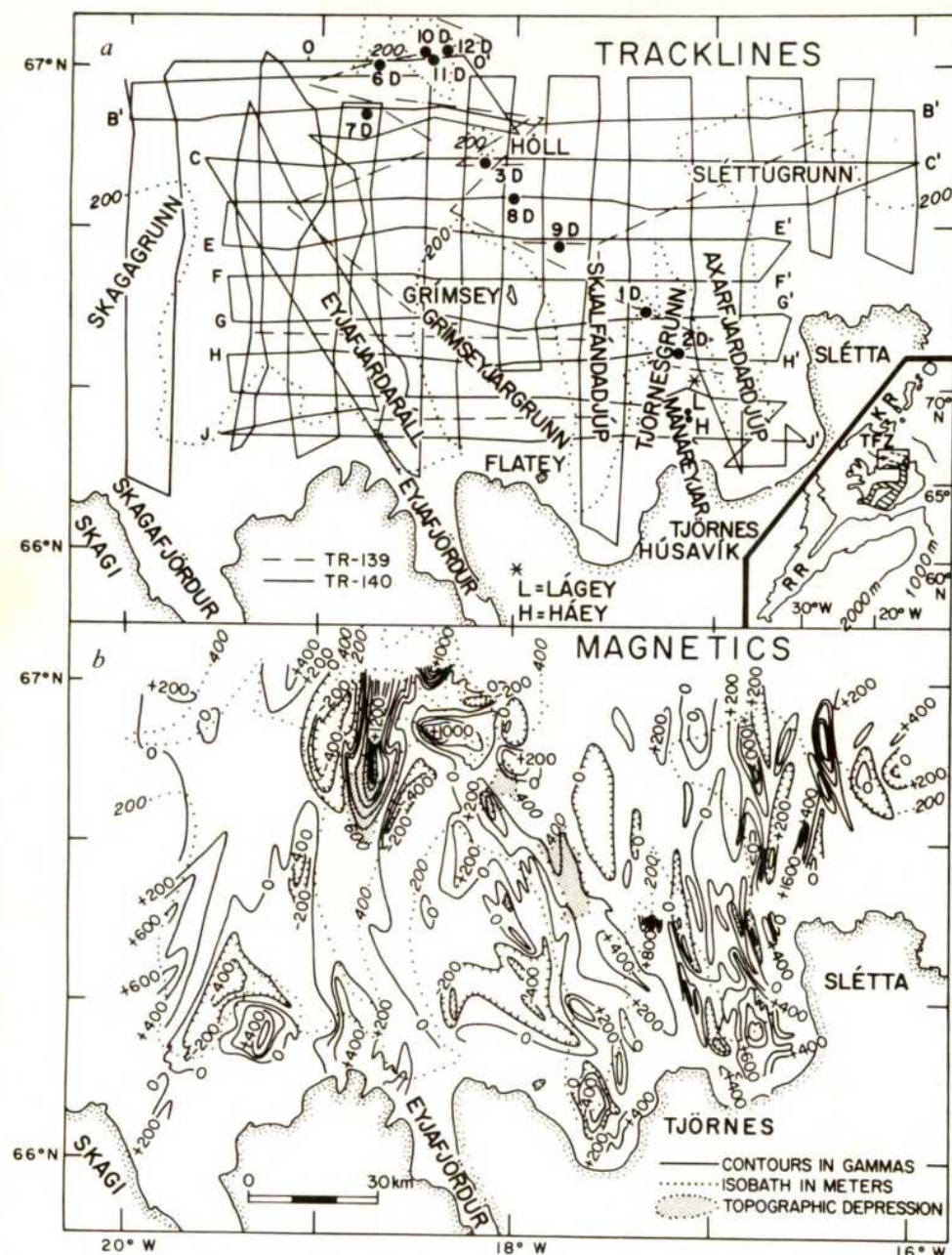


Fig. 1 Insular margin, northern Iceland; a, locations of R/V Trident's TR-140 tracklines and TR-139 tracklines and dredge stations; b, magnetic anomalies. On index map KR, Kolbeinsey Ridge; RR, Reykjanes Ridge; TFZ, Tjörnes Fracture Zone.

This report focuses on the offset zone between the Kolbeinsey Ridge^{3,4,5} and the north-east neovolcanic zone (NENZ) (ref. 6) of northern Iceland's insular shelf, commonly referred to as the Tjörnes Fracture Zone (TFZ) a right-lateral transform fault^{3-5,7-9} (Fig. 1a). Not only is the existence of the transform fault questioned^{2,10} but proponents^{4,5,8,9} acknowledge that topographic expressions paralleling the transform direction fail to control the offset zone.

We report here new evidence derived from a detailed field investigation involving bathymetry, magnetics, seismic reflection and rock dredging on the insular margin. The survey was conducted during the University of Rhode Island's R/V Trident cruises 139 and 140, in summer of 1973. The ship's tracks shown in Fig. 1a were controlled by satellite navigation.

Our results reveal that morphologically and tectonically the TFZ is dominated by three N-S striking lineaments, namely Kolbeinsey Ridge—Eyjafjardaráll Trough Complex (KRETC), Kolbeinsey Ridge—Skjálfandadjúp Trough Complex (KRSTC) and the Axarfjardardjúp Trough. These features display remarkable continuity across the shelf and overlap each other towards the north-west. The three troughs are distinguished by faulting with post-glacial volcanic activity occurring within

both Eyjafjardaráll and Skjálfandadjúp rifting zones but not along their entire lengths (Fig. 2). In contrast the zones of recent volcanic activity are arranged in a NNW-SSE *en echelon* fashion from the Kolbeinsey Ridge towards the axis of the NENZ (Fig. 2) and thus do not imbricate significantly as the rift zones do.

Kolbeinsey Ridge—Eyjafjardaráll Trough Complex

The KRETC is governed by N-S dip-slip faulting. On the north the ridge is symmetrical, remarkably regular, and broken only by the small persistent rift valley that is offset westward from the crestal axis (Fig. 3, profile O). A strong positive magnetic anomaly is aligned along the valley trend, flanked on the west by a weaker but equally well-defined negative anomaly (Fig. 1b). Small eruptive fissures typify the graben's floor which is shown by dredging to consist of fresh pillow basalts (Fig. 2, Table 1, station 6 and 7). Onlapping sediments thicken away from the axis as expected for normal spreading ridges. Southwards the character of the ridge is altered significantly: the elevation decreases as the width increases even though the

Table 1 TR 139 rock dredging across Tjörnes Fracture Zone

Station	Latitude and Longitude	Depth (m)	Feature	Recovery	Inference
1D	66°30.5'N 17°20.5'W	65–80	Flat-topped seamount; NW Tjörnesgrunn	Fresh glassy pillow basalt fragments	Post glacial submarine eruption (possibly 1868)
2D	66°25.5'N 17°10'W	16–25	Mánareyjar ridge crest; Lágey	Poorly sorted palagonitized tuff fragments	Not far from source vent
3D	66°48.5'N 18°09'W	180–205	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex: Höll Seamount	Rounded and jointed cobbles; oxidised scoriaceous vesicular basalt	Subaerial or very shallow depth submarine eruption, near vent, wave erosion and subsidence—post glacial
6D	67°00.5'N 18°42.5'W	270–310	Kolbeinsey Ridge— Eyjafjardaráll Trough Complex—eruptive fissure on graben floor	Fresh glassy pillow basalt	Typical submarine ridge eruption—post-glacial
7D	66°54.5'N 18°46.5'W	400–410	Kolbeinsey Ridge— Eyjafjardaráll Trough Complex—eruptive fissure on graben floor	Fresh glassy pillow basalt with brown crust	Typical submarine ridge eruption—post-glacial
8D	66°44.5'N 18°00.5'W	340–350	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex—small graben	Tuff fragments, 3 small pebbles of massive basalt, indurated balls of mud with glass shards (one surrounded with a small film of lava)	Subaerial volcanic material with some recent submarine volcanic activity
9D	66°38.5'N 17°47'W	180–210	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex—isolated seamount within graben complex—with sediment disturbance	Large vesicular basalt, slightly glassy—small fresh highly vesicular pillows with palagonite crust; rounded cobbles—small fist-size grabbo nodules	Post glacial subaerial or shallow submarine eruption—inconclusive
10D	67°02.5'N 18°28.5'W	65–80	Kolbeinsey Ridge— Skjálfandadjúp Trough Complex. Large flat-topped volcano with two distinct terraces at 55 m and 90 m	0.3 m rounded vesicular basalt	Post glacial subaerial eruption—wave erosion
11D	67°01.5'N 18°26'W	90–100	Same feature as 10D	Rounded vesicular basalt; banded tuff, near vent, few erratics	Post glacial or inter-glacial subaerial eruption, near vent, wave erosion, subsidence
12D	67°02.5'N 18°22'W	90–100	Same feature as 10D (90 m terrace)	Cobbles and pebbles of vesicular basalt; 1 limestone fragment	Post glacial or inter-glacial subaerial, volcanic island; surf zone type deposit

acoustic basement configuration and sediment accumulations remain symmetrical about the ridge axis (Fig. 3, profile B). The subsiding effect serves to promote fracturing. The south-eastern ridge extension, marked by peaks and small grabens maintains its ridge profile but the southern ridge branch gradually changes to a linear depression. In the Eyjafjardaráll Trough at 66°40'N, the coincidence of intersecting faulting, the limit of axial magnetic stripes and sediment deposits⁵ coupled with the site of the closed 40 mgal free-air gravity anomaly contour¹¹ define a structural-volcanic discontinuity (Fig. 2). Southwards the trough is bordered and apparently controlled by N–S dip-slip faults which at the trough's shoreward end bend toward the SE to join the WNW Húsvík fault trend⁹. Sediments more than 0.3 s thick fill the trough and no evidence of volcanic activity is observed. Thus the 66°40'N structural-volcanic discontinuity clearly represents the southernmost extent of spreading and rifting in the TFZ which is in direct continuity with the present Kolbeinsey Ridge spreading axis.

Kolbeinsey Ridge–Skjálfandadjúp Trough Complex

The SE curving KRSTC shows numerous dip-slip faults that follow the trend (Fig. 2). The south-eastern ridge extension contains a series of discontinuous volcanic peaks, including Höll, and small grabens along the trend that disappear at 66°49'N. Only the south-eastern end of a negative anomaly

band is evident due to its disruption by a strong positive anomaly west of Höll (Fig. 1b). Overlapping sediment deposits typify the extension's eastern flank (Fig. 3, profile C). The adjoining Skjálfandadjúp's borders are controlled by normal faulting except over its southernmost reach where downwarping seems to govern its western flank (Fig. 2). A distinct NW–SE trending positive anomaly can be traced from Höll to Tjörnesgrunn (Fig. 1b). The north-western half of this anomaly overlies the trough's floor. East and south of Grímsey the trough turns to the south but the positive anomaly maintains its SE strike, cutting across the trough's eastern wall and intersecting the western flank of Tjörnesgrunn at a high angle. The most intense faulting occurs north, east and south-east of Grímsey (Fig. 2). The direction and style of faulting fail to support the presence of a major WNW right-lateral strike-slip fault in this area previously inferred¹². We also feel that the distribution and precision of earthquake epicentre locations are insufficient to resolve the two interpretations (Fig. 2). Furthermore the trough's northern section contains numerous submarine eruptive features where little or no sediment is accumulating (Fig. 2, Table 1, stations 8 and 9). It is clearly evident that volcanic activity in the Skjálfandadjúp rifting zone is restricted to the segment north and north-east of Grímsey. Both subaerial and fresh submarine volcanic products, apparently post-glacial, are present. Rocks from Höll Peak and southwards are dominated by vesicular and scoriaceous basalts rounded by wave action suggesting subsidence of the ridge extension (Fig. 2,

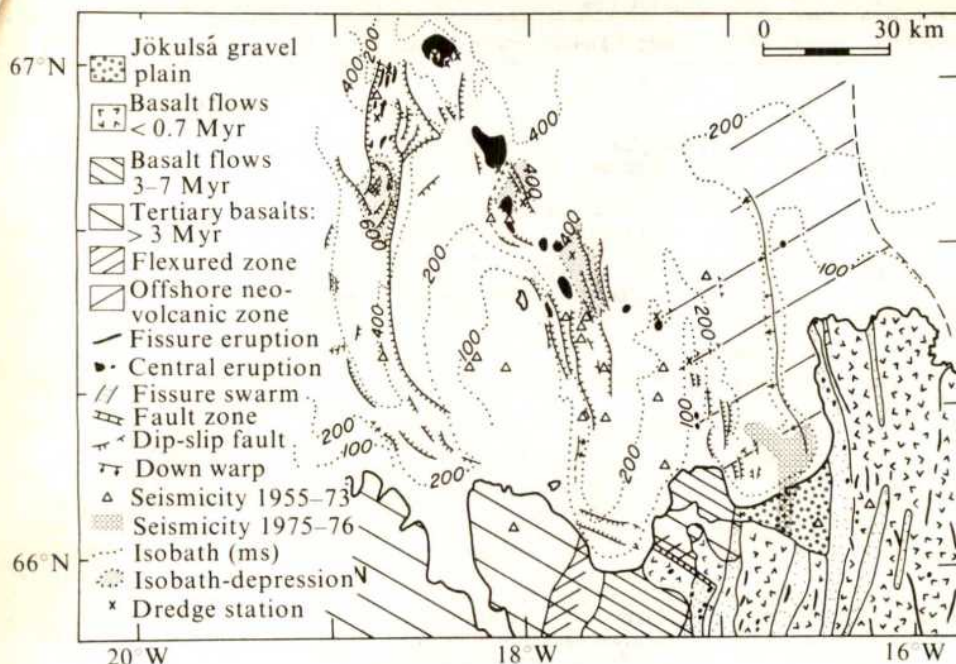


Fig. 2 Summary geology map. Earthquake epicentre locations (refs 13, 21); 1964-73 epicentres from Preliminary Determination of Epicenters Monthly Listing published by US Coast and Geodetic Survey and successor organisations through 1972 and jointly by NOAA/USGS thereafter.

Table 1, stations 3, 10, 11 and 12). South of 66°31'N no recent volcanic activity is observed as sediment covers the trough's floor reaching a thickness of 0.13 s towards the coast.

Axarfjardardjúp Trough

This trough is a downwarp modified by faulting, rifting and volcanism (Fig. 2). The predominant direction of the dip-slip faults is parallel to the trough's long axis. Normal faulting is associated with the eastern flank of the apparently older Mánareyjar volcanic chain and can be traced southwards to the Tjörnes Peninsula's eastern border scarp¹³. The trough's magnetics show a series of narrow, elongated anomalies which differ markedly from the Kolbeinsey Ridge anomaly bands (Fig. 1b). Notably the western edge of this anomaly series strikes NNW-SSE and diagonally crosses the Tjörnesgrunn trend⁶. Recent undeformed sediments up to 0.1 s thick, partially or completely cover the trough's floor (Fig. 3, profiles H and J). Although no morphological indications of volcanism are manifest and no search for evidence of recent volcanic activity was possible due to time limitations, pre-depositional rifting is distinctly revealed at the trough's southern end alongside Tjörnes Peninsula (Fig. 2 and Fig. 3, profile J). Moreover, since our survey's completion, significant recent rifting¹² is reported in the same part of the trough covered by our seismic reflection lines but lies immediately eastward of the pre-depositional rift zone. Hence recent rifting occurs in the offshore NENZ⁶ and is not found on the Mánareyjar chain as indicated previously².

Subdued shelf ridges

The three tectonic trends discussed above are separated by Sléttugrunn, Tjörnesgrunn and Grímseygrunn (Fig. 1a). On the east Sléttugrunn is the northward lobate projection of Slétta Peninsula (Fig. 1a). It is characterised by the same magnetic anomalous pattern observed in the Axarfjardardjúp Trough (Fig. 1b). Alignment of these anomalies with fissure swarms on land as well as their general N-S strike and high magnetic intensity suggest an offshore continuation of the NENZ (ref. 6). Some volcanism is revealed by the presence of a single cone and apparent flows, indicated by the lack of sub-bottom penetration, that cover the surface immediately seaward of the Slétta Peninsula but no seismicity is reported⁹ (Fig. 2). Thus volcanically and tectonically most of Sléttugrunn may represent a presently dormant zone.

Tjörnesgrunn is a tapering N-S submarine extension of the Tjörnes Peninsula that includes the Mánareyjar volcanic

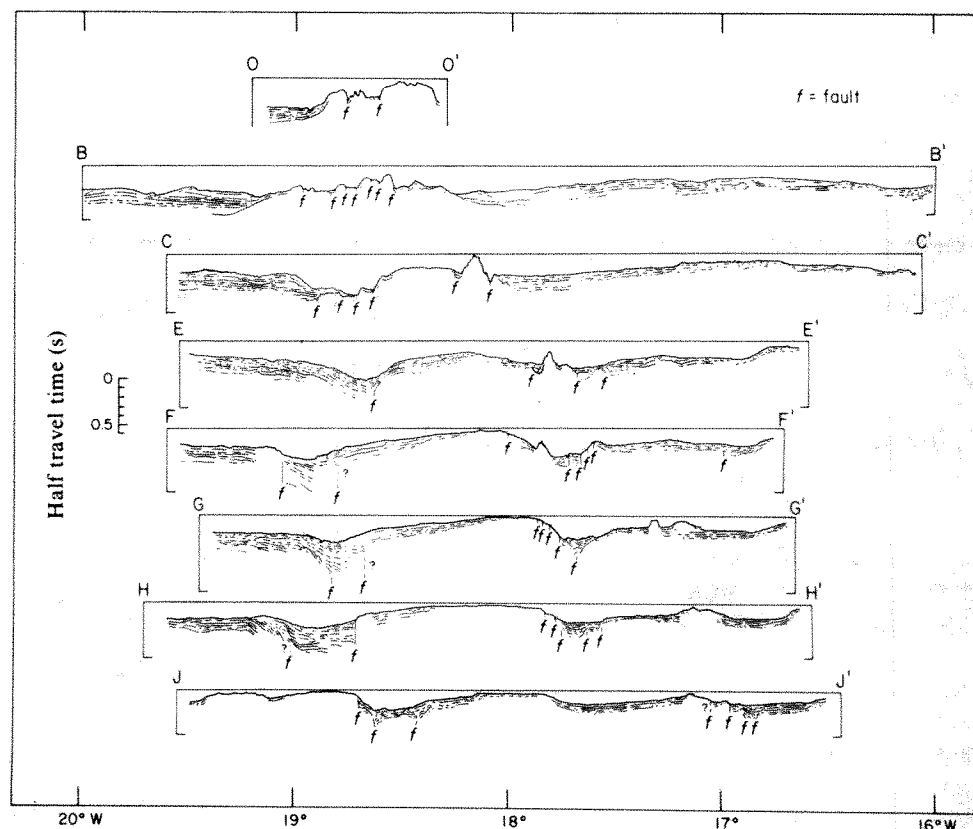
chain (Fig. 1a). Its eastern flank, partly fault controlled, is steeper and more irregular than its western flank. The western edge of the magnetic anomaly series that distinguishes Axarfjardardjúp and Sléttugrunn, passes beneath the ridge's outer reach (Fig. 1b). No evidence of recent submarine volcanic activity is encountered along the N-S Mánareyjar ridge crest, which is probably formed of relatively older subaerial volcanics. Only palagonitized tuff and interbedded vesicular basalt pebbles are dredged from the crest at the ridge north of Lágey (Fig. 1a and Table 1, station 2). The material is similar to that found on Háey, or overlapping the grey basalt flow of Lágey islets. Near the NW end of Tjörnesgrunn, however, (Fig. 1a, Table 1, station 1) very fresh glassy vesicular pillow basalts is recovered from a flat-topped seamount. The seamount definitely represents a recent submarine eruption at very shallow depth, perhaps representing the 1868 volcanic activity reported in Icelandic history. This volcanism, lying along the NENZ's western border may suggest another small rift segment linking the general NNW *en echelon* pattern of rifting and latest volcanic activity that occurs in the Skjálfandadjúp.

Grimseyjargrunn is a tabular block that shows no internal acoustic reflectors (Fig. 1a and Fig. 3, profiles F, G, H and J). The block is negatively magnetised crust except for its northern and southern edges which display weak positive anomalies (Fig. 1b). The ridge's northern and eastern walls descend steeply into Skjálfandadjúp while its western flank with on-lapping sediments slopes gently westwards into Eyjafjardaráll. The westward dip is the same as that reported for Tertiary flood basalts of the adjacent coastal area⁹. Grimsey Island, laying atop of Grimseyjargrunn, also consists of SW dipping plateau basalts with a suggested age younger than mid-Gauss⁹.

Húsavík faults

The Húsavík faults⁹ are extended west-north-westward across Skjálfandadjúp's southernmost reach (Fig. 2). Between Húsavík and Eyjafjardaráll, the axis of a gravity low¹¹ lies immediately north of the apparent trace of the offshore fault zone. At the southern end of Eyjafjardaráll, the centre of the gravity low is elongated WNW-ESE and the trough's faults bend towards the south-east. These deviations from the N-S trend may reflect strike-slip dislocations along the seaward extent of the Húsavík faults between 4-1 Myr age⁹. Thus the Húsavík fault zone is unrelated to present rifting of the Mid-Atlantic Ridge as activity along these faults is believed to have diminished about 1 Myr ago¹⁴.

Fig. 3 Line drawings of selected seismic reflection profiles. For locations see Fig. 1a. f, Fault.



Discussion

From these preliminary results, we infer that no transverse tectonic features common to transform faults^{15,16} are recognised over the TFZ with the exception of the Húsavík faults which are believed to be presently inactive and unrelated to the plate boundary. Instead the TFZ is characterised by three distinct and overlapping tectonically active zones associated with the KRETC, KRSTC, and Axarfjardardjúp Trough (NENZ). Seismicity shows no simple and direct relationship with these active zones except for the post 1973 earthquakes at Axarfjardardjúp's southern end¹² (Fig. 2). Recent volcanic activity along eruptive fissures between the Kolbeinsey Ridge and NENZ occurs in NNW-SSE *en echelon* manner (Fig. 2) and defines the most recent plate boundary between Eurasia and Greenland. Practically no overlap of volcanic activity is found except perhaps immediately south of 67°N where Eyjafjardaráll and Skjálíandadjúp connect with the Kolbeinsey Ridge. The *en echelon* arrangement of recent eruptive fissures is further corroborated by magnetic anomaly lineaments and to some extent by the distribution of recent earthquake epicentres in Axarfjardardjúp Trough¹³.

The surface structural character of the TFZ revealed in our survey may be compared profitably with the transform tectonic expressions produced by a simple experimental clay model for the transient stage of discontinuous deformation¹⁷. During this stage the azimuth of fault traces develops oblique to the offset direction. Clear similarities are found but discrepancies are also evident. Analogies include: (a) the assumed displacement direction being roughly 65° with respect to the normal faults of the Kolbeinsey Ridge and Axarfjardardjúp Trough (NENZ); (b) *en echelon* dip-slip faults and eruptive fissures in Skjálíandadjúp striking 30°–40° relative to the presumed displacement trend; (c) intersecting rift zone and oblique fault systems forming crude diamond-shaped blocks in the Kolbeinsey Ridge-Eyjafjardaráll Trough transition. Significant dissimilarities are that *en echelon* oblique faults are not distributed uniformly across the assumed displacement zone, major strike-slip movement is not documented along these faults although near Grimsey strike-slip motion has been determined recently from a fault plane solution¹² and no intersecting fault

pattern is found where the inferred activity meets the NENZ. Despite these discrepancies we believe that the arrangement of volcano-tectonic features within the TFZ is best explained by discontinuous transformation as expressed by the clay model¹⁷. Perhaps the transient stage is somewhat mature judging from the 30°–40° angle that faulting and eruptive fissures form with the presumed displacement of the transform fault.

These types of transient or leaky transform faults are also noted south of Iceland, the Afar, and south of the Azores Platform at 37°N (refs 17, 18). The three areas occur at the loci of proposed mantle plumes and major directional changes of the mid-ocean ridge. Independently, Burke *et al.*¹⁹ suggest that mid-ocean ridges located near mantle plumes will tend to jump back over the plume during a transient stage thus generating multiple spreading ridge axes and consequent fracture zones, overlapping to some extent in space and time. Recent detailed spatial and temporal analyses of active rift zones throughout Iceland^{2,20} seem to indicate such rift jumping. Our proposed transient stage of discontinuous deformation for the TFZ, therefore, seems to be consistent with such a model. Further Walker's² conjectured *en echelon* active rift zone configuration for the TFZ, based on meagre data, is essentially confirmed.

Finally, tensional fissures removed from the major plate boundary are clearly documented in the clay model experiment for the transient stage of discontinuous deformation¹⁷. Therefore we suggest that the active eruptive centres along Sléttá's western coast (Fig. 2) which remain unexplained² as well as the recent linear submarine volcanic zone of the KRETC are identical features. As such these kinds of eruptive fissures may have a similar origin to that proposed for the Vestmann Islands¹⁷. In these cases the fissures are considered to lie along prolongation of normal faults that extend into the plate for limited distances on diagonally opposite sides of the offset.

We acknowledge support (NSF Grant DES 72-01705) and are grateful to Iceland's National Research Council for permitting the field work to be undertaken.

¹ Jakobsson, S. P. *Lithos* 5, 365–386 (1972).

² Walker, G. P. L. *Nature* 255, 468–471 (1975).

³ Sigurdsson, H. & Brown, M. G. *J. Petrol* 11, 205–220 (1970).

- ⁴ Meyer, O., Voppel, D., Fleishcher, U., Closs, H. & Gerke, K. *Deutsch. Hydrogr. Z.* **25**, 193–201 (1972).
- ⁵ Johnson, G. L. in *Geodynamics of Iceland and the North Atlantic Area* (ed. Kristjánsson, L.), 49–62 (Reidel, Dordrecht, 1974).
- ⁶ Robinson, N. M. thesis, Univ. Georgia (1975).
- ⁷ Sykes, L. R. *J. geophys. Res.* **72**, 2131–2153 (1967).
- ⁸ Ward, P. L. *Geol. Soc. Am. Bull.* **82**, 2991–3012 (1971).
- ⁹ Saemundsson, K. *Geol. Soc. Am. Bull.* **85**, 495–504 (1974).
- ¹⁰ Tryggvason, E. *Bull. Seism. Soc. Am.* **63**, 1327–1348 (1973).
- ¹¹ Palmason, G. in *The Geology of Continental Margins* (eds Burk, C. A. & Drake, C. L.) 375–380 (Springer-Verlag, New York, 1974).

- ¹² Björnsson, A., Saemundsson, K., Einarsson, P., Tryggvason, E. & Grönvold, K. *Nature* **266**, 318–323 (1977).
- ¹³ Einarsson, Tr. *Soc. Sci. Islandica* **32**, 1–79 (1958).
- ¹⁴ Aronson, J. L. & Saemundsson, K. *Earth planet. Sci. Lett.* **28**, 83–97 (1975).
- ¹⁵ van Andel, T. J. H. & Corliss, J. B. *J. mar. Res.* **25**, 343–351 (1967).
- ¹⁶ Menard, H. W. & Chase, T. E. in *The Sea* **4**, pt. 1 (ed. Maxwell, A. E.), 421–444 (Wiley-Interscience, New York, 1970).
- ¹⁷ Courtillot, V., Tapponnier, P. & Varet, J. *Tectonophysics* **24**, 317–329 (1974).
- ¹⁸ Klein, F. W., Einarsson, P. & Wyss, M. *J. geophys. Res.* **82**, 865–888 (1977).
- ¹⁹ Burke, K., Kidd, W. S. F. & Wilson, J. T. *Nature phys. Sci.* **241**, 128–129 (1973).
- ²⁰ Palmason, G. *Geophys. J. R. astr. Soc.* **33**, 451–481 (1973).
- ²¹ Sykes, L. R. *Bull. Seism. Soc. Am.* **55**, 501–518 (1965).

Amino-terminal fragments of *Escherichia coli* *lac* repressor bind to DNA

Thomas M. Jovin, Norbert Geisler & Klaus Weber

Max-Planck Institut für Biophysikalische Chemie D-34 Göttingen, FRG

The N-terminal fragments (residues 1–51 and 1–59) obtained by selective tryptic cleavage of native lac repressor retain the ability to bind DNA. These fragments (headpieces) are monomeric and form complexes which resemble those of tetrameric repressor with non-operator DNA. But, they do not show the high specificity of repressor for operator sequences. The DNA binding has been demonstrated by filter-binding assay as well as in solution using absorption, circular dichroism, and fluorescence measurements.

There is overwhelming genetic and biochemical evidence to indicate that the integrity of the amino-terminal segments of the *Escherichia coli* *lac* repressor subunits is a necessary requirement for the weak binding to non-operator DNA as well as the highly specific and strong binding to operator DNA^{1–6} (for reviews and discussion, see refs 7–9). Geisler and Weber¹⁰ have reported the selective cleavage by trypsin of the N-terminal region of repressor at Arg₅₁ and Lys₅₉ so as to liberate approximately equal amounts of 'short' and 'long' headpiece peptides (designated here as SH and LH) comprising residues 1–51 and 1–59. The tetrameric core no longer binds DNA (ref. 3) but the headpiece preparations retain affinity for phosphocellulose and DNA–cellulose, a criterion for nonspecific DNA binding^{7,9}.

The aim of this study was the initial characterisation of the DNA-binding properties of the two headpiece fragments and their correlation with known features of native repressor. The presumption that the characteristics of headpiece are related to those of the parent repressor and are thus biologically relevant is based on the following considerations: (1) isolated headpiece possesses secondary structure revealed by circular dichroism¹⁰. (2). The pattern of susceptibility to trypsin digestion is the same for isolated headpiece and the corresponding region in native repressor. Thus, in 1 M Tris-HCl, pH 7.5, 30% glycerol, repressor is cleaved so as to generate headpiece but the latter is not susceptible to further digestion¹⁰. We have established that the isolated headpiece is also resistant to trypsin in the same conditions (data not shown). In 0.05 M Tris buffer, however, both isolated headpiece and the corresponding region in intact repressor rapidly undergo further proteolysis to peptides (data not shown). (3). The capacity of repressor to bind DNA nonspecifically is transferred to β -galactosidase in chimaeric molecules produced by certain gene fusions between the *i*- and *z*-genes of the *lac* operon⁶. These molecules carry about 59–80 residues of the N terminus of repressor. Further evidence for the close relationship between the properties of the isolated headpiece and those of native repressor was obtained in the present study.

In interpreting these findings, one should note the close correlation between the operator-specific and the nonspecific modes of binding of *lac* repressor to DNA and the important role ascribed to the sequence-independent interactions in the regulation of repression^{6–9,11}.

Determination of DNA binding by filter assay

The two headpieces LH and SH can be separated on DNA–cellulose columns (Fig. 1a). SH elutes before LH, as might be expected due to its smaller size and charge. Both fragments are active in binding assays based on the retention of protein–DNA complexes on nitrocellulose filters (Fig. 1b). The markedly sigmoidal binding isotherms denote the existence of real (or apparent) co-operativity, a feature not seen with native repressor. While the binding of DNA is quantitative at high concentration of either headpiece, the points for 50% retention and the corresponding slopes differ markedly (0.8 μ M and 3 μ M for LH and SH, respectively). The mixed headpiece preparation shows an intermediate behaviour consistent with the superposition of LH and SH properties, the former being dominant.

Additional features revealed by the filter assay are given in Table 1. In standard conditions, various natural and synthetic DNA's are bound in a similar fashion. Thus, headpiece does not demonstrate a sequence-specific (operator) interaction with DNA, in contrast to the parent repressor. Denaturation of the DNA slightly potentiates binding by headpiece while fragmentation by sonication diminishes it only to a limited degree (note also the large difference in chain lengths between the phage DNA and poly d(A–s⁴T) used in Table 1). Salt manipulations lead to complex and somewhat DNA-dependent effects. Eliminating Mg²⁺ or raising the KCl concentration increases binding at low headpiece concentrations but reduces the maximal extent of retention, especially in the case of phage DNA. One is undoubtedly perturbing the thermodynamic¹⁷ and kinetic⁷ parameters governing the formation of the binary complex and/or its interactions with the filter. Finally, the kinetics of association and dissociation are relatively rapid. With poly d(A–s⁴T) and headpiece at 50 nM and 2.5 μ M, respectively, a filterable complex is formed within 10 s (a value which only sets a lower limit for k_{on} of about 10⁴ M^{–1} s^{–1}). Similarly, challenging a preformed complex with an 100-fold excess of non-radioactive poly d(A–s⁴T) leads to dissociation within seconds. Taken together, these data establish that the headpiece of *lac* repressor possesses the inherent property to bind DNA and that the observed phenomena are not due to contamination with residual functional repressor.

Spectroscopic measurements using poly d(A–s⁴T)

Four of the eight tyrosine residues in each repressor subunit are located in the N-terminal region (residues 7, 12, 17 and 47) (ref. 16). Genetic analysis of repressor mutants has shown that Tyr₁₇ is essential for specific operator binding¹⁸. The spectral properties of the tyrosines are particularly suitable for study since no other chromophoric amino acid side chains are present in the headpiece. Figure 2A(a) shows the absorption spectrum of LH which corresponds closely to that of unionised unperturbed (exposed) tyrosine. Thus, the buried and ionised (or hydrogen-bonded) tyrosine residues observed in intact repressor by ¹⁹F nuclear

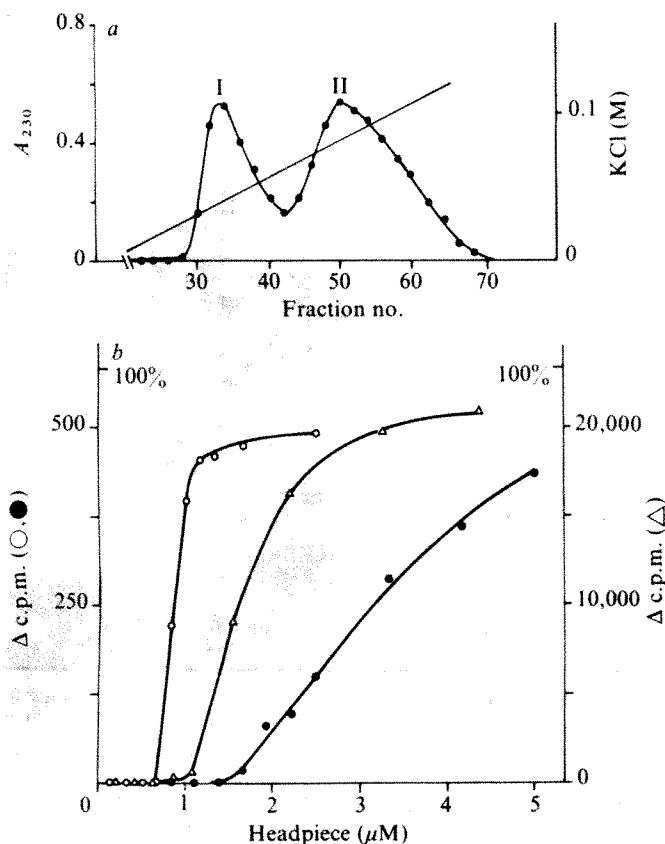


Fig. 1 Purification and DNA-binding properties of *lac* repressor headpieces. *a*, Separation of LH (long headpiece: residues 1-59) and SH (short headpiece: residues 1-51) on a DNA-cellulose column. Tryptic digests of repressor were prepared and purified by chromatography on Sephadex G-150 and G-25 according to Geisler and Weber¹⁰. The headpiece mixture was applied to a native calf thymus DNA-cellulose column¹² equilibrated with 50 mM Tris-HCl, pH 7.8 (4 °C), 1 mM EDTA, 5% glycerol and eluted with a 300 ml linear gradient of buffer containing 0-0.15 M KCl. Peaks I and II were identified as SH and LH, respectively, by amino acid analysis and C-terminal determination with carboxypeptidase B. The molecular weights of SH and LH are 5,615 and 6,395, respectively, from the amino acid composition. *b*, DNA binding by the two headpiece preparations and their unfractionated mixture. Slight modifications of the standard assay¹³ for the operator binding activity of repressor were used to increase retention and reproducibility: the 25-mm Millipore HAWP (0.8- μ m pore size) filters are supported on a glass holder, the outlet of which is filled with liquid and attached to a peristaltic pump. The binding buffer (BB) (0.6 ml) contained 10 mM KCl, 10 mM Tris-HCl, pH 7.4, 3 mM magnesium acetate, 0.1 mM EDTA, 5% dimethyl sulfoxide (DMSO), 0.1 mM dithiothreitol (DTT), and 50 μ g ml⁻¹ BSA. ³²P-labelled λ phage DNA (see Table 1) was present at a nucleotide concentration of 0.9 μ M (specific activity 1300 c.p.m. nmol⁻¹ in the case of LH and SH, 40,000 c.p.m. nmol⁻¹ in the case of the mixture). After addition of protein and incubation at about 25 °C for 30 min, 0.58 ml was filtered together with 0.5 pre- and post-washes of FB (filtering buffer, same as BB but lacking BSA and DTT) at a constant rate of 1 ml min⁻¹. The air-dried filters were counted in a scintillation counter. The background level of DNA binding to the filter was 2-3% and the 100% retention points (indicated on the ordinates) were calculated from aliquots of the DNA stock solution applied directly to the filters. Long headpiece LH (O), short headpiece SH (\bullet), and an approximately equimolar mixture (Δ).

magnetic resonance (NMR) spectroscopy¹⁹ are most likely in the core of the molecule. The accessibility of the amino-terminal tyrosines of native repressor (residues 7, 12, and 17) has also been established in iodination studies²⁰.

The synthetic polynucleotide poly d(A-s⁴T) was selected for further physical measurements because of its unique spectral properties¹⁵. The thioketo substitution in the position 4 of thymidine produces a strong absorption band in the near ultraviolet (Fig. 2A(b)), well isolated from the protein spectrum, and it also makes possible the efficient photoaffinity labelling of protein-nucleic acid complexes²¹. Poly d(A-s⁴T) forms a helical

structure with Watson-Crick base pairing and features intermediate between the C and D conformations. The pitch height and axial rise are about 10% greater than in the case of poly d(A-s⁴T) (W. Saenger, personal communication). Poly d(A-s⁴T) has been used in studies of *lac* repressor analogous to those described here for the headpiece (T.M.J., in preparation).

The binding of headpiece (LH) to poly d(A-s⁴T) results in altered absorption spectra (Fig. 2B). Hypochromic changes are seen in the s⁴T (360 nm) and, possibly, the dA (260 nm) regions. These cannot be attributed to a disruption of the DNA helix since the latter process leads to a hyperchromic blue shift¹⁵. The sign (positive) and positions of the peaks in the difference spectrum below 300 nm are strongly suggestive of perturbations in the tyrosine spectra, such as from ionisation (or hydrogen-bonding) of the phenolic OH²² or transfer from an aqueous environment to one of higher refractive index²³ (for example, through stacking interactions). If we attribute the change at 286 nm exclusively to tyrosine ionisation and assume that 50% of the headpiece is bound (from data in Fig. 4), then the observed magnitude would be compatible with the complete ionisation of two of the four

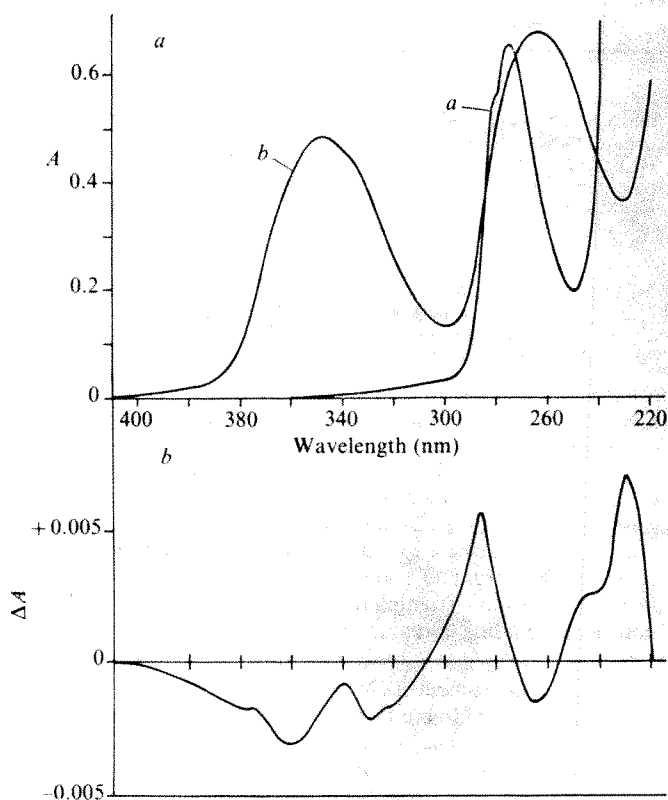


Fig. 2 Absorption and difference absorption spectra of repressor headpiece LH and poly d(A-s⁴T). *A*, Absorption spectra of *a*, 12 μ M LH and *b*, 51 μ M poly d(A-s⁴T) at 20 °C in BB buffer lacking BSA and DMSO. The instrument was a Cary 16. The LH spectrum is 10-fold expanded for clarity. The extinction coefficients for headpiece, based on amino acid analysis, are 4,800 M⁻¹ cm⁻¹ and 5,000 M⁻¹ cm⁻¹ at 280 and 276 nm. ($A_{280}^{1\%} = 7.6$), in agreement with corresponding values for tyrosine. The maxima of the DNA are at 347 and 263 nm and of the protein at 276 nm (minimum at 245 nm). *B*, Difference spectrum between the mixed and unmixed DNA and protein solutions. Tandem cuvettes with 0.438-cm path length in each compartment were used in both sample and reference beams. The solutions shown in (*A*) were used. The difference spectrum was obtained after mixing the sample cuvette. The maxima are at 230 and 286 nm and the minima at 266, 330, and 361 nm. The latter peak corresponds to a 2% decrease in absorbance. The peak at 286 nm represents a 37%, or a 4% increase if referred to the protein or DNA contributions alone, respectively. Isosbestic points are at 255, 273, and 307 nm. The difference spectrum in this and other experiments was virtually abolished by addition of 0.2-0.4 M KCl. The difference spectrum with the same concentration of SH was similar but 30% lower in magnitude.

Table 1 DNA binding properties of *lac* repressor headpiece by filter assay

Expt no.	DNA	Concentration (μm)	Additions (mM)*		% DNA retained								
					Headpiece† concentration (μg ml ⁻¹)								
					1	2	3	4	5	6.7	10	15	20
1	λ plac	1			0.2	0.3	1.4	10	48			94	96
		10			0.3	1.0	1.3	6	25	44	83	95	96
2	λ	0.2				0.7				22		77	83
3	Poly d(A-T)	0.5				<1				60		91	96
4	Poly d(A-s ⁴ T)	0.01			<1	<1			40		72		77
		0.05			<1	1.2			32		73		76
5	λ (heat denatured)	1				0.4			34‡	44	71	89	75
6	λ plac intact	0.9										64(64)§	
	sonicated											44(12)§	
7	λ plac	1	KCl	Mg(Ac) ₂									
			—	—		<1				53		93	
			—	Removed		10				70		95	
			50	—		1				4		61	
			100	—		1				2		4	
			200	—		3				1		6	
	Poly d(A-s ⁴ T)	0.05		—		1				57		80	
				Removed		5				43		80	
			50	—		6				28		73	
			100	—		9				11		51	
			200	—		11				11		11	
					Headpiece derived from BG2 i ^{-d} mutant repressor								
5	λ	0.2				2.8			7	10	17	26	38

Assay conditions were as in Fig. 1 except that half volumes were sometimes used. The binding at pH 8.6 was 20% lower at saturation than at pH 7.4 and 8.0. Labelled DNA's were obtained by induction and purification of phage DNA¹⁴ or synthesised by *E. coli* DNA polymerase I and extracted with sodium dodecyl sulphate and chloroform-isoamyl alcohol. The specific activities in c.p.m. nmol⁻¹ and background levels in the filter retention assay in % are given in parentheses: ³²P- λ *plac*: [*lac*, *pro*]₂ (λ Cl₈₅₋₈₇-*plac*5i⁻o⁺z⁺y⁻a⁻), strain BMH 782 (10³-10⁴, 1-5); ³²P- λ : W8 (λ Cl₈₅₋₈₇) (10⁴, 1.5); poly d(A-s⁴H-T) (5000, 4), poly d(³²P-A-s⁴T) (10³-10⁴, 3-6). The phage DNA's with or without the *lac* operator have chain lengths of 48,000 compared to 100-200 base pairs for poly d(A-s⁴T). Saturating concentrations of headpiece bound at least 6 nmol of phage DNA and up to 2 nmol of poly d(A-s⁴T). Denaturation in expt 5 was for 2 min at 100 °C. Sonication in expt 6 was for 10 min and led to an approximately 80% reduction in the native molecular weight.

*The additions were made only to BB except in expt 7 in which they were added to FB as well.

†The headpiece preparation was a mixture of SH and LH. There is an uncertainty of about 10% in the concentrations given for different experiments.

‡The corresponding value for native DNA was 1.3%.

§The numbers in parentheses correspond to parallel experiments with 0.17 nM native *lac* repressor.

||*i*^{-d} constitutive mutant with Val₉ replaced by isoleucine⁹. The tetrameric repressor does not bind to operator and shows a reduced affinity for phosphocellulose from which it is inferred that nonspecific DNA binding is impaired as well⁹. The headpiece isolated from another *i*^{-d} mutant repressor AP309 (Ser₁₆→Pro) binds normally to phosphocellulose¹⁰ and functions like the wild-type molecule in our filter retention assay.

tyrosines in the headpiece on binding to poly d(A-s⁴T). The isosbestic points are similar to those reported in model oligopeptide studies²⁴ in which various physical methods have provided evidence for both kinds of interactions between tyrosine side groups and DNA (ref. 24 and references therein). The difference spectra are abolished by added salt (0.2 M), attesting to the reversibility of complex formation. This finding is also in agreement with the ionic strength dependence of both the specific and nonspecific binding of repressor to DNA²⁵.

Poly d(A-s⁴T) has a circular dichroism (CD) spectrum with pronounced Cotton effects in the near ultraviolet (Fig. 3A(b)). The binding of headpiece leads to a positive displacement of the entire spectrum extending down to 265 nm, below which the protein contribution is dominant¹⁰. Native repressor produces similar effects. The titration of (mixed) headpiece with the synthetic DNA yields an apparent stoichiometry *n* of ≤10-14 basepairs per headpiece molecule (Fig. 3B). As in the case of absorption, the perturbation of the CD spectrum is incompatible with a denaturation of the DNA (which gives rise to decreased ellipticity at 340 nm). It is more probable that changes in helical parameters, also seen in the binding of repressor to poly d(A-s⁴T)^{27,28}, are involved⁷.

The luminescence properties of headpiece can also be exploited in combination with poly d(A-s⁴T). The excitation and emission spectra of headpiece are very similar to those of free tyrosine (Fig. 4A). Addition of poly d(A-s⁴T) leads to extensive quenching, the extent of which depends on the polynucleotide-to-headpiece ratio (Fig. 4B). The quantitative evaluation of such data is complicated by the phenomenon of excluded binding²⁶ especially in conditions of protein excess. A reciprocal titration is shown in Fig. 4C; in this case, the DNA is initially in excess and the parameters obtained (especially the dissociation constant *K*) should correspond to binding with minimal interaction between headpiece molecules (*K* > 10 μM , *n* ≤ 12, and the quenching is about 50%).

Discussion

The quenching of tyrosine fluorescence by poly d(A-s⁴T) could be due to hydrogen-bonding and proton transfer in the excited state³⁰, stacking interactions^{24,30}, and/or energy transfer to the bases^{30,31}. The latter process would be favoured by the spectral overlap between tyrosine emission (Fig. 4A) and 4-thiothymidine absorption (Fig. 2A). Many models for the operator-specific interaction of *lac* repressor and its amino terminus with DNA have discussed hydrogen-bonding and/or hydrophobic (intercalative) contacts between tyrosine side groups and the edges of bases exposed in the DNA grooves⁷. Structural features of the protein which have been proposed include protrusions¹, β structure^{32,33}, and antiparallel β -pleated sheets^{33,34}. The binding mechanisms could include chelation³⁵ or release¹⁷ of ions bound to the DNA and the dehydration of sterically specific carbonyl oxygens³⁶ in the minor groove on coordination with (cationic) groups of the repressor. The combined evidence^{7,28,37} is that binding is to the fully-paired DNA helical duplex, centrosymmetric with respect to the operator sequence, yet involving asymmetric contacts with bases in both strands.

Our results are consistent with the above concepts and also relevant to the question of the number of subunits involved. Several studies have established that (at least) two subunits of tetrameric repressor are required for the extremely specific and stable binding to operator^{6,7,38-40}. The interaction of repressor with natural or synthetic non-operator DNA is characterised by a much lower affinity (*K* = 10⁻⁴-10⁻⁵ M)^{25,40} and an apparent binding length *n* of 11-16^{27,28,42}. These values are similar to those reported here for headpiece (although we emphasise their tentative nature and can only set an upper limit for *n* of about 12). The probable correlation between the structure of the headpiece region integrated into the intact repressor protomer and that in the free state after proteolytic cleavage has already been discussed and is further supported by the available data for mutationally-altered

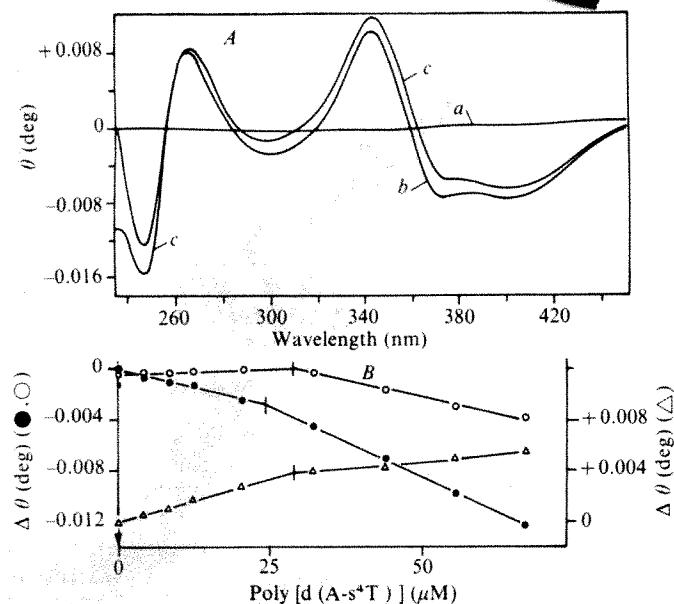


Fig. 3 Circular dichroism spectra of poly d(A-s⁴T) in the absence and presence of headpiece. *A*, CD spectrum of *a*, buffer, as in Fig. 2, at 19.3 °C; *b*, 40 μ M poly d(A-s⁴T); *c*, same solution as in (*b*) plus 26 μ M headpiece. The spectra were measured in selected 1-cm path length absorption cuvettes using the 0.04° range of a Cary 60 equipped with CD attachment. *B*, Titration of headpiece with poly d(A-s⁴T). A 2.3 μ M solution of mixed headpiece in a 1-cm cuvette was placed in the CD spectrometer and measured at 10 discrete wavelengths (only three are shown \circ , 300 nm; Δ , 357 nm; \bullet , 370 nm). Additions of poly d(A-s⁴T) were made and the cuvette mixed using a pipette but without repositioning. Two linear regions were obtained in the curves at the various wavelengths with intersections denoted by vertical ticks. The data were analysed according to the formalism for ligand lattice interactions of McGhee and von Hippel²⁵. We assume initially the case of a non-co-operative ligand (the protein) for which the binding isotherm is given by the master equation (our nomenclature) $r = L \cdot f(r)/K$ where r is the ratio of bound protein to total lattice sites S_0 (in this case taken as DNA basepairs). K is the intrinsic dissociation constant for an isolated site, L is the free ligand (protein) concentration, and $f(r) = (1 - nr)^n / [1 - (n-1)r]^{n-1}$, a function which takes into account the statistical exclusion of several potential binding sites by the attachment of a single protein molecule covering n consecutive lattice residues. In the initial region of the titration here, the ligand is in excess and the slope is given by $\alpha[1 - (1-\beta)nr]$ where α is the increment in CD signal per unit concentration of free DNA and β is the signal due to complexed DNA relative to that of free DNA residues. From the master equation, r is constant and may approach the value $1/n$ if L/K is large enough (a useful approximation for $r > n/(1+n)$ is given by $r \approx (1/n)[1 - (1/n)(K/L)^{1/n}]$). In the second linear region, the lattice is in excess and the slope is equal to α (the observed values are compatible with the spectral changes in (*A*)) and the finite x intercept is given by $\alpha n(1-\beta)L_0$ if the protein is completely bound. It follows that the intersection points defined by the extrapolations of the linear regions obey the relationship $S_0 = L_0/r$. If we assume from Fig. 1 that the LH but not the SH species was quantitatively bound ($L_0(\text{LH}) = L_0/2, r = 1/n$), then from these data $n \approx 10-14$. If these conditions were not fulfilled, n would be lower.

molecules (Table 1). The results open the possibility that the nonspecific DNA binding of native repressor in certain circumstances may involve only a single subunit of the tetramer.

We now re-examine the apparent co-operativity in headpiece binding to DNA as seen in the filter assay (Fig. 1*b*). This phenomenon could arise from (1) a concentration-dependent aggregation of headpiece to form a species with higher affinity for the filter and/or DNA; (2) a requirement for several bound protein molecules per DNA chain for successful fixation to the filter, either because of weak interactions with the latter or rapid dissociation of the binary or tertiary complexes; and (3) true thermodynamic co-operativity, that is, strong positive interaction between adjacent bound protein molecules. The chromatographic characterisation of isolated headpiece provides no evidence for (1)⁴⁰. Some possibilities under (2) are excluded by the stability of the filter-bound complex to moderate washing and the absence of a strong

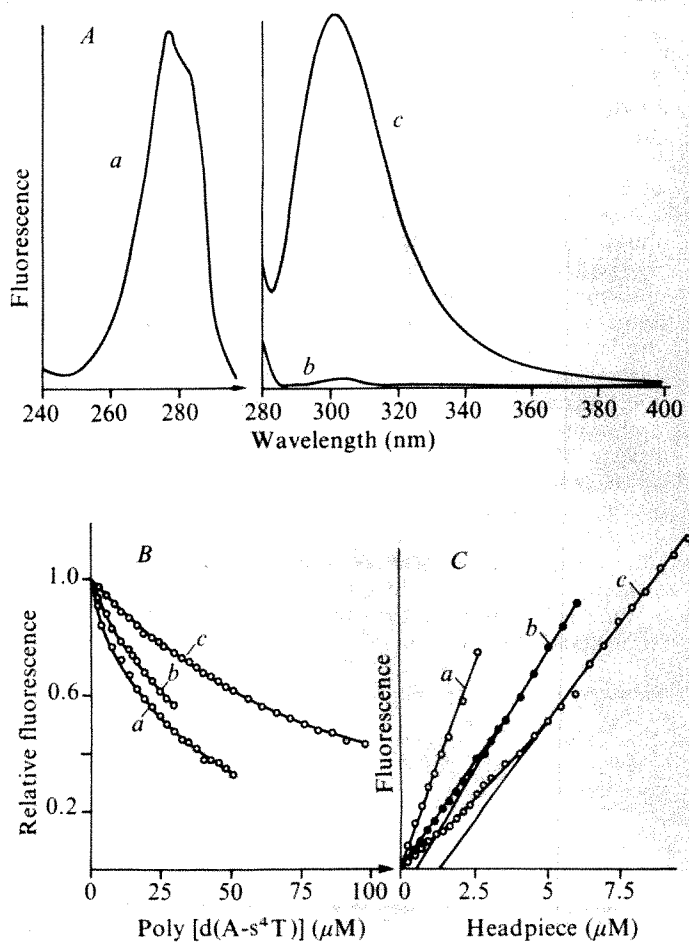


Fig. 4 Fluorescence spectra of headpiece and titration with poly d(A-s⁴T). *A*, Excitation (*a*) and emission (*c*) spectra of 2 μ M LH at 19.5 °C in the buffer given in Fig. 2. A Perkin Elmer MPF4 fluorimeter and 5-mm pathlength cuvettes were used. The spectra were recorded with a 4 nm bandwidth and are uncorrected. The excitation and emission maxima are at 275 and 303 nm, respectively. Curve (*b*) is the buffer blank emission. The spectra are virtually identical with those of tyrosine in the same conditions (the quantum yield of the headpiece, however, has not been measured). *B*, Titration of LH solutions of different concentrations with poly d(A-s⁴T). *a*, 2 μ M LH; *b*, 4 μ M LH; *c*, 13 μ M LH. Excitation was at 275 nm and emission was measured at 303 nm. The emission values after stepwise additions of DNA were corrected for dilution and for inner filter effects due to the DNA absorption (the correction factors were obtained in parallel titrations of tyrosine solutions) and normalised to the corresponding initial intensity. Pronounced curvature is evident and at the highest protein concentration the apparent values of r are improbably high (> 0.23 , corresponding to $n < 4$). In similar experiments with shorter pathlengths and using mixed headpiece preparations (4–7 μ M), the fluorescence quenching was $50 \pm 10\%$ and the apparent stoichiometry 10 ± 2 from the initial slopes. *C*, Titration of poly d(A-s⁴T) solutions of different concentration with LH. *a*, No DNA; *b*, 30 μ M poly d(A-s⁴T); *c*, 48 μ M poly d(A-s⁴T). The titrations were performed in 7-mm path length cuvettes mounted in a temperature-jump kinetic fluorimeter²⁹ using phase-sensitive detection. Excitation was at 280 nm and the emission above 305 nm was collected. Several additions of concentrated headpiece were made from a microsyringe and mixed with a magnetic bar. In contradistinction to the experiments in (*B*), the initial phases in these titrations are characterised by excess lattice (DNA) and thus protein-protein interactions are minimised. Ultimately, headpiece is in excess and the final linear slopes result from the addition of free protein (they are not identical in the three curves due to the different, but constant, inner filter effects caused by the DNA). The ratio γ of the initial to the final linear slopes of each curve obeys the relationship $1/(1-\gamma) = (K/S_0 + 1)/(1-\beta)$ from which K (the dissociation constant for an isolated complex) has a value $> 10 \mu$ M and β (the relative fluorescence of the complex) ≈ 0.5 . The x intercepts of curves (*b*) and (*c*) are given by $S_0(1-\beta)/n$, assuming saturation of the DNA, from which $n \approx 12$. The true value may be lower, however, since saturation will not have been achieved unless co-operative binding at the higher protein concentration occurs and is characterised by a much lower value of K .

chain length dependence. Certain indications for co-operativity (3) exist and are discussed in the figure legends. It has not been possible to check for sigmoidicity in solution studies by measuring a property of the DNA during addition of protein. The signals are simply too small (Figs 2, 3). Yet the behaviour at low concentration of the headpiece derived from the BG2 mutant repressor (Table 1) is most easily understood in terms of reduced co-operativity and thus a more uniform distribution of bound molecules. Retention by the filter may also require 'clusters' of headpiece or a given threshold level of complex formation. It is possible that the low values of n seen at high protein/DNA ratios (Fig. 4B) result from overlapping between binding sites, that is, occupancy by more than one headpiece molecule of the same stretch of duplex DNA. Electron microscopic evidence has been obtained recently for such a phenomenon in the case of native repressor bound nonspecifically to DNA at low ionic strength^{4,5}.

The system described here is ideal for probing general features of protein-nucleic acid interaction due to its inherent properties and the potential for extensive biochemical⁷ and genetic^{5,9} manipulations. Further physical studies using dynamic luminescence and high resolution NMR techniques are in progress.

We thank Dr B. Müller-Hill for supplying some of the bacterial strains, and Maria van der Ploeg for technical assistance.

Received 25 March; accepted 10 August 1977.

1. Adler, K. *et al.* *Nature* **237**, 322-327 (1972).
2. Platt, T., Weber, K., Ganem, D. & Miller, J. H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 897-901 (1972).
3. Platt, T., Files, J. G. & Weber, K. *J. biol. Chem.* **248**, 110-121 (1973).
4. Lin, S. & Riggs, A. D. *Biochem. biophys. Res. Commun.* **62**, 704-710 (1975).
5. Miller, J. H., Ganem, D., Lu, P. & Schmitz, A. *J. molec. Biol.* **109**, 275-298 (1977).
6. Müller-Hill, B., Gronenborn, B., Kania, J., Schlottmann, M. & Beyreuther, K. *P + S biomed. Sci. Symp. Nucleic Acid-Protein Recognition* (ed. Vogel, H.) (Academic, New York, in the press).

7. Bourgeois, S. *Proc. Chem.* **30**, 1-99 (1976).
8. Barkle, S. *in Symp. Regulatory Biol.* (eds Marzlu, G. A. & Copeland, J. C.) (Ohio State Univ. Press, Athens, 1976).
9. Müller, B. *Prog. Biophys. molec. Biol.* **30**, 227-252 (1975).
10. Geisler, N. & Weber, K. *Biochemistry* **16**, 938-943 (1977).
11. von Hippel, P. H., Revzin, A., Gross, C. A. & Wang, A. C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4808-4812 (1974).
12. Alberts, B. M., Amodio, F. J., Jenkins, M., Gutmann, E. D. & Ferris, F. L. *Cold Spring Harb. Symp. quant. Biol.* **33**, 289-305 (1968).
13. Bourgeois, S. *Meth. Enzym.* **21**, 491-500 (1971).
14. Miller, J. H. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, New York, 1972).
15. Eckstein, F. & Scheit, K. H., in *Procedures in Nucleic Acid Research 2* (eds Cantoni, G. L. & Davies, D. R.) 665-679 (Harper and Row, New York, 1971).
16. Weber, K., Platt, T., Ganem, D. & Miller, J. H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3624-3628 (1972).
17. Record, M. T., Jr., Lohman, T. M. & de Haseth, P. J. *molec. Biol.* **107**, 145-158 (1976).
18. Miller, J. H., Coulondre, C., Schmeisser, U., Schmitz, A. & Lu, P. in *Protein-Ligand Interactions* (eds Sund, H. & Blauer, G.) 231-252 (de Gruyter, Berlin, 1975).
19. Lu, P., Jarema, M., Mosser, K. & Daniel, W. E., Jr *Proc. natn. Acad. Sci. U.S.A.* **73**, 3471-3475 (1976).
20. Fanning, T. G. *Biochemistry* **14**, 2512-2520 (1975).
21. Frischauf, A. M. & Scheit, K. H. *Biochem. biophys. Res. Commun.* **53**, 1227-1233 (1974).
22. Wetlauffer, D. B. *Adv. Prot. Chem.* **17**, 303-390 (1962).
23. Donovan, J. W. *Meth. Enzym.* **27**, 497-525 (1973).
24. Brun, F., Toulme, J. J. & Hélène, C. *Biochemistry* **14**, 558-563 (1975).
25. Lin, S. & Riggs, A. D. *Cell* **4**, 107-111 (1975).
26. McGhee, J. D. & von Hippel, P. H. *J. molec. Biol.* **86**, 469-489 (1974).
27. Maurizot, J. C., Charlier, M. & Hélène, C. *Biochem. biophys. Res. Commun.* **60**, 951-957 (1974).
28. Butler, A. P., Revzin, A. & von Hippel, P. *Biochemistry* (in the press).
29. Jovin, T. M. in *Biochemical Fluorescence: Concepts* 1 (eds Chen, R. G. & Edelhoch, H.) 305-374 (Dekker, New York, 1976).
30. Cowgill, R. W. in *Biochemical Fluorescence: Concepts* 2 (eds Chen, R. G. & Edelhoch, H.) 441-486 (Dekker, New York, 1976).
31. Hélène, C., Montenay-Garestier, T. & Charlier, M. *An. Acad. Bras. Ciênc.* **45** suppl., 59-62 (1973).
32. Chou, P. Y., Adler, A. J. & Fasman, G. D. *J. molec. Biol.* **96**, 29-45 (1975).
33. Patel, D. J. *Biochemistry* **14**, 1057-1059 (1975).
34. Gursky, G. V. *et al. Molec. Biol. Rep.* **2**, 427-434 (1976).
35. Seeman, N. C., Rosenberg, J. M. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 804-808 (1976).
36. Manning, G. S. *Colston Pap. No. 29* (Scientifica, Bristol); Record, M. T., Jr & Manning, G. S. *Q. Rev. Biophys.* (in the press).
37. Gilbert, W., Maxam, A. & Mirzabekov, A. in *Control of Ribosome Synthesis* (eds Kjeldgaard, N. C. & Maaløe, O.) 139-143 (Munksgaard, Copenhagen, 1976).
38. Sadler, J. R. & Tecklenburg, M. *Biochemistry* **15**, 4355-4356 (1976).
39. Kania, J. & Brown, D. T. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3529-3533 (1976).
40. Geisler, N. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3103-3106 (1976).
41. Revzin, A. & von Hippel, P. *Biochemistry* (in the press).
42. Richmond, T. & Steitz, T. A. *J. molec. Biol.* **103**, 35-38 (1976).
43. Zingsheim, H. P., Geisler, N., Mayer, F. & Weber, K. *J. molec. Biol.* (in the press).

letters to nature

Electron scattering in X ray-emitting galaxies

SEVERAL galactic nuclei have been identified as luminous sources of X rays. These include the radio galaxy Cen A (refs 1 and 2), several type I Seyfert galaxies^{1,3-5} such as NGC 4151, 3C120 and 3C390.3. The quasar 3C273 has also been detected^{1,3}. Most of these sources have been observed to vary and have, therefore, been considered compact. Their X-ray spectra, where measured, seem to indicate significant low energy absorption intrinsic to that galaxy. Examples are Cen A (ref. 2) for which the line of sight hydrogen column density (assuming solar abundances) $N_H \sim 10^{23} \text{ cm}^{-2}$ and NGC4151 (ref. 6) where $N_H \sim 5 \times 10^{22} \text{ cm}^{-2}$.

The optical depth to electron scattering, τ_{es} , is unity when $N_H \sim 1.5 \times 10^{24} \text{ cm}^{-2}$. Consequently τ_{es} is ~ 10 and 5% for Cen A and NGC4151 respectively. Higher values of τ_{es} may apply if the heavy elements are underabundant, or if a significant fraction of them is completely ionised. A scattered flux of X rays is thus to be expected from such galaxies. This flux may be detected in the time variations of the compact source if the bulk of the scattering material lies in the nucleus. A fraction $\sim \tau_{es}$ of the variations are broadened by the light travel time across the scattering region. It is likely, however, that much of the gas lies in the body, or disk, of that galaxy. In this case the scattered flux may be spatially resolvable, appearing as a diffuse extended source surrounding the nucleus. The total intensity of this extended source, I_s , is $\sim \tau_{es}$ times the mean intensity of the compact source, I_c (provided that $\tau_{es} \lesssim 1$). τ_{es} is the electron scattering optical depth weighted as to

luminosity and averaged over all directions. Note that it need not be equal to τ_{es} deduced from X-ray absorption measurements.

The spatial structure of the scattered flux depends on the underlying gas distribution and the past activity of the compact source over a time equal to that taken by light to cross that region ($\lesssim 10^4$ yr). The variability of the compact source implies that its observed intensity, I_c , need not equal \bar{I}_c , and thus I_s need not equal $\tau_{es} I_c$. I_s will, of course, exceed $\tau_{es} I_c$ in approximately half of any sample of galaxies. It is in principle possible that the past X-ray history of the nucleus can be determined by comparing independent measurements of the gas density (for example, 21 cm if the gas is predominantly neutral) with the spatial structure of the extended source.

The spectrum of the scattered X rays should be similar to that of the compact source above ~ 8 keV, but a low energy cut off due to photoelectric absorption at energy E_a should occur at $E_a \approx 8 \tau_{es} \text{ keV}$. This cutoff is related to the observed cutoff, E_{ao} , by

$$E_a \approx \left(\frac{\tau_{es}}{\tau_{es}} \right)^{1/3} E_{ao}$$

This may allow τ_{es} to be determined. Grazing-incidence telescopes such as are to be carried on HEAO-B are ineffective above ~ 4 keV, and may only detect the innermost scattered flux. Modulation collimators (such as on HEAO-A), or the lunar occultation technique (for example, EXOSAT), may prove more useful in mapping the complete scattering region. Photoelectric absorption by iron results in an absorption edge

and fluorescence emission line in the observed spectrum. The equivalent width of this line (in the scattered spectrum) is ~ 900 eV, assuming solar abundances. The scattered X rays should be highly polarised, the degree of polarisation being maximum ($\sim 100\%$) perpendicular to an edge on disk.

Both Cen A and NGC4151 are good candidates for the detection of scattered X rays. The scattered flux may already have been detected in the case of Cen A, for Delvaile⁷ reports the detection of a 3 arc min diameter X-ray source coincident with the compact source. Cen A bears some resemblance to an edge-on spiral⁸, and might therefore be a candidate for polarisation studies. The disk of NGC4151 is observed nearly face on^{9,10}, with a mean neutral hydrogen surface density over the optical spiral structure (8 arc min diameter) of $\sim 7 \times 10^{-4}$ g cm⁻² (ref. 8). τ_{es} perpendicular to the plane of the disk is thus $\sim 5 \times 10^{-4}$, which is $\sim \tau_{\text{es}}$ for the case of a thin disk containing a central source radiating isotropically. This indicates a very weak scattered flux, I_s , from most of the disk, if $I_c \sim \bar{I}_c$. τ_{es} and thus I_s may be considerably greater within the central few kpc, especially if much of the reported low energy absorption⁶ of the presumed compact source occurs in this region. Note that this compact central X-ray source may maintain a considerable fraction of the lightest elements in the inner parts of NGC4151 in an ionised state. The electron scattered flux in the infrared, optical and ultraviolet wavebands should be reddened in a similar manner to the direct flux that is detected from the galactic centre. This assumes that NGC4151 contains a distribution of dust similar to that in our Galaxy. The continuous optical spectrum of the bar in NGC4151 does, however, more closely resemble that of its nucleus than that expected from blue stars¹⁰. Optical polarisation studies could rule out electron scattering, which would imply a high degree of polarisation perpendicular to the bar.

Finally, it is worth noting that the extended X-ray source at the galactic centre¹¹ may in part be due to scattering of the X rays from the strong sources in that region.

I thank J. E. Pringle and M. J. Rees for helpful discussions.

A. C. FABIAN

*Institute of Astronomy,
Madingley Road,
Cambridge, UK*

Received 25 July; accepted 30 August 1977.

1. Giacconi, R. *et al. Astrophys. J. Suppl.* **27**, 37–64 (1974).
2. Stark, J. P., Davison, P. J. N. & Culhane, J. L. *Mon. Not. R. astr. Soc.* **174**, 35P–40P (1976).
3. Cooke, B. A. *et al. Mon. Not. R. astr. Soc.* (in the press).
4. Schnopper, H. W. *et al. Astrophys. J.* **215**, L7–L12 (1977).
5. Charles, P. A., Longair, M. S. & Sanford, P. W. *Mon. Not. R. astr. Soc.* **170**, 17P–22P (1975).
6. Ives, J. C., Sanford, P. W. & Penston, M. V. *Astrophys. J. Lett.* **207**, L159–L162 (1976).
7. Delvaile, J. P. *Bull. Am. astr. Soc.* **9**, 323 (1977).
8. Whiteoak, J. B. & Gardner, F. F. *Astrophys. Lett.* **8**, 57–60 (1971).
9. Davies, R. D. *Mon. Not. R. astr. Soc.* **161**, 25P–30P (1973).
10. Simkin, S. *Astrophys. J.* **200**, 567–573 (1975).
11. Kellogg, E. *et al. Astrophys. J.* **169**, L99–L103 (1971).

Absorption lines in the optical spectrum of quasar AO0827+24

In January 1977 the radio source AO0827+24 (ref. 1) was observed at 90 GHz by F. Owen, S. Mufson, R. Porcas and T. Moffett with the NRAO 36-foot antenna at Kitt Peak, during observations of several flat spectrum sources selected from the Green Bank 5-GHz survey². Among these sources, 34, including AO0827+24, were found to have flux densities greater than 1 Jy at 90 GHz. Spectrographic observations of the optical counterparts of these 34 sources are in progress. We report here observations of the Mg(II) and Fe(II) absorption line system, at a redshift $z = 0.525$, in the optical spectrum of the 17.5-mag stellar object associated with AO0827+24 because of the similarity with the absorption line system of the two BL Lacertae objects PKS0735+178 (ref. 3) and AO0235+164 (ref. 4) which also have flat radio spectra.

We obtained trailed image tube spectra of AO0827+24 with a moonlight eliminator, using the 2.1-m and 2.7-m telescopes at McDonald Observatory in February and March 1977. We first took two spectra at 230 Å mm^{-1} covering the wavelength range 3,500–6,200 Å. These spectra showed a low contrast feature in emission near 3,700 Å. We later realised that a line at this wavelength had been seen in previous observations of AO0827+24 (refs 5 and 6). Wills and Wills⁶ propose an emission line redshift of 2.046 based on the line at 3,700 Å being identified with La and another emission line which they observe at 4,063 Å being identified with O(II)λ1335; they also note that against this interpretation is the apparent absence of C(IV)λ1549 and that the emission line redshift of this object is still in doubt.

The most conspicuous feature on our low-dispersion spectra was an absorption line at 4,270 Å. We therefore obtained spectra with a higher dispersion, 103 Å mm^{-1} , in the range 3,500–5,400 Å. On the best spectrum the feature at 4,270 Å is clearly resolved into two components at 4,262.5 and 4,273.5 Å and another absorption line is observed at 3,966.8 Å.

The identification of the doublet with Mg(II) 2,795.53, 2,802.70 Å gives a redshift of 0.5247. Using this value the line at 3,966 Å corresponds to a rest wavelength of 2,601.7 Å and is identified with Fe(II) 2,599.40. This line may be contaminated by the interstellar line Ca(II) 3,968 but is probably not caused solely by it otherwise we would expect to see Ca(II) 3,933 with a comparable strength, and this is not observed on our spectra.

Since the Mg(II) doublet is resolved, the full line width in the rest frame of the object is less than 7 Å or 750 km s^{-1} . The real width of the redshifted lines is not known and may be much smaller than the resolution of our spectra, which is about 8 Å. Clearly the absorption line spectrum of AO0827+24 deserves further observation, however, the available data suggest that the absorption spectrum of AO0827+24 has marked similarities with that of PKS0735+17 and AO0235+16. Since there is at least one emission line in the spectrum of 0827+24, the object is not a true BL Lacertae object. But recent results indicate that the spectrographic differences between BL Lacertae objects and quasars are not as clear cut as previously thought⁷.

In PKS0735+17 (ref. 3) only the Mg(II) doublet has been so far detected and gives a redshift $z = 0.424$. In AO0235+16 (ref. 4), there are two redshift systems, one at $z = 0.851$ determined from the Mg(II) doublet, the other one at $z = 0.524$ (which is strikingly close to the redshift of AO0827+24) determined from the Mg(II) doublet and lines of Mg(I), Fe(II) and Mn.

Interestingly, the four absorption-line redshifts in the optical spectra of these objects are all between 0.4 and 0.85. This situation may be caused in part by selection effects inherent to the spectrographic observations: the range of redshift 0.4 to 0.85, corresponds to the easily recognisable Mg(II) doublet falling between 3,900 and 5,200 Å where the search for absorption lines is the easiest.

The sample mentioned above, comprising 34 sources stronger than 1 Jy at 90 GHz, includes AO0827+24 and also the two BL Lacertae objects PK20735+17 and AO0235+16. Thus at least three out of 34, or about 10% of the objects, show similar absorption line systems with $z < 0.9$; the incidence of such absorption systems is therefore not uncommon. It is not known at present whether this phenomenon is caused by cold gas clouds ejected by the continuum emitting source or by intervening galaxies. If these absorption lines are caused by intervening galaxies, they should be present in the same percentage of the spectra of all QSOs with $z > 0.9$ regardless of their optical and radio properties. The incidence of absorption lines in the spectra of QSOs with emission lines of width and strength similar to those found in BL Lacertae objects is not known since up to now QSOs have generally been observed with a low dispersion sufficient to detect broad emission lines but inadequate to resolve and thus recognise the Mg(II) doublet; this is in contrast to the spectra of the BL Lacertae objects, especially the bright ones, which are thoroughly searched for

absorption lines. Clearly a systematic search for absorption lines in the spectra of QSOs will be useful for determining their origin.

NRAO is operated by Associated Universities, Inc., under contract with the NSF.

M.-H. ULRICH*

McDonald Observatory,
Department of Astronomy,
University of Texas,
Austin, Texas 78712

F. N. OWEN

National Radio Astronomy Observatory,
Edgemont Road,
Charlottesville, Virginia 22901

Received 21 April; accepted 30 August 1977.

Present address: ESO c/o CERN Geneva, Switzerland.

1. Hazard, C., Gulkis, S. & Sutton, J. *Astrophys. J.* **154**, 413–422 (1968).
2. Pauliny-Toth, I. I. K. & Kellermann, K. I. *Astr. J.* **77**, 797–809 (1972).
3. Carswell, R. F., Strittmatter, P. A., Williams, R. E., Kinman, T. D. & Serkowski, K. *Astrophys. J. Lett.* **190**, L101–104 (1974).
4. Burbidge, E. M., Caldwell, R. D., Smith, H. E., Liebert, J. & Spinrad, H. *Astrophys. J. Lett.* **205**, L117–120 (1976).
5. Wills, D. & Wills, B. J. *Astrophys. J. Suppl.* **31**, 143–164 (1976).
6. Strittmatter, P. A., Carswell, R. F., Gilbert, G. & Burbidge, E. M. *Astrophys. J.* **190**, 509–514 (1974).
7. Miller, J. S. & Hawley, S. A. *Astrophys. J. Lett.* **212**, L47–51 (1977).

Prebiotic polymers and infrared spectra of galactic sources

INFRARED absorption features characteristic of molecular dust clouds in the Galaxy may be assigned to complex organic polymers or prebiotic polymers. It could be argued that such highly stable, complex polymers evolve due to radiation processing of molecular mantles on interstellar grains—essentially by a type of natural selection which operates in the interstellar medium. A large fraction of all C, N, O elements in the interstellar medium could be condensed in the form of these stable polymers. Such interstellar material may also account for a significant fraction of the 'insoluble organic matter' which is found in carbonaceous chondrites.

The chemical composition of interstellar dust is uncertain. Observations of infrared excesses in cool stars combined with appropriate thermodynamic calculations support the view that grains comprised of silicates, iron and graphite are produced in these stars¹. It is unlikely, however, that these materials alone can satisfactorily explain the entire range of optical data relating to interstellar grains². Measurements of interstellar linear and circular polarisation point strongly to the dominance of a dielectric grain material at optical wavelengths³. Furthermore, estimates of the mean extinction coefficient of interstellar matter together with cosmic abundance data imply that the large bulk of grain material is comprised of C, N, O elements². These elements, in appropriate combinations with hydrogen, may exist as molecular mantles on grains. Mixtures of inorganic ices were proposed by van de Hulst⁴,

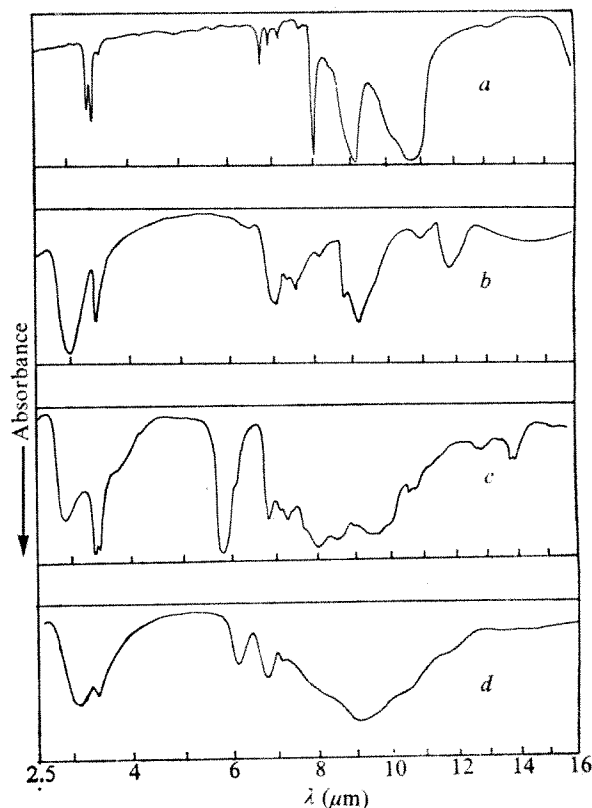


Fig. 2 Absorbance (A) of organic polymers as a function of wavelength. *a*, Polyformaldehyde (Delrin acetal resin); *b*, polyvinyl alcohol; *c*, natural resin: Shellac—regular bone dry, bleached—FEXO type¹². *d*, Average absorbance for 18 selected polymers of various types.

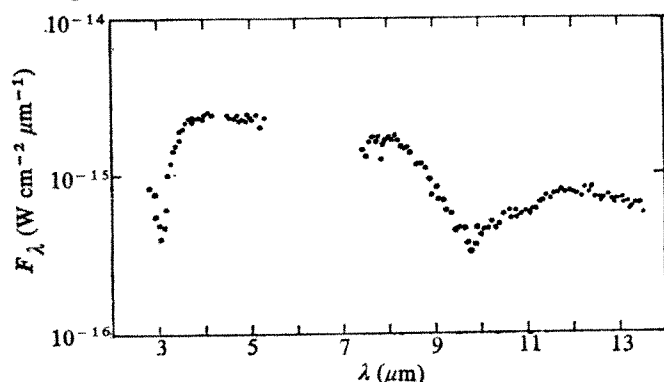
and N.C.W.⁵ has discussed the possibility of formaldehyde polymers. These two types of composition could represent intermediate stages in a progression towards an increasingly complex macromolecular structure for grain mantles.

We envisage the evolution of grain mantles in two distinct steps. First a mixture of relatively simple organic and inorganic ices (for example, CO, H₂O, NH₃, H₂CO) condenses on silicate grains in interstellar clouds. The combined effect of interstellar cosmic rays, X rays and ultraviolet photons on these grains will be to initiate solid-state polymerisation reactions of the type discussed by Goldanskii⁶. The end product of such processing of grain mantles is a stable, radiation-resistant organic polymer or prebiotic polymer. Such polymers may be thought of as evolving according to a type of natural selection in response to a wide range of selective pressures operative in the interstellar medium. It is possible that the low abundances of simple molecules and radicals (for example, OH, CH, CN) observed in diffuse interstellar clouds is maintained by a very slow degradation of these polymers.

Infrared absorption features observed in galactic sources, include bands centred on 3.1, 3.4 and 8–12 and ~20 μm. The most extensive observational data are available for the 8–12 μm feature. This band is seen in emission by hot dust in HII regions and circumstellar shells, as well as in absorption against sources of continuum infrared radiation. A silicate explanation for the 10 μm band seems natural for many sources exhibiting this feature, particularly in cases where dust is observed in circumstellar shells around oxygen-rich M giant stars⁷. Similarly, an H₂O–ice explanation for the 3.1-μm band may be tenable in some cases. In sources of exceptionally strong absorption, for example, the BN object in the Orion nebula, there are inadequacies in this conventional point of view.

Figure 1 shows the infrared spectrum of the BN object, which is probably one of the most heavily reddened objects in the Galaxy⁸. Here we see absorption bands centred on wavelengths close to 3

Fig. 1 Infrared spectrum on the BN object in the Orion Nebula⁸.



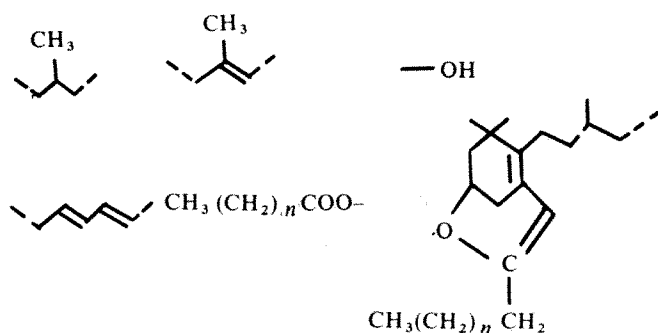
and 10 μm . The 10- μm band is usually attributed to silicates, and the 3- μm bands to H_2O -ice. Both these compositional assignments present problems. If we assume that the overall composition of dust which obscures the BN object is representative of grains in the general interstellar medium, the mass ratio of ice to silicates which is consistent with the observed relative strengths of the two bands in Fig. 1 is inconsistent with cosmic abundance criteria^{2,9}. Also, the detailed shape and width of this 10 μm band does not give a satisfactory fit with the absorption spectrum of any known silicate.

Table 1 Vibrational frequencies for various linkages in organic molecules¹¹

Bond	Wavelengths (μm)
C-C, C-O, C-N	7.7-12.5
C=C, C=O, C=N, N=O	5.3-6.7
C \equiv C, C \equiv N	4.4-5.0
C-H, O-H, N-H	2.6-3.7

Another case which presents difficulties for simple silicate-ice models is the infrared spectrum of a source at the galactic centre. A broad 3- μm band, which includes a weak feature at 3.4 μm , cannot be explained in terms of a simple model of H_2O -ice grains¹⁰. Likewise, the deep, narrow absorption band centred on 9.7 μm in this source⁷ cannot readily be explained by silicate-type grains.

The possibility that organic polymers could contribute to absorptions at 3, 3.4 μm , and over the 8-12- and 18-24- μm wavebands in galactic sources suggests that organic polymers could provide a potentially more flexible and viable explanation of astronomical absorption spectra at near and mid-infrared wavelengths compared with silicate grains. The mass density of absorbing material in this former category may be higher by an order of magnitude, because of the higher cosmic abundance of C, N, O elements.

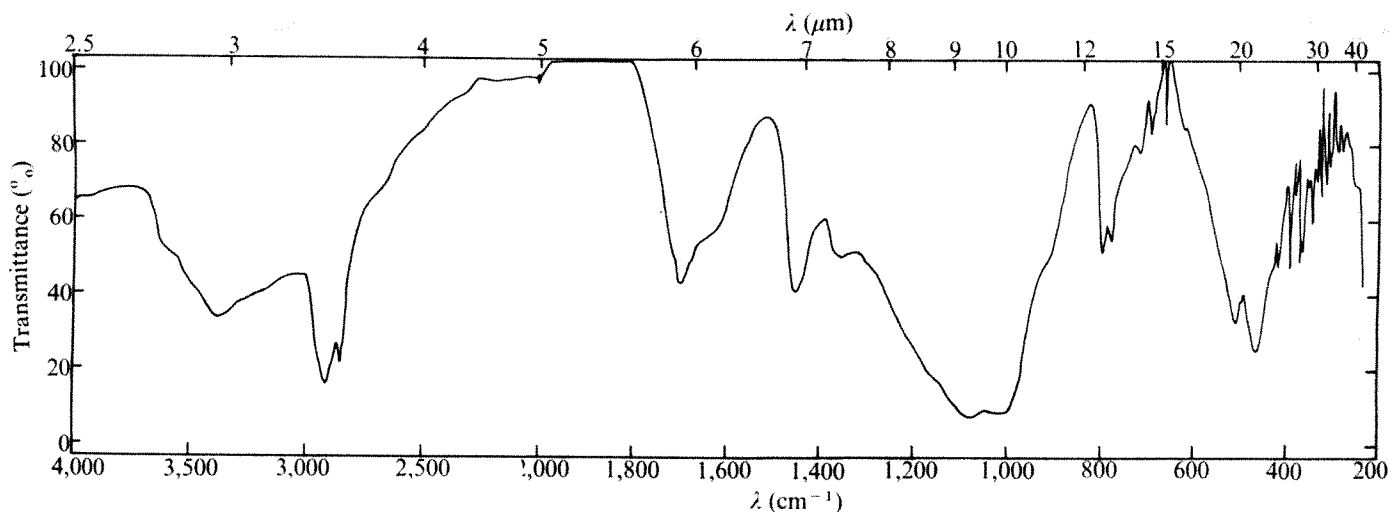


Organic molecules and polymers are known to have broad absorption bands in the wavelength region 1-100 μm due to transitions between vibrational-rotational levels. Over the waveband 2.5-13 μm considered above, stretching modes of elementary bonds which contribute to absorptions are listed in Table 1.

The precise positions of band centres and band widths appropriate to individual groups depend on associated linkages and on the particular molecular environment in which these groups are placed. From extensive spectral data¹² on organic polymers, absorption features in the wavelength ranges 8-12 and 2.5-3.5 μm are seen to be the most persistent properties encompassing natural and synthetic polymeric compounds of widely ranging types.

Figure 2 shows the absorbance spectra of an acetal resin (a), polyvinyl alcohol (b) and a natural resin (c). While we could not find a single spectrum of a simple polymer which provides a satisfactory fit to the data in Fig. 1, the average spectrum of a number of different types of polymer tends to produce a relatively structureless, broad 10- μm band giving better agreement with this astronomical absorption feature. Figure 2d represents a synthetic spectrum obtained simply by averaging absorbance data^{11,12} for a selection of 18 polymeric species. The selection was made so as to include polymers of a wide variety of types, but with a bias towards those which exhibited broad bands centred near 3 μm due to either O-H or N-H stretching modes. This 'synthetic' curve is fairly close to a smoothed out mean of the three absorbance spectra Fig. 2a, b and c. Although we cannot claim any uniqueness for our synthetic spectrum (d), this curve provides significantly better agreement with the BN source data (Fig. 1) than any model involving silicate-ice grains. This cannot be dismissed as fortuitous. A smooth absorption feature of appreciable strength in the 8-12 μm waveband arises quite naturally for an average polymer spectrum, if we take a large enough number of polymers of various types^{11,12}. But the requirement of either a strong 3- μm band or a 3.4- μm band demands a more restrictive choice. In this context it is worth noting that some galactic sources show strong bands at 3.4 μm , but not the usual 8-12- μm feature. A case in point is the emission spectrum of the planetary nebula NGC7027 (ref. 13). It is also interesting that some astronomical data, including the extended infrared emission source in the Trapezium nebula¹⁴, indicate a significant absorption coefficient in the '10- μm band' of interstellar dust extending to $\lambda \geq 13 \mu\text{m}$. There are other cases where a 3- μm absorption band, wider than one which can usually be attributed to ices, occurs together with a broad 8-13- μm band¹⁵. Such absorptions cannot readily be explained by silicates and H_2O -ice but could be obtained by biasing our organic compound mix to include a substantial fraction of amides. These compounds have a broad 3- μm feature due to N-H stretching as well as a broad absorption feature in the 12.5-15- μm region due to out-of-plane NH wagging¹¹; a typical case is the spectrum of isobutyramide¹¹.

Fig. 3 Infrared absorption spectrum of sporopollenin from *Tasmanites punctatus* fossil planktonic algae. Sporopollenin is an oxidative co-polymer of carotenoid-carotenoid esters containing the molecular and macromolecular groupings shown above.



An exceedingly complex organic or prebiotic polymer which evolves in the interstellar medium could well possess absorption properties similar to those of the synthetic polymer spectrum Fig. 2d. Alternatively, the average interstellar absorption curve may be due to an ensemble of less complex polymers which has an average spectrum similar to Fig. 2d. In the context of the former possibility, it may be of interest to consider the properties of highly stable naturally occurring biopolymers.

A particularly stable polymer of this type is sporopollenin, an oxidative copolymer of carotenoid-carotenoid esters, which forms a major component of pollen and many spore walls. Figure 3 shows the infrared absorption spectrum of sporopollenin from *Tasmanites* fossil planktonic algae over the wavelength region 2.5 to 40 μm . This spectrum shows a broad absorption feature at 3 μm , a narrower 3.4- μm band, and a deep and somewhat narrow absorption band at 9.7 μm generally similar to data for the galactic centre infrared source^{7,10}. In Fig. 3 there is also a band at 20 μm similar to one which is regarded as providing additional evidence for interstellar silicates. Sources where the detection of a 20- μm band has been claimed include the Trapezium and KL nebulae¹⁶.

Although these spectral similarities do not prove the presence of sporopollenin in interstellar space, nor indeed of any biopolymer, there is an indication here of a fairly complex, radiation-resistant prebiological polymer associated with interstellar grains. It is possible that the conversion of interstellar grain mantles into radiation resistant polymers, and the formation of protective coatings on pollens and spores followed similar chemical pathways, in response to broadly similar selective pressures. Prebiotic polymers with large-scale molecular groupings similar to those occurring in sporopollenin (but less complex) may be the net result of such an evolution. Infrared spectra of these two systems could be fairly similar.

We have already suggested an identification of the λ 2,200 Å interstellar absorption band with electronic transitions in conjugated multiple bonds of prebiotic molecules¹⁷—such molecules being supposedly trapped within polymeric matrices of the type considered here. Sakata *et al.*¹⁸ have also shown that a similar ultraviolet absorption feature occurs in the soluble organic matter extracted from a carbonaceous chondrite. This similarity in ultraviolet spectra was interpreted as support for the inclusion of primitive grain clump material in meteorites. In view of the infrared spectral identification suggested here, we might further argue that the insoluble organic matter of carbonaceous chondrites may also have an interstellar origin. This material is too complex for detailed identification, but is believed to be comprised of highly condensed aromatic polymers which account for nearly 70% of the total carbon content in these meteorites¹⁹. Such prebiological polymers, processed in the interstellar medium, could have played a crucial part in the evolution of terrestrial life.

We (F.H. and N.C.W.)²⁰ have also shown a strong case for the class of biopolymer—polysaccharides—in interstellar dust. Other organic polymers such as discussed above would probably be less abundant than polysaccharides.

N. C. WICKRAMASINGHE
F. HOYLE*

Department of Applied Mathematics and Astronomy,
University College,
Cardiff, UK

J. BROOKS
G. SHAW

School of Chemistry,
University of Bradford,
Bradford, UK

Received 18 April; accepted 19 August 1977.

*Honorary Professional Fellow. Present address: Coxley Moor, Dockray, Penrith, Cumbria.

1. Hoyle, F. & Wickramasinghe, N. C. *Nature* **223**, 459 (1969).
2. Wickramasinghe, N. C. in *Solid State Astrophysics* (eds Wickramasinghe, N. C. & Morgan, D. J.) (Reidel, Dordrecht, 1975).
3. Martin, P. G., Illing, R. & Angel, J. R. P. in *Interstellar Dust and Related Topics* (eds Greenberg, J. M. & van de Hulst, H. C.) (Reidel, Dordrecht, 1973).
4. van de Hulst, H. C. *Rech. astr. Obs. Utrecht* **11**, Part 1 (1946).
5. Wickramasinghe, N. C. *Nature* **252**, 462 (1974).
6. Goldanskii, V. I. *A. Rev. phys. Chem.* **27**, 85 (1976).

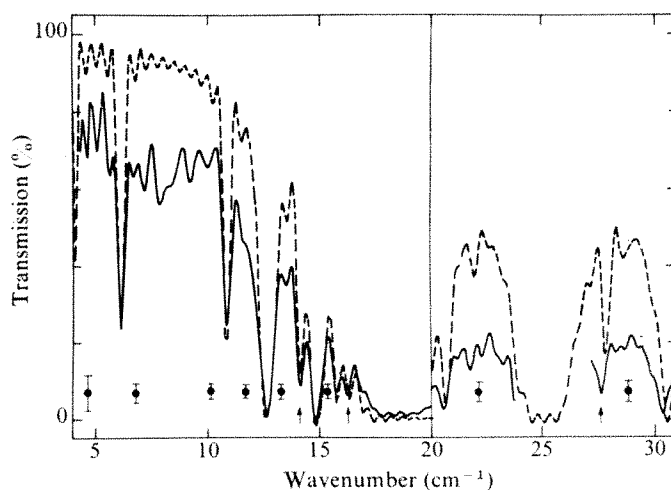
7. Woolf, N. J. in *Interstellar Dust and Related Topics* (eds Greenberg, J. M. & van de Hulst, H. C.) (Reidel, Dordrecht, 1973).
8. Gillett, F. C. & Forrest, W. J. *Astrophys. J.* **179**, 483 (1973).
9. Dempsey, M. J. & Wickramasinghe, N. C. *Astrophys. Space Sci.* **34**, 185 (1975).
10. Soifer, B. T., Russell, R. W. & Merrill, K. M. *Astrophys. J.* **207**, L83 (1976).
11. Silverstein, R. M., Clayton Bassler, G. & Morrill, T. C. *Spectrometric Identification of Organic Compounds* (Wiley, New York, 1974).
12. Nyquist, R. A. *Infrared Spectra of Polymers and Resins* (Dow Chemical Co., 1961).
13. Merrill, K. M., Soifer, B. T. & Russell, R. W. *Astrophys. J.* **200**, L37 (1975).
14. Forrest, W. J., Gillett, F. C. & Stein, W. A. *Astrophys. J.* **192**, 351 (1975).
15. Gillett, F. C. & Soifer, B. T. *Astrophys. J.* **207**, 780 (1976).
16. Forrest, W. J. & Soifer, B. T. *Astrophys. J.* **208**, L129 (1976).
17. Hoyle, F. & Wickramasinghe, N. C. *Nature* **266**, 241 (1977).
18. Sakata, A. *et al.* *Nature* **266**, 241 (1977).
19. Nagy, B. *Carbonaceous Meteorites* (Elsevier, Amsterdam, 1975).
20. Hoyle, F. & Wickramasinghe, N. C. *Nature* **268**, 610 (1977).
21. Brooks, J. & Shaw, G. *Origin and Development in living systems* (Academic, New York, 1973).

Atmospheric absorption between 4 and 30 cm^{-1} measured above Mauna Kea

CONSIDERABLE attention is being given to the construction of new telescopes which will extend ground-based astronomical observations to millimetre and sub-millimetre wavelengths, and so it is essential to have a better understanding of atmospheric absorption at these wavelengths. We report here measurements made from the summit of Mauna Kea in Hawaii of the absorption of radiation coming through the atmosphere in slant paths from the sun. These have extended previous knowledge of absorption at this site^{1,2} to lower frequencies and have confirmed that a little understood phenomenon which we call anomalous absorption can greatly reduce atmospheric transparency at some millimetre wavelengths theoretically predicted to be good windows. To compare our measurements with theoretical predictions, particular attention was paid to determinations of atmospheric water content in the same path from the sun. A complete absorption band near 1.4 μm was scanned, therefore, using a monochromator and values of precipitable water were derived by well known formulae³.

Spectra in the wavenumber range 4–30 cm^{-1} were obtained by Fourier inversion of the output of a Michelson interferometer which was illuminated by a 50-cm diameter telescope and used a Golay detector. Interferograms giving a resolution of 0.5 cm^{-1} were recorded in 30 min. As the sun is nearly a black body at these frequencies, a local black-body source was used

Fig. 1 Measurements in January–February 1976 at Mauna Kea (elevation 4.2 km, latitude 19°50', longitude 155°28'). The solid line is an average transmission spectrum for which the mean zenith angle is 55°. The resolution is 0.5 cm^{-1} . Below 20 cm^{-1} the average is from nine days of observation with a mean water vapour content of 1.9 mm precipitable in the path. Above 20 cm^{-1} the average is from the four driest days, with 0.9 mm precipitable. The displaced error bars represent the standard deviation of the noise on the spectra. The dotted line shows theoretical predictions in which the small oscillations between known lines come from including the effects of the instrumental 'spectral windows'. Arrows mark O_2 lines, other lines are due to water.



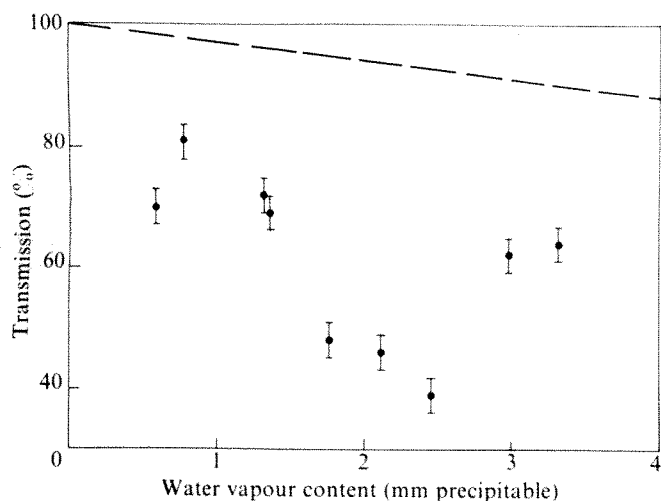


Fig. 2 Daily averages of transmission at 7.7 cm^{-1} plotted against the measured water vapour content. Mean zenith angles are in the range $47\text{--}61^\circ$. The dashed line shows predicted transmission.

to provide a calibration by which day-to-day changes in radiometric sensitivity could be followed and also give a reference spectrum by which the solar spectra were divided to measure atmospheric transmission. As we could not record a solar signal without absorption, measurements for one day of particularly high atmospheric transparency were used to establish the transmission scale. We selected a wavenumber of 10.3 cm^{-1} where there is low atmospheric absorption and assumed that the only loss was that predicted by the theoretical model corresponding to the measured water content. We found that on that day the signal strength varied with zenith angle by the expected amount. Figure 1 shows a composite spectrum from several observations averaged as described in the legend. These data were obtained in clear conditions and for the wavelengths under discussion this means that attenuation by particles could be neglected. Theoretical spectra were calculated from AFRL tabulations of molecular line parameters⁴.

As well as the expected lines of water and oxygen, additional absorption was observed which seriously affects 'windows' used for astronomical observation. The extra component is described as anomalous absorption and no simple correlation between its strength and atmospheric water content was found. Figure 2 shows this in the behaviour of the atmospheric window near the astronomically important $J = 1 \rightarrow 2$ pure rotation line of carbon monoxide at 7.7 cm^{-1} . At this frequency the average absorption was 2.1 dB where the water vapour and oxygen model predicts 0.23 dB for the mean water content of 1.9 mm precipitable.

As an alternative to radiometric calibration, it is common practice to assess atmospheric absorption by measuring signals as a function of the zenith angle from an astronomical source. Assuming that the absorbers are horizontally stratified and do not vary in concentration during a sequence of measurements, a plot of absorption against airmass would be a straight line through the origin. Plotting absorption values for the interval 7 cm^{-1} to 9.5 cm^{-1} measured in this way, we found that the intercept at zero airmass was significantly greater than zero on most days, as shown in Fig. 3. This suggests that stratification of the molecules giving rise to anomalous absorption may be affected by the presence of the mountain and that the method of calibration based on zenith angle dependence, though widely used, may not apply in such circumstances.

We conclude that anomalous absorption, frequently reported⁵ in qualitative terms, is a major phenomenon at Mauna

Kea. The observed magnitudes are similar to values previously obtained at Mauna Kea¹, and other mountain sites^{6,7}. The variability in the effect which we observed is sufficiently great that these observations are not necessarily in conflict with the low values recorded at White Mountain, California⁸. The amount of anomalous absorption in the range $7\text{--}9.5 \text{ cm}^{-1}$ is consistent with what has been found in horizontal atmospheric paths⁹ if reasonable values of scale height for absorbers are assumed. The non-zero intercept on the absorption against

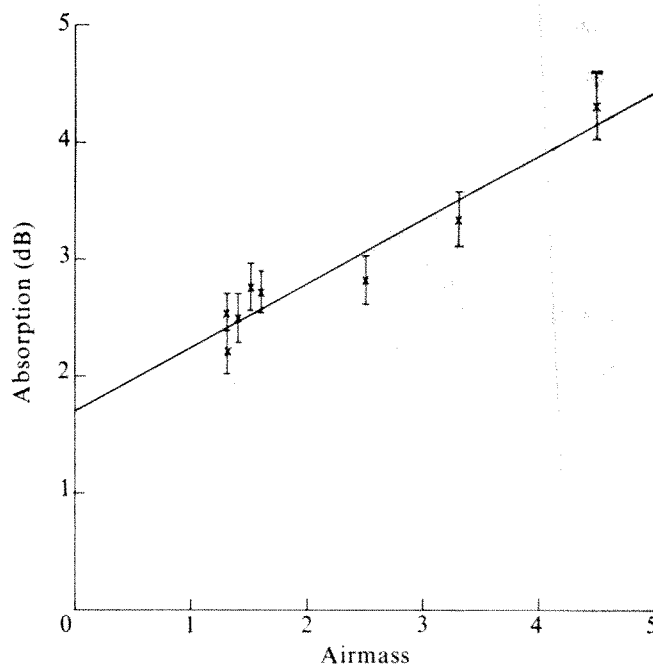


Fig. 3 Each point, which represents absorption averaged over the 7.0 cm^{-1} to 9.5 cm^{-1} interval, was measured in the same day. The error bars give an estimate of the standard deviation of the noise in this interval. Airmass is defined as the ratio of the amount of atmosphere along the observing path to the amount in the zenith direction. The line fitted to the data gives an intercept of $1.7 \pm 0.2 \text{ dB}$.

airmass plot suggests that there may be factors specific to a site which can enhance anomalous absorption.

We thank Professor J. T. Jefferies for the use of Mauna Kea Observatory, University of Hawaii, also Mr T. Krieger and his staff at the Observatory for their assistance.

P. H. MOFFAT
R. A. BOHLANDER
W. R. MACRAE
H. A. GEBBIE

SRC, Appleton Laboratory,
Ditton Park, Slough, UK

Received 27 April; accepted 30 August 1977.

1. Nolt, I. G., Martin, T. Z., Wood, C. W. & Sinton, W. M. *J. atmos. Sci.* **28**, 238–241 (1971).
2. Morrison, D., Murphy, R. E., Cruickshank, D. P., Sinton, W. M. & Martin, T. Z. *Pub. astr. Soc. Pacif.* **85**, 255–267 (1973).
3. Howard, J. N., Burch, D. E. & Williams, D. J. *opt. Soc. Am.* **46**, 242–245 (1956).
4. McClatchey, R. A. et al. *AFRL Atmospheric Absorption Line Parameters Compilation AFRL-TR-73-0096*, 1973.
5. Gebbie, H. A., Chamberlain, J. & Burroughs, W. J. *Nature* **220**, 893–895 (1968).
6. Gimmestad, G. G., Gebbie, H. A., Bohlander, R. A. & Mendoza, V. E. *E. Astrophys. J.* **178**, 267–270 (1972).
7. Gimmestad, G. G., Ware, R. H., Bohlander, R. A. & Gebbie, H. A. *Astrophys. J.* (in the press).
8. Mather, J. C., Werner, M. W. & Richards, P. L. *Astrophys. J.* **170**, L59–65 (1971).
9. Emery, R. J., Moffat, P., Bohlander, R. A. & Gebbie, H. A. *J. atmos. terr. Phys.* **37**, 587–594 (1975).

Carbonate mineral detection by variable atmosphere differential thermal analysis

GREATLY improved detection limits for the presence of anhydrous carbonate minerals in mixtures with other types of minerals can be obtained by variable atmosphere differential thermal analysis (DTA) using dynamic furnace atmospheres of CO_2 (flow rate 100 ml min^{-1})¹. Details will be published elsewhere^{2,3} (see ref. 1 for details of the Du Pont unit and conditions of analysis). These carbonates fall into two groups: those which decompose in CO_2 with single, or multiple⁴ endothermic dissociation reactions liberating CO_2 . The first group contains, calcite CaCO_3 , magnesite MgCO_3 , smithsonite ZnCO_3 , and siderite FeCO_3 (ref. 5); while the second includes dolomite $\text{CaMg}(\text{CO}_3)_2$, ankerite $\text{Ca}(\text{MgFe})(\text{CO}_3)_2$ and cerussite PbCO_3 (ref. 4). Here calcite and dolomite are taken as typical examples and their behaviour described.

In conditions of dynamic CO_2 compared with static air, the single endothermic peaks of group one members show several important DTA curve modifications (Fig. 1*b* and *c*): peaks become narrower, and more sharply defined with much increased peak heights: the complete peaks, including initial, peak and final temperatures, move up scale to occur at considerably higher temperatures.

The multiple endothermic peaked second group behave differently in the same conditions (Fig. 1*d* and *f*): the initial peak becomes displaced down scale^{2,6} (sometimes, due to crystallinity and equipment, for low concentrations, the known fall in peak temperatures due to dilution when determined in air⁶ and their relative stability in dynamic CO_2 may cause both dolomite peaks to appear up scale from the fused composite peak representing them in air (Fig. 1*d* and *f*)). The higher temperature peak (dolomite) or peaks (ankerite) move up scale to occur at higher temperatures with considerably increased and decreased peak heights and widths respectively: markedly increased peak sep-

aration and definition results. Individual peaks remain recognisable down to the limits of detection (see Fig. 1*e*) and do not, with progressive content dilution coalesce into the single broad similar (difficult to identify) features which typically result from determinations in air (compare Fig. 1*d* and *f*). The size of the now clearly resolved middle endothermic peak of ankerite, varies with iron content².

The DTA of such carbonates in these conditions enables their greatly improved detection down to contents in the order of 0.25% ³, the identification of members of the dolomite-ferroan dolomite-ankerite series² and the detection of 'iron carbonate' components, present either as siderite or ankerite-ferroan dolomite with detection limits and content evaluations² which seem considerably superior to those obtainable by routinely available X-ray diffraction data, which emphasises the potential of DTA in this aspect of determinative mineralogy.

I thank the University of Bristol (Geology) and the Macaulay Institute for Soil Research (Pedology) for the opportunity to undertake this work during the tenure of a visiting professorship.

S. ST J. WARNE

Department of Geology,
The University of Newcastle,
Shortland, New South Wales 2308,
Australia

Received 28 July; accepted 19 August 1977.

1. Warne, S. St J. & Mackenzie, R. C. *J. therm. Anal.* **3**, 49-55 (1971).
2. Warne, S. St J. *5th Int. Confed. therm. Anal.* Japan (1977).
3. Warne, S. St J. & Mitchell, B. D. *Br. J. Soil Sci.* (submitted).
4. Warne, S. St J. & Bayliss, P. *Am. Miner.* **47**, 1011 (1962).
5. Warne, S. St J. *Chem. Erde*, **35**, 251 (1976).
6. Wolf, K. H., Easton, A. J. & Warne, S. St J. *Carbonate Rocks* B, 253-341 (Elsevier, Amsterdam, 1967).

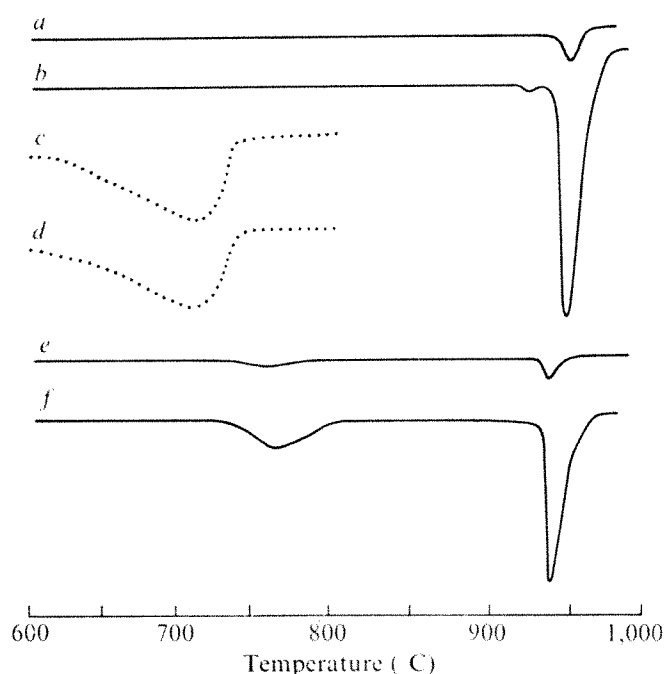
Δ^2 -Sterenes as diagenetic intermediates in sediments

A SMALL fraction of the sterols derived from living organisms is found intact or partially degraded in the geological environment. Seawater and many recent or relatively immature older sediments contain unaltered, or only slightly altered sterols¹⁻⁸, whereas in older sediments and petroleum these compounds have usually been transformed by redox reactions into their saturated (steranes) or partially aromatised counterparts⁹⁻¹². Due to the high stability of the steroid skeleton, products derived from steroids can be detected in consolidated sediments far into an advanced stage of maturation. We have examined 11 recent sediments and suggest here that the Δ^2 -sterenes which they contain are degradation intermediates of the precursor sterols.

The characterisation of degradation intermediates, for example, unsaturated hydrocarbons, can bring a better understanding of the nature of the evolution pathways undergone by the sterols in the sedimentary environment, as well as useful information on the stages of evolution at which various degradation reactions take place¹³. Mono- and di-unsaturated steroid alkenes have been tentatively detected in various sediments, but these compounds have not been conclusively identified¹⁴⁻¹⁶.

We have now studied the unsaturated hydrocarbon fraction of 11 recent sediments—five from the sea of Norway, one from the Baltic sea, two from the vicinity of the Amazon estuary, one from the Cariaco Trench near Venezuela and two continental muds from the immediate vicinity of Strasbourg. The samples were frozen and freeze-dried immediately after collection. The dry samples were then extracted with freshly-distilled chloroform in a Soxhlet apparatus or under ultrasonics. When necessary the total extracts were desulphurised on active copper and separated by thin-layer chromatography (TLC) on SiO_2 with hexane elution. The unsaturated hydrocarbons (10-20% of the total saturated + unsaturated hydrocarbons) were further separated by Ag^+/SiO_2 TLC with hexane elution yielding 1-2 p.p.m. by weight based on

Fig. 1 Differential thermal analysis curves of calcite and dolomite obtained from artificial mixtures of *a*, 0.5% calcite; *b*, 6% calcite; *c*, 6% calcite; *d*, 6% dolomite; *e*, 0.5% dolomite; *f*, 6% dolomite by weight with calcined alumina. Determinations being made in furnace atmosphere conditions of static air (dotted line) or dynamic carbon dioxide (solid line). The much improved peak definition, resolution and detection limits are clearly shown by the curves determined in dynamic carbon dioxide.



dry sediment (standard precautions against contamination necessary in organic geochemical studies were taken throughout). Gas chromatographic (GC) analyses were carried out on 1% Dexsil-packed glass columns (2.5 m \times 3 mm) and OV-101 and Apiezon L capillary columns (25 m \times 0.5 mm i.d.) using a GC-mass spectrometry-computer set-up (LKB 9000 S/PDP 11E 10). In control experiments a solution of 70 mg of pure cholesterol in chloroform was treated with 50 g of a chloroform pre-extracted sediment under ultrasonics: no trace of Δ^2 -sterenes could be detected after extraction and separation following the analytical procedure described above.

We have identified, from the unsaturated fractions of the recent sediments studied, three major constituents (Fig. 1) as the C_{27} , C_{28} and C_{29} $5(\alpha H)$ Δ^2 -sterenes *a-c* by the following criteria: mass spectral fragmentation patterns identical with those of the corresponding Δ^2 -sterenes which we and others¹⁷ had prepared, and co-elution with the reference compounds (C_{27} and C_{29}) on two capillary columns (OV-101 and Apiezon L). The mass spectra of the Δ^2 -sterenes show significant differences from those of Δ^4 - or Δ^5 -sterenes, since the former are characterised by an important fragment at *m/z* 54 (loss of butadiene due to a retro-Diels reaction)¹⁸. Stereochemistry at C_{24} remains undetermined.

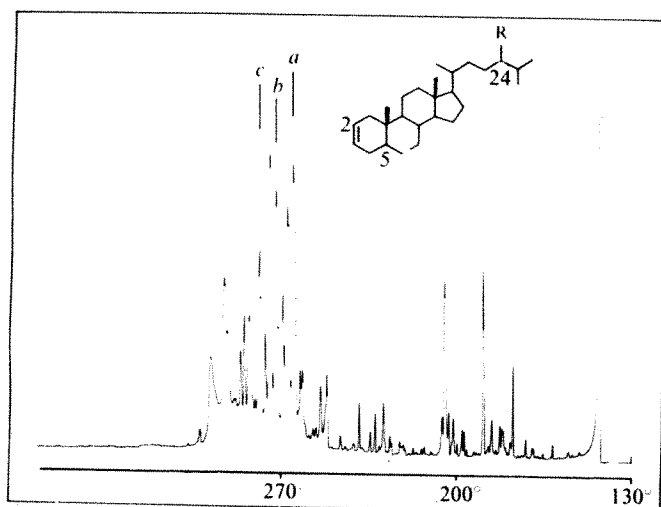


Fig. 1 Gas chromatogram of the unsaturated hydrocarbons of a recent marine sediment (5,000 yr) from the Cariaco Trench, near Venezuela. Conditions: OV 101, 25 m \times 0.5 mm i.d., 130–270 $^{\circ}C$, 3 $^{\circ}C$ min⁻¹. Peaks correspond to the Δ^2 -sterenes: *a*, $R = H$; *b*, $R = CH_3$; *c*, $R = C_2H_5$.

Recent results are good evidence for the microbiological reduction of Δ^2 -sterols into the corresponding $5\alpha H$ (and $5\beta H$) stanols in the first stages of diagenesis in surface sediments^{7,19,20}. The Δ^2 -sterenes (which have so far only been reported in non-fat dry milk¹⁷) may be formed in a further stage by dehydration of the corresponding stanols. In one of our samples, from the sea of Norway, which showed a slightly acidic pH, we noticed the concomitant presence of a series of $\Delta^{13(17)}$ -sterenes (C_{27} – C_{29}) with a backbone-rearranged skeleton. It is reasonable to assume that these compounds, which we have previously identified in several older shales, are formed from stanols by an acid-catalysed skeletal rearrangement through Δ^2 -sterene intermediates. This process has been clearly demonstrated by simulation experiments with cholesterol or Δ^2 -cholestene and montmorillonite clay²¹. Further studies, however, are still needed to confirm that the isolation of rearranged sterenes is really due to the observed low pH.

The identification of Δ^2 -sterenes in various recent sediments deposited in marine or continental environments suggests that these compounds are degradation intermediates of the precursor sterols, via the corresponding stanols, in the sedimentary environment. It is also possible that they arise directly from stanols, since

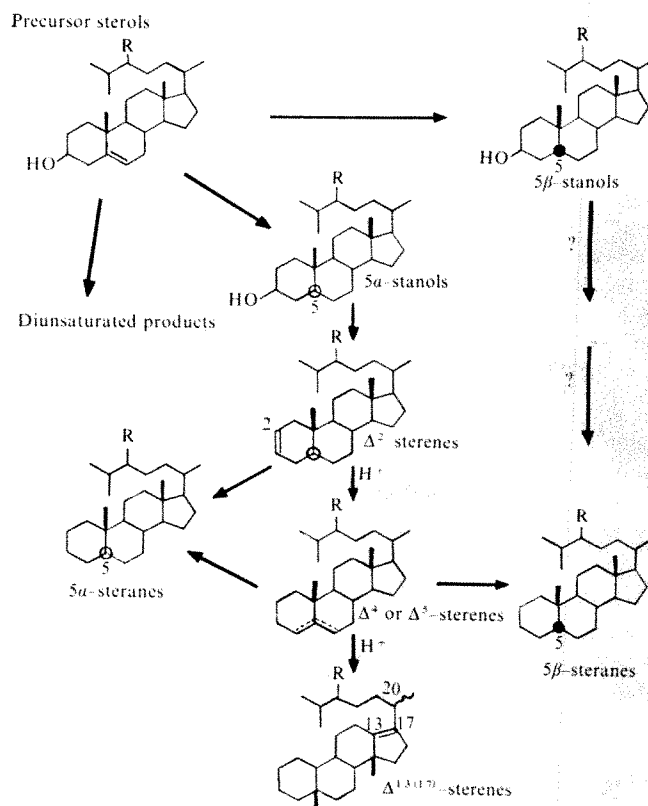


Fig. 2 Early transformations of sterols in the geological environment, based on laboratory and field results. Processes marked ? are only inferred (see refs 8, 9, 20, 21 and refs therein) $R = H, CH_3, C_2H_5$.

the latter have been detected in several living organisms⁸. These alterations start at a very early stage of diagenesis in surface sediments. These results, combined with our, and other authors' previous results, increase our knowledge of the first geochemical evolution pathways undergone by the sterols in the geological environment, as shown in Fig. 2.

This work constitutes a contribution to the 'Projet ORGON', an interdisciplinary approach study of recent sediments undertaken by several French laboratories. We thank ELF-Aquitaine for financial support.

MICHEL DASTILLUNG
PIERRE ALBRECHT

Laboratoire de Chimie Organique des

Substances Naturelles,

Associé au C.N.R.S.,

Institut de Chimie,

1, rue Blaise Pascal, 67008 Strasbourg, France

Received 29 March; accepted 8 August 1977.

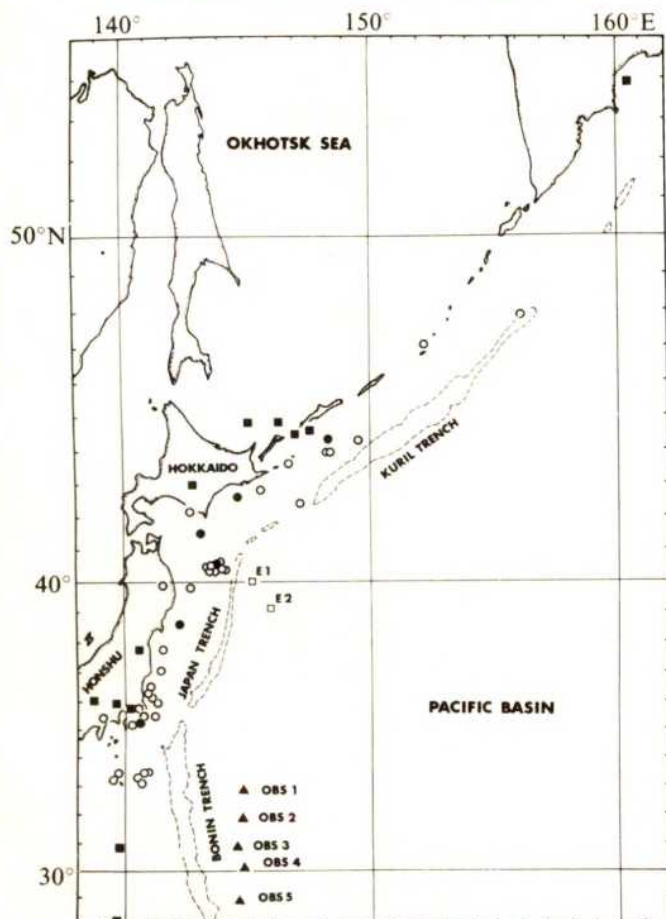
- Gagosian, R. B. *Geochim. cosmochim. Acta* **39**, 1443–1454 (1975).
- Salot, A. & Barbier, M. *Deep-Sea Res.* **20**, 1077–1082 (1973).
- Attaway, D. & Parker, P. L. *Science* **169**, 674–675 (1970).
- Ogura, K. & Hanya, T. *Proc. Japan Acad.* **49**, 201–204 (1973).
- Mattern, G., Albrecht, P. & Ourisson, G. *Chem. Commun.* 1570–1571 (1970).
- Wang, W. E. & Meinschein, W. G. *Geochim. cosmochim. Acta* **40**, 323–330 (1976).
- Nishimura, M. & Koyama, T. *Chem. Geol.* **17**, 229–239 (1976).
- Nishimura, M. & Koyama, T. *Geochim. cosmochim. Acta* **41**, 379–385 (1977).
- Henderson, W., Reed, W. E. & Steel, G. in *Advances in Organic Geochemistry 1971* (eds von Gaertner, H. R. & Wehner, H.) 335–352 (Pergamon, Oxford, 1972).
- Anderson, P. C., Gardner, P. M., Whitehead, E. V., Anders, D. E. & Robinson, W. E. *Geochim. cosmochim. Acta* **33**, 1304–1307 (1969).
- Hills, I. R., Smith, G. W. & Whitehead, E. V. *J. Inst. Petrol.* **56**, 127–137 (1970).
- Rubinstein, I. & Albrecht, P. *JCS Chem. Commun.* 957–958 (1975).
- Albrecht, P. & Ourisson, G. *Angew. Chem.* **83**, 221–238 (1971); *Angew. Chem. Internat. ed.* **10**, 209–225 (1971).
- Steel, G., Reed, W. E. & Henderson, E. in *Advances in Organic Geochemistry 1971* (eds von Gaertner, H. R. & Wehner, H.) 353–364 (Pergamon, Oxford, 1972).
- Ikan, R., Baedeker, M. J. & Kaplan, I. R. *Geochim. cosmochim. Acta* **39**, 195–203 (1975).
- Cardoso, J. et al. in *Environmental Biogeochemistry*, **1**, 149–174 (ed. Nriagu, J. O.) (Science, Ann Arbor, 1976).
- Flanagan, V. P. & Ferretti, A. *Lipids* **9**, 471–475 (1974).
- Budzikiewicz, H. in *Biochemical Applications of Mass Spectrometry* (ed. Waller, G. R.) 251 (Wiley, New York, 1972).
- Gaskell, S. J. & Eglinton, G. *Nature* **254**, 209–211 (1975).
- Gaskell, S. J. & Eglinton, G. *Geochim. cosmochim. Acta* **40**, 1221–1228 (1976).
- Rubinstein, I., Sieskind, O. & Albrecht, P. *JCS Perkin I* 1833–1836 (1975).

High shear velocity layer in the upper mantle of the Western Pacific

SHEAR waves have been only rarely observed in explosion seismology studies at sea, so their precise velocities have not been extensively studied. An inversion method from seismic surface wave studies has been used to measure the shear wave velocities of the oceanic upper mantle¹⁻³. But, the values obtained, generally 4.6 km s^{-1} , are inherently an average of a path from a seismic source to a seismic recording site on land, so that the method can not give a 'pure' value for the upper oceanic mantle. Shear wave velocity is, however, important for the physical property of the upper mantle as well as for discriminating the minerals which make up the oceanic upper mantle. We describe here our measurements of apparent shear wave velocities, using 61 natural earthquakes with epicentral distances ranging from 400 to 2,900 km. Results indicate that the lower part of the oceanic lithosphere contains a large amount of garnet.

A linear array of high sensitive ocean bottom seismographs (OBSs) was positioned on the Western Pacific Basin, where the Pacific lithosphere is thought to be very old and about to subside (Fig. 1). The water depth is between 5,500 and 5,600 m. The linear dimension of the array is about

Fig. 1 A map showing the array of ocean bottom seismographs (▲) and precisely located epicentres of earthquakes, having focal depths shallower than 40 km (○), 41–70 km (●), and 71–190 km (■). Note that most of the wave paths from the foci to the OBS array pass through the upper mantle of the Pacific lithosphere (if we consider that the lithosphere is subducting north-westerly); hence the obtained apparent velocities represent those of Pacific upper mantle. □, 7- and 5-t explosions which were used for the explosion seismology study⁴ where P-wave structure has been obtained for the region.



400 km, so that the apparent velocities were accurately measured within the array⁴.

The observations were carried out during a two-week period in October 1974, when 61 shallow earthquakes were precisely located by various land seismograph networks (Japan Meteorological Agency, USGS, the seismological network of Sakhalin Complex Scientific Institute USSR, seismological networks operated by Tokyo University and Hokkaido University in Japan). Most of the earthquakes occurred along the Kuril trench and Japan trench which are, seismically, the most active regions in the world. Since the OBSs are sensitive, earthquakes with magnitude 3 or 4 were clearly recorded at distances of 1,000 or 1,600 km, respectively.

Our observed S travel times, from earthquake foci to the OBSs, are shown in Fig. 2. These are markedly smaller than those of the Jeffreys–Bullen travel time tables. The differences are 10–30 s for shallow earthquakes with 400 to 1,800 km range of epicentral distances. The results indicate that the structure of the oceanic upper mantle at the Western Pacific is unusual. Travel times, however, have inherent limitations in resolution since they are strongly affected by errors in their origin times which are given by seismograph networks on land.

Apparent velocities, which are measured from passing times of seismic waves which cross the OBS array and indicate the velocities of the rocks at the deepest points of seismic rays, are accurate, since they are free from errors in the origin times. Although they give useful information, the apparent velocities are the least sensitive quantity to the complexity of the structure.

The region west of the trenches probably has a complicated structure that includes a continental shelf overlying a subducting lithosphere. The structure may cause errors in the determination of hypocentres and deflections of seismic waves which are incident to the OBS array.

Some calculations on ray paths, using plausible velocity models for the region, reveal that the complexity of the region's structure cannot affect the obtained apparent velocities by more than 1%. The size of the error is the result of choosing earthquakes with small errors in focal positions for our analysis and also that the earthquake foci are very close, in a three-dimensional sense, to the plausible subducting lithosphere, which is considered to subduct towards the north-westerly from the trenches.

The apparent velocities were calculated from pairs of readings by adjacent OBSs, which are indicated by line segments in Fig. 2. Figure 3 demonstrates that V_s values are faster than 4.6 km s^{-1} . The average value, $4.88 \pm 0.12 \text{ km s}^{-1}$, is obtained from 59 data for shallow earthquakes, since calculated velocities seem to have little dependency on epicentral distances within the range of the figure. The small standard deviation suggests the accuracy of overall measurements of this analysis, which are composites of errors in source locations, errors in OBS locations, timing errors in OBS and the possible inhomogeneities of structures beneath the OBSs sites. The effect of incident angle to the array has been compensated.

The obtained value of S-wave velocities, which show a predominance at 4.9 km s^{-1} , in all probability corresponds to the P-wave velocity of 8.6 km s^{-1} , which has been obtained by a long-range explosion seismology study³ and apparent velocity measurements for P waves⁴, at the same region. Hence from a probable analogy to the P-wave structure which was measured by the explosion experiment, the measured S value, 4.9 km s^{-1} for the range 500 through 1,800 km of epicentral distances, indicates the S-wave velocities in layers from 60 km to about 150 km depths, except for the low velocity layer.

The existence of the low velocity layer, which is observed by the explosion experiment and apparent velocity measure-

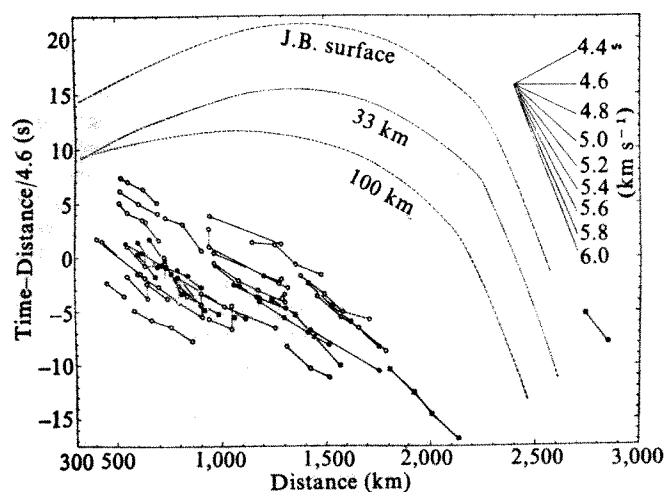


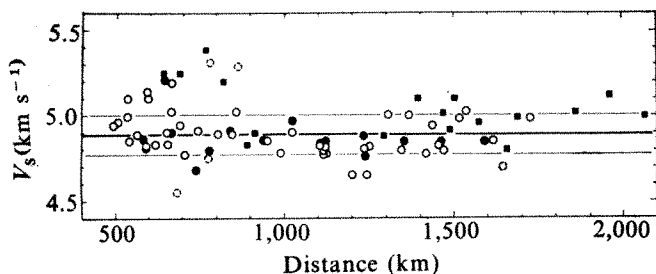
Fig. 2 Travel times of earthquakes, whose epicentres are shown in Fig. 1, observed by the OBS array. \circ , \bullet and \blacksquare as for Fig. 1. Each group of short line segments denotes an event of an earthquake. Travel times of Jeffreys-Bullen, for focal depths of 0, 33, and 100 km, are also shown. The observed travel times are remarkably faster. Vertical dotted lines are possible offsets.

ments for P waves, is also suggested by the present S-wave study. The effect of the low velocity layer beneath the oceanic lithosphere is demonstrated by offsets of travel times⁴. This is the case for S waves. The observed offsets are indicated in Fig. 2 by dotted lines. Although we could not obtain the V_s value in the low velocity layer since it is not observed as an apparent velocity, the thickness of the low S-velocity layer is estimated to be as thin as that of the low P-velocity layer which was measured to be less than 50 km (ref. 5), if we consider the travel time differences at the observed offsets. We conclude that the oceanic upper mantle at the Western Pacific mainly consists of 4.9 km s^{-1} layer, except for the uppermost 4.6 km s^{-1} layer with a probable small thickness and the low velocity channel which also has a small thickness. The depth of the bottom of the 4.9 km s^{-1} layer was not known accurately.

The V_s value of 4.9 km s^{-1} sets an important limitation on the candidate minerals which could make up the oceanic upper mantle. M. Kumazawa has measured V_p and V_s for the various minerals which have been considered as components of the oceanic upper mantles, under various temperatures and pressures (personal communication). A description of the pressure at the lower part of oceanic lithospheres has thus been obtained, although some ambiguities which are mainly effects of temperature and pressure, have been introduced. A three-component composite rock—the most probable composition for the oceanic lithosphere—is assumed (see Fig. 4).

Figure 4 indicates that pure olivine, pure pyroxene or a mixture of olivine and pyroxene fails to meet the seismo-

Fig. 3 Apparent velocities obtained from Fig. 2. Only shallow earthquakes with focal depths less than 40 km are used to obtain an average velocity of $4.88 \pm 0.12 \text{ km s}^{-1}$. \circ , \bullet and \blacksquare as for Fig. 1; broken circles are less reliable, data are probably contaminated by a possible inclusion of offsets in the span of the OBS array.



logically-obtained values, unless an impossible temperature, such as lower than 250°C , is assumed. Therefore, a considerable amount of garnet is necessary to explain the high velocities of P and S, despite the fact that garnet has not been widely accepted to be a major component of oceanic lithosphere (except by Ito⁶). The content of garnet in the lower part of lithosphere is also suggested by Fig. 4 to be probably more than half, possibly 60 to 80% of all the minerals present, under any assumption of temperature which has been generally accepted, that is $500\text{--}1,000^\circ\text{C}$.

Although the finer structure of the lower part of the oceanic lithosphere has not been elucidated in this study, a probable mechanism to compensate for the increase of V_p and V_s due to higher pressure towards the bottom of the

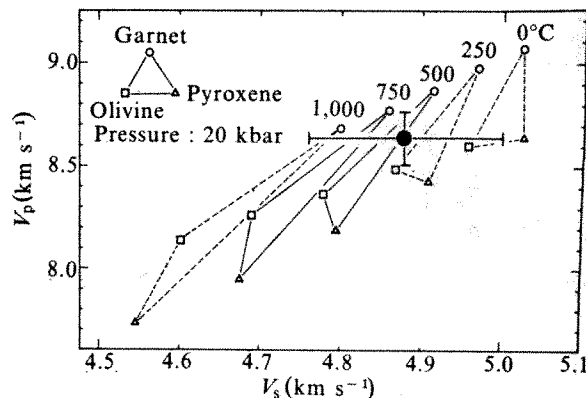


Fig. 4 Seismic wave velocities of the three-component composite rock at a pressure of the top of the high velocity layer in the oceanic lithosphere, for various conditions of temperature. The postulated components are garnet [70% pyrope ($\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$) + 30% almandine ($\text{Fe}_3\text{Al}_2\text{Si}_3\text{O}_{12}$)], pyroxene [90% enstatite (MgSiO_3) + 10% ferrosilite (FeSiO_3)] and olivine [90% forsterite (Mg_2SiO_4) + 10% fayalite (FeSiO_4)]. The measured velocities, $8.64 \pm 0.13 \text{ km s}^{-1}$ for V_p (ref. 4) and $4.88 \pm 0.12 \text{ km s}^{-1}$ for V_s , from ocean bottom seismometry are also shown.

oceanic lithosphere by the decrease of V_p and V_s due to increase of temperature has been suggested, which results in the fairly uniform values of V_p and V_s for a wide range of depths, that corresponds to a wide range of the epicentral distances (Fig. 3).

Another layer of 4.9 km s^{-1} which lies beneath the low velocity layer, may also be accounted for by the identical garnet-rich rock because of a similar compensation. But uncertainties of high temperatures and pressures effects in the range obstruct any definite interpretation.

We are planning another experiment in which OBS array will be placed perpendicular to that of the present study, which means an array perpendicular to the local magnetic anomaly: this should show whether or not V_p and V_s values larger than $8.6\text{--}4.9 \text{ km s}^{-1}$ can be obtained.

We thank Dr K. Ito for helpful discussions.

HIDEKI SHIMAMURA

Geophysical Institute,
Hokkaido University,
Sapporo 060, Japan

TOSHI ASADA

Geophysical Institute,
Tokyo University,
Tokyo 113, Japan

MINEO KUMAZAWA

Department of Earth Sciences,
Nagoya University,
Nagoya 464, Japan

Received 31 December 1976; accepted 8 August 1977.

1 Kanamori, H. & Press, F. *Nature* 226, 330 (1970).

2 Mizutani, H. & Abe, K. *Phys. Earth planet. Int.* 5, 345 (1972).

³ Leeds, A. R., Knopoff, L. & Kausel, E. G. *Science* **186**, 141 (1974).

⁴ Shimamura, H. & Asada, T. *Phys. Earth planet. Int.* **13**, 15 (1976).

⁵ Asada, T. & Shimamura, H. in *The Geophysics of the Pacific Ocean Basin and its Margin* (eds. Sutton, G. et al.), Geophysical Monograph 19, 135 (American Geophysical Union, Washington, D.C., 1976).

⁶ Ito, K. in *High-Pressure Research: Application to Geophysics* (eds. Manghnani, M. H. & Akimoto, S.) (Academic, New York) (in the press).

Uranium series ages and late Quaternary uplift in the New Hebrides

CURRENT interest in geodynamics has stimulated research into late Quaternary uplift rates of the Earth's crust from uranium series age determinations of elevated coral reefs^{1,2}. Of particular interest is the subduction rate of the Australian Plate beneath the New Hebridean Island Arc along the New Hebrides Trench (Fig. 1) derived using the uplift rate of a pre-subduction lithosphere flexure near the south-eastern Loyalty Islands^{3,4}. We report here seven uranium series ages of elevated coral reefs in the New Hebridean Islands in the hope that estimates of uplift rate in the island arc will provide additional constraints for Quaternary plate-tectonic models.

The history of the New Hebridean islands since their formation in the Oligocene is relatively well known⁵⁻⁷. Before the Pliocene, the rocks deposited were largely basalt and volcanoclastic sediment. In the late Pliocene substantial deposition of reef limestone became common⁸, especially in the eastern and western belts of the archipelago indicating slow sinking of most of the archipelago for 1 or 2 Myr. Since about the mid Pleistocene, however, many islands of the group rose progressively above sea level, as is indicated by flights of raised reefs along their coasts.

Raised reef sequences have been described on Malekula⁸, Pentecost⁹, Efate⁷, and Malo Island¹⁰ (Fig. 1). The general appearance and sedimentary analysis of the reef capped terraces on Malekula suggest that they were formed by transgressive-regressive cycles, probably resulting from eustatic sea-level oscillations, combined with continuous uplift of the coastal section concerned⁸. Similar models, supported by uranium series age, have been advanced for raised reefs on Barbados^{11,12}, the Ryukyu Islands¹, the Huon Peninsula of New Guinea^{13,14} and Timor and Atauro in the Banda Arc².

The ages presented here are from samples collected (by G.N.) on Efate and Malo Islands during May 1974. The raised reefs form good topographical features but they are rarely well exposed and road cuts are the best collecting sites. Heights were estimated by two pocket altimeters and these readings are consistent with contours of the 1:100,000 topographical maps published by the Institut Géographique National, Paris. Radiometric analyses have been carried out (by H.H.V.) using methods previously described². The results are shown in Table 1.

Although only a limited number of the elevated reef terraces yielded coral samples suitable for age determinations, all terrace levels recognised in the field are indicated in Table 1, to aid in the interpretation and correlation of dated terraces. The ages were calculated from the measured $^{230}\text{Th}/^{234}\text{U}$ ratios, assuming initial isotopic activity ratios of $^{234}\text{U}/^{238}\text{U} = 1.15$ and $^{230}\text{Th}/^{234}\text{U} = 0^{2,11-14}$. The ^{232}Th present in the corals was negligible, and no corrections have been made for possible secondary addition of common ^{230}Th .

On Malo Island, 40 km north of Malekula, five raised reefs are gently tilted to the east¹⁰. Four of these raised reefs are present along an east-trending track near Ana settlement and two raised reefs have provided ages. Another sample, Ma 1, collected from a track between Abountari settlement and Pic Malo is probably the same age as the highest raised reef on the Ana sequence. Unlike Malo, North-West Efate is cut by several late

Quaternary faults which trend westerly and north-westerly¹⁵. A Holocene raised reef is mapped¹⁵ and above this at least three raised reefs are recognised on air photographs between Creek Ai and the air strip. One of the samples (Efate X) is from the much dissected highest raised reef and samples Efate 235 and Efate 180 from north-west of Port Havannah, lie adjacent to a late Quaternary fault on the 'upthrow' side. Because of poor exposures and late Quaternary diastrophism, then, correlation of raised reefs developed in the New Hebrides is not yet possible. Also using altimeters for height measurement can lead to significant errors. Age assignment to undated raised reefs on the basis of sea-level maxima recognised elsewhere is therefore not reliable, and has not been attempted here.

There is little doubt, however, that the terrace represented by Malo LT-1 and Efate 20 corresponds to reef complex I of Huon Peninsula, dated at 5,000 to 9,000 yr¹⁴. Similarly, the raised reefs represented by samples Efate 330 and Ma 1, both dated at 134,000 yr, and Efate X and Efate 235 both dated at about 120,000 yr may well correspond to sea-level stands VIIa (140,000 yr) and VIIb (120,000 yr), respectively, of Huon Peninsula^{13,14}. The ages of Efate 330 and Ma 1 are judged to be more reliable than those of Efate X and Efate 235, on the basis of mineralogical criteria used in the evaluation of dated

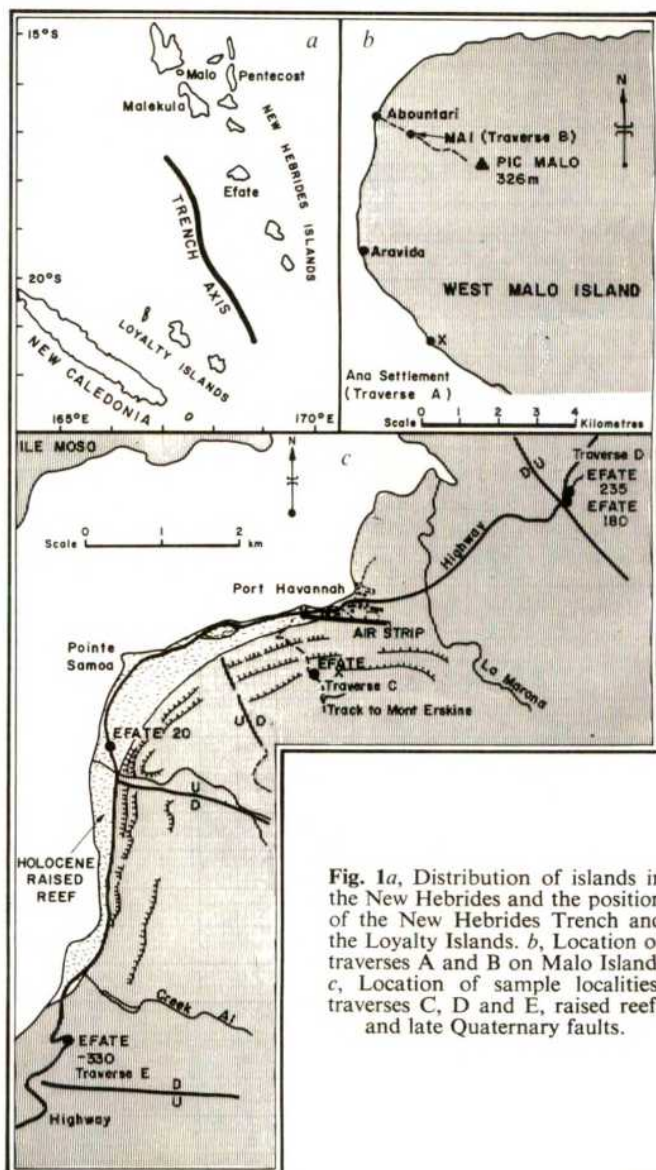


Fig. 1a, Distribution of islands in the New Hebrides and the position of the New Hebrides Trench and the Loyalty Islands. b, Location of traverses A and B on Malo Island. c, Location of sample localities, traverses C, D and E, raised reefs and late Quaternary faults.

Table 1 Raised reef elevations and radiometric ages of dated samples

Traverse	Elevation of terrace (m)	Sample no.	Calcite* (%)	U (p.p.m.)	U^{234}/U^{238}	Th^{230}/U^{234}	Age ($\times 10^3$ yr)
Malo A	94-98† 49-55 35-41 17 4.5	Malo LT-4B	< 2	2.57	1.08 ± 0.03	0.43 ± 0.02	60 ± 4
B	96	Malo LT-1 Ma 1	< 2 3	2.47 2.56	1.15 ± 0.03 1.10 ± 0.02	0.035 ± 0.004 0.72 ± 0.03	4 ± 0.5 134 ± 10
Efate C	c 100 c 75 c 40 6	Efate X	5	2.37	1.08 ± 0.02	0.67 ± 0.02	118 ± 7
D	c 140 c 100 72 55 c 40 6	Efate 235 Efate 180	< 2‡ < 2	2.80 2.20	1.09 ± 0.02 1.12 ± 0.01	0.68 ± 0.02 0.53 ± 0.02	120 ± 7 81 ± 4
E	c 230 c 170 c 130 100 c 75 c 45 6	Efate 330 Efate 20	< 2 < 2	2.94 2.56	1.13 ± 0.02 1.12 ± 0.02	0.72 ± 0.02 0.066 ± 0.005	134 ± 8 7 ± 0.6

The errors shown are based on counting statistics ($\pm 1\sigma$).

*Based on X-ray diffraction analysis of representative coral sample after mechanical cleaning with dentist drill and/or ultrasonic vibration.

†Range in elevation between front and back of terrace.

‡Contained void filling micrite cement before clean-up.

samples^{2,14}. Efate X and Efate 235 may be suspect, the former because of its relatively high calcite content, suggesting incipient recrystallisation, the latter because it contained some secondary micrite cement which may not have been completely removed during the mechanical clean-up procedure. Furthermore, a distinction between the two age groups solely on the basis of radiometric ages may not be warranted, considering that the ages are not significantly different at the 95% confidence level. Two separate and stratigraphically well defined transgressions at 134,000 yr and 119,000 yr have also been recognised on the island of Atauro, north of Timor², supporting the view that the '125,000 yr sea-level stand' may in fact be the mean of two closely spaced, yet separate, stands of roughly the same elevation, the recognition of which depends on uplift rate and/or stratigraphic definition of a given raised reef sequence^{2,16,17}.

Because our data are limited, with only one age determination per individual terrace site, or none at all, we are not yet prepared to discuss the fine structure of palaeosea level in the New Hebrides over the past 140,000 yr. The ages given here for several of the raised reefs are useful, however, as they provide a more realistic time base for the late Pleistocene tectonic history of the area than has so far been available. For example, previous age estimates of the raised reefs on Malekula were obtained by assuming that the highest of the six tilted raised reefs present corresponds to the interglacial following the first cold period of the Pleistocene, hence it should be ~2 Myr old, with the ages of the other raised reefs, in descending order, calculated to be 1.8, 1.5, 1.0, 0.6 and 0.4 Myr on the basis of a steady uplift rate⁸. Mallick and Neef⁹ considered these age estimates far too old in comparison with the eustatic sea-level data from dated raised reefs on Huon Peninsula, a view which is supported by our much younger ages for terraces of similar elevation on nearby Malo Island, lying only 40 km from north-west Malekula.

The question of uniform or non-uniform uplift rate with time at a given location cannot be resolved without further field work and additional age determinations in the New Hebrides.

We propose that the mean uplift rate over the past 140,000 yr was at least 0.67 m per 1,000 yr on Malo, and between 0.55 and 0.77 m per 1,000 yr on North-West Efate—based on the data shown in Table 1 and the best estimates of palaeo sea levels at 120,000 and 134,000 yr (refs 2, 17). These values are not substantially different from those determined by similar methods in other active island arcs and orogenic belts along zones of lithosphere plate convergence^{1,2,11,13,14} but significantly higher than the uplift rate of 0.13 m per 1,000 yr estimated for the pre-subduction bulge west of the New Hebrides Trench⁴.

We thank Dr D. I. J. Mallick for advice on potential sample locations and Mrs G. Halliday for drafting Fig. 1. Financial assistance to G.N. was provided by the University of New South Wales and the Mine Managers' Association of Broken Hill and to H.H.V. by the Australian Research Grants Commission.

G. NEEF

W.S. & L.B. Robinson University College,
Broken Hill, Australia

H. HERBERT VEEH

School of Earth Sciences,
Flinders University of South Australia,
Bedford Park, S.A. 5042, Australia

Received 6 May; accepted 9 August 1977.

- Konishi, K., Schlanger, S. O. & Omura, A. *Mar. Geol.* **9**, 225 (1970).
- Chappell, J. & Veeh, H. H. *Bull. geol. Soc. Am.* (in the press).
- Dubois, J., Launay, J. & Recy, J. *Tectonophysics* **24**, 133 (1974).
- Dubois, J., Launay, J., Marshall, J. & Recy, J. *13th Pacific Sci. Cong.* (Vancouver, 1975).
- Mitchell, A. H. G. & Warden, A. J. *J. geol. Soc., Lond.* **127**, 501 (1971).
- Mallick, D. I. J. *Phil. Trans. R. Soc. B272*, 277 (1975).
- Mallick, D. I. J. *Ann. rep. New Heb. Geol. Survey for 1969* 10 (1970).
- Mitchell, A. H. G. *J. Geol.* **77**, 56 (1969).
- Mallick, D. I. J. & Neef, G. *New Heb. Geol. Survey Regional Rept* (1974).
- Mallick, D. I. J. *Ann. rep. New Heb. Geol. Survey for 1970* 20 (1971).
- Broecker, W. S. *et al. Science* **159**, 297 (1968).
- Mesolella, K. J., Matthews, R. K., Broecker, W. S. & Thurber, D. L. *J. Geol.* **77**, 250 (1969).
- Chappell, J. *Bull. geol. Soc. Am.* **85**, 553 (1974).
- Bloom, A. L., Broecker, W. S., Chappell, J. M. A., Matthews, R. K. & Mesolella, K. J. *Quat. Res.* **4**, 185 (1974).
- Ash, R. P. *New Heb. Geol. Survey, Sheet 9* (1974).
- Marshall, J. F. & Thom, B. G. *Nature* **263**, 120 (1976).
- Stearns, C. E. *Quat. Res.* **6**, 445 (1976).

New evidence and possible origin of native iron in ophiolites of eastern Canada

THERMOMAGNETIC analysis has been used to detect traces of submicroscopic elemental iron in four bodies of Early Palaeozoic oceanic metabasalt (Fig. 1). The initial finding of iron in Newfoundland ophiolites¹ is believed to be the first terrestrial discovery of native ferromagnetic metal through magnetism. A few rock samples in these four areas have unusually high Curie points indicating pure or nearly pure iron. In one basaltic pillow, previous magnetic evidence² was confirmed by electron microprobe showing very fine Fe particles embedded in chlorite. We propose that such particles formed by reduction of olivine under water and became trapped in the chlorite host. Although native iron seems rare on Earth^{3,4}, further suboptical discoveries through rock magnetism may be predicted. Such findings could prove valuable in identifying ancient oceanic crust generated in a reducing environment.

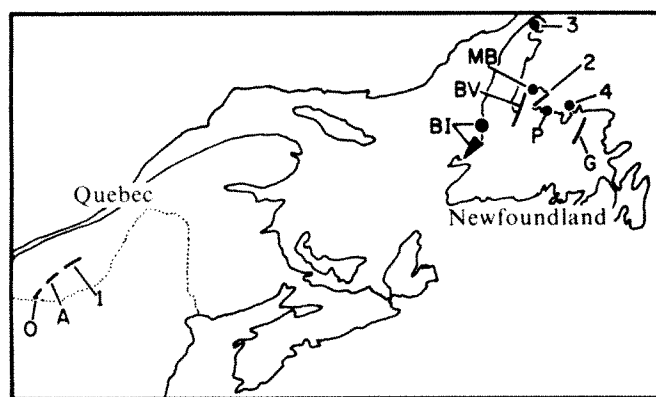


Fig. 1 Ophiolites and related rocks in Quebec and Newfoundland. A, Asbestos; BV, Baie Verte; G, Gander River; MB, Ming's Bight; O, Orford; P, Pilleys Island. Bodies in which native iron was identified are numbered: 1, Thetford Mines; 2, Betts Cove; 3, Hare Bay; 4, Moreton's Harbour.

The Thetford Mines⁵ and Betts Cove⁶ ophiolites (Fig. 1, areas 1, 2) are closely similar⁷. The former is middle Cambrian or older and was emplaced in the early Ordovician⁵, while Betts Cove may have originated and been emplaced somewhat later^{8,9}. The early Ordovician^{10,11} transported ophiolite at Hare Bay (area 3) lacks gabbro and sheeted dikes. At Moreton's Harbour (area 4) an 8-km thick volcanic sequence is attributed¹² to Ordovician island arc volcanism. No iron has been detected outside of areas 1–4, nor in gabbros or ultramafics.

Curie points (T_c) were obtained from curves of high-field magnetic moment plotted against temperature ($M-T$). Powders were heated in vacuum² or air¹³ to 800 or 900 °C (Fig. 2a, c–e). Also initial susceptibility variation with temperature ($K-T$) of whole rock was measured in air¹⁴ (Fig. 2b). Iron contamination

can be ruled out as all specimens were prepared by rigorously avoiding contact with ferromagnetic materials.

Two of the heating curves (Fig. 2a, b) indicate magnetite, but all five show high T_c values in the range 755–775 °C. This corresponds to¹⁵ the Curie point of α -iron (770 °C) or of iron alloyed with less than 5% nickel (770–750 °C). The trends of the irreversible curves obtained in air seem to be better explained by oxidation of iron, initially to magnetite, than by α , γ phase transitions¹⁵. Figure 2c, d resembles curves for lunar samples which likewise may have been oxidised, though heated in a vacuum^{16,17}. The smaller saturation magnetisation of magnetite ($4.80 \times 10^5 \text{ A m}^{-1}$) than of iron ($17.0 \times 10^5 \text{ A m}^{-1}$) is consistent with this. Still, some samples became more magnetic upon cooling (Fig. 2b, c). Lunar samples (see ref. 17) show similar variations which suggest that grain-size effects are important. The large susceptibility increase on cooling in Fig. 2b may be due to superparamagnetic magnetite produced by heating the iron¹⁷.

Table 1 shows values of natural remanence, susceptibility and Koenigsberger ratio of Thetford Mines pillows. For any pillow zone the mean values are respectively three, one and two orders of magnitude less than world-wide averages for young drilled deep-sea basalts¹⁸. Low, widely variable Koenigsberger ratios (0.5 to 0.001) predominated also in Newfoundland. Very unevenly distributed secondary magnetite and/or iron could account for these findings.

Estimated elemental iron in samples does not exceed a few tenths of one weight percent. At Thetford Mines, iron was detected in 13 out of 51 thermomagnetic analyses made on 14 pillows (Table 1), with mean Curie point 756 ± 4 °C (standard deviation). In Newfoundland, 28 samples each had yielded at least one $M-T$ curve showing significant iron (total 56 analyses). Mean Curie points are: Betts Cove pillows, 760 ± 11 °C (15 samples); Betts Cove dikes, 759 ± 7 °C (four samples); Hare Bay pillows, 761 ± 11 °C (six samples); Moreton's Harbour pillows, 750 ± 5 °C (three samples). Three reliable $K-T$ curves, for Hare Bay (Fig. 2b) and Betts Cove, gave $T_c = 760-775$ °C.

At Laval University a Thetford Mines pillow that had produced $M-T$ curves similar to Fig. 2a was shown by electron microprobe to contain small ($< 5 \mu\text{m}$), lamellar particles of 96–98% pure Fe: also chalcopyrite, sphene, magnetite and chromite. The opaques occur as minute inclusions in the fractures of former olivine microphenocrysts entirely pseudomorphosed by magnesian-chlorite. A single iron particle was just large enough to be seen with the reflecting microscope. In four samples from areas 2–4 in Fig. 1 having 'iron' but not 'magnetite' Curie points, microprobe analysis showed abundant sulphides, also sometimes limonite, hematite, iron oxyhydroxides or sphene, but neither magnetite nor Fe. This is compatible with the presence of extremely fine-grained iron.

Terrestrial nickel and iron has been described from less than 24 localities^{3,4} where nickel-iron containing 65–75% Ni always occurs only in serpentinised ultramafics. Nickel-poor iron ($> 90\%$ Fe), however, usually occurs in mafic rock^{3,4,19–22}. A second significant fact is that, wherever we inferred iron, the pillow lava or dike rock has undergone greenschist metamorphism. The pillow lava is of two types²³, a dark splititised metatholeiite rich in chlorite and a light green olivine metatholeiite rich in actinolite and chlorite pseudomorphs. At Thetford Mines, but not Betts Cove, native iron seems to be confined to the olivine metatholeiites².

Table 1 Magnetic properties and ferromagnetic constituents according to pillow zones (Thetford Mines, Quebec)

Pillow zone	<i>n</i>	$J_n (\times 10^{-3} \text{ A m}^{-1})$		$K (\times 4\pi \times 10^{-6})$		Range Q_n	Mean	n_M	n_{Fe}
		Range	Mean	Range	Mean				
Margin	15	1.15–4.25	1.95	18.0–57.3	45.2	0.03–0.13	0.05	15	0
Intermediate	22	0.56–3.26	1.42	19.9–35.9	31.1	0.05–0.14	0.08	22	9
Core	14	0.78–4.41	1.81	12.5–33.3	25.0	0.07–0.27	0.12	14	4

n, No. of whole-rock specimens (total 51) cut from 14 samples of pillow lava; J_n , intensity of NRM; K , initial susceptibility; $Q_n = J_n KH$, natural Koenigsberger ratio, where H is the Earth's present field. Geometric means are quoted. One powder per whole-rock specimen was prepared for thermomagnetic analysis to identify the magnetic constituents (Fig. 2a, ref. 2); n_M , n_{Fe} , number of analyses indicating magnetite or (additionally) iron. Note that magnetite is inferred in all powders.

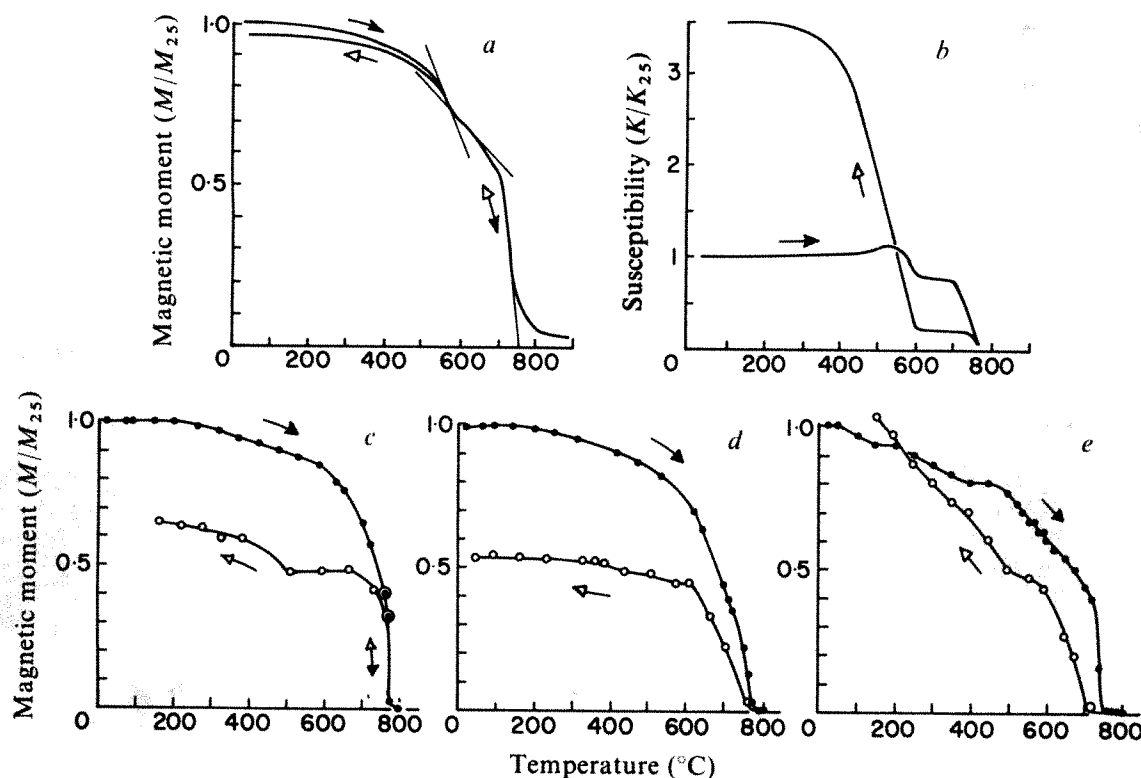
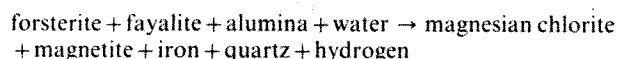


Fig. 2 Thermomagnetic curves for pillow lava from areas 1-4 (Fig. 1), normalised to 25 °C before heating. Specimens were heated and cooled in a vacuum of 10^{-3} torr (a) or in air (b-e). b, K - T curves (see text) for whole rock fragments up to 1 cm diameter, measured in a peak field $H = 0.031$ mT. a, c-e are M - T curves for coarse powders up to 1 mm, measured in fields of 0.10-0.13 T. Curie points (T_c) were obtained to better than ± 15 °C and are quoted to 5 °C. a, Thetford Mines, intermediate zone of pillow A (Table 1, ref. 2), showing two-component M - T curves, $T_c = 580$ °C, 755 °C. b, Hare Bay, intermediate zone of pillow HB 1, showing two-component K - T curves: $T_c = 595$ °C, 770 °C. c, Hare Bay, intermediate zone of pillow HB 1, M - T curves, $T_c = 770$ °C. d, Betts Cove, core of pillow BC 9, M - T curves, $T_c = 775$ °C. e, Moreton's Harbour, small pillow MH 33, M - T curves: $T_c = 755$ °C.

The iron could have originated (1) as dusts with magnetite in the rock ground-mass; (2) as reaction product between magnetite and iron sulphide in the ground-mass; or (3) as by-product of alteration of the olivine microphenocrysts. Mode (1) was not confirmed by microprobe and in any case the tiny, unprotected iron particles would have been rapidly oxidised. Mode (2) also seems unsupported since the iron identified by microprobe occurs without sulphide-magnetite; in the Newfoundland samples Fe tends to occur without magnetite; the association sulphide-magnetite is frequent in basalts while native iron is rare.

Alternative (3) assumes a paragenetic relationship between olivine and native iron. During serpentinisation of olivine, iron-related redox mechanisms may have controlled the formation in serpentine of magnetite and nickel-iron in equilibrium⁴. Serpentinisation itself might generate a highly reducing environment by the dissociation of water to provide the oxygen required to form magnetite, thereby releasing hydrogen^{24,25}. The same process can tentatively be applied to chloritisation of olivine in our rocks. The equilibrium assemblage resulting from the reaction



would comprise the minerals that actually replaced the olivine phenocrysts in the pillow lava. With sufficient hydrogen the magnetite can be further reduced to iron and water, which may explain its paucity in Newfoundland samples. The iron encapsulated in chlorite crystals would be protected against oxidation as long as the chlorite remained unaltered.

If this explanation is correct, then elemental iron should occur also in rocks of similar genesis elsewhere; for example, Curie points suggesting Fe have been reported²² from the greenschist facies of Archaean metavolcanics. Because of typically minute grain sizes, rock magnetism remains the most promising method for discovering native ferromagnetic metal.

We thank Dr M. B. Berubé, Dr L. G. Kristjansson, R. R. Pätzold, J. P. Ricbourg, Dr H. Upadhyay and T. Vallis for assistance. This work was supported by grants to E. R. D., R. L., and M. K.-S. from the NRC of Canada.

E. R. DEUTSCH
K. V. RAO

Geomagnetic Research Laboratory,
Department of Physics,
Memorial University of Newfoundland,
St John's, Newfoundland, Canada

R. LAURENT
M. K.-SEGUIN

Département de Géologie,
Université Laval,
Québec, Canada

Received 28 April; accepted 19 August 1977.

1. Deutsch, E. R. & Rao, K. V. in *Implications of Continental Drift to the Earth Sciences* (eds Tarling, D. H. & Runcorn, S. K.) 2, 987-992 (Academic, London, 1973).
2. Seguin, M. K. & Laurent, R. *Can. J. Earth Sci.* **12**, 1406-1420 (1975).
3. Ramdohr, P. *The Ore Minerals and their Intergrowths* (Pergamon, New York, 1969).
4. Chamberlain, J. A., McLeod, C. R., Traill, R. J. & Lachance, G. R. *Can. J. Earth Sci.* **2**, 188-215 (1965).
5. Laurent, R. *Can. J. Earth Sci.* **12**, 443-455 (1975).
6. Upadhyay, H. D., Dewey, J. F. & Neale, E. R. W. *Proc. Geol. Ass. Canada* **24**, 27-34 (1971).
7. Church, W. R. *Can. J. Earth Sci.* **14**, 1668-1673 (1977).
8. Dewey, J. F. & Bird, J. M. *J. geophys. Res.* **76**, 3179-3206 (1971).
9. Snelgrove, A. K. *Bull. Can. Inst. Min. Metal.* **228**, 447-519 (1931).
10. Williams, H. *Can. J. Earth Sci.* **12**, 1874-1894 (1975).
11. Dallmeyer, R. D. *Am. J. Sci.* **277**, 61-72 (1977).
12. Keen, B. F. & Strong, D. F. *Am. J. Sci.* **275**, 97-118 (1975).
13. Deutsch, E. R., Kristjansson, L. G. & May, B. T. *Can. J. Earth Sci.* **8**, 1542-1552 (1971).
14. Pätzold, R. R. *thesis*, Memorial Univ. Newfoundland (1972).
15. Bozorth, R. M. *Ferromagnetism* (Van Nostrand, New York, 1951).
16. Strangway, D. W., Larson, E. E. & Pearce, G. W. *Proc. Apollo 11 Lunar Science Conf.* **3**, 2435-2451 (1970).
17. Schwarz, E. J. *Proc. Apollo 11 Lunar Science Conf.* **3**, 2389-2397 (1970).
18. Lowrie, W. *J. geol. Soc. Lond.* **133**, 61-82 (1977).
19. Bird, J. M. & Weathers, M. S. *J. Geol.* **85**, 359-371 (1977).
20. Momose, K. in *Rock Magnetism and Paleogeophysics* (ed Kono, M.) **2**, 1 (Tokyo, 1974).
21. Verma, R. K. & Prasad, S. N. *J. geophys. Res.* **80**, 3755-3756 (1975).
22. Park, J. K. *Can. J. Earth Sci.* **12**, 1272-1290 (1975).
23. Laurent, R. & Hébert, Y. in *Volcanic Regimes in Canada* (ed Baragar, W. R. A.) (in the press).
24. Eckstrand, O. R. *Econ. Geol.* **70**, 183-201 (1975).
25. Nitsan, U. *J. geophys. Res.* **79**, 706-711 (1974).

Mesozoic sea floor off Dronning Maud Land, Antarctica

THE accreting margin between the African and Antarctic plates is now well understood between the Bouvet triple junction and Marion Island (Fig. 1). Published data obtained from the ridge axis near 1°W^1 , 15°E^2 , and 36°E^3 can be combined to yield a relative rotation pole at 11°N , 41°W with an equatorial half spreading rate of 0.8 cm yr^{-1} . Away from the spreading axis, very little is known apart from a narrow region to the north in the Mozambique Basin where Bergh and Norton³ have shown that the present spreading regime commenced at least as far back as the Late Cretaceous. This is the only region where transform fault traces have been extended for more than 100 km from the spreading centre and where magnetic lineations have been related to the present tectonic pattern. I describe here a group of lineations recently mapped in the Antarctic Basin off Dronning Maud Land, interpreted as being due to sea floor generated at the South-west Indian Ocean Ridge in the Early Cretaceous.

Two curtailed excursions in this area were carried out during the summers of 1976 and 1977. Figure 2 shows magnetic anomalies plotted perpendicular to ship's tracks. Anomaly identification was made by projecting the profiles on to 30°E (Fig. 3) and comparing with simulated profiles based on the reversal timescale of Larson and Hilde⁴. The Mesozoic sequence M-1 to M-9 (113 to 121 Myr.) is considered to be well matched, with M-2, M-4 and the diminutive M-6 showing up clearly. A spreading rate change from 2.0 to 1.5 cm yr^{-1} at anomaly M-5 time is apparent from the matching to the two theoretical profiles in Fig. 3. Anomalies older than M-9 are included only tentatively in the present interpretation.

The area surveyed is characterised by a generally featureless bottom topography with the regional depth sloping from 4 km at about 68°S to 5 km at 64°S . Limited seismic reflection profiling has revealed a flat-lying acoustic basement with sediment thickness of about 1.6 s of double travel time. A striking basement outcrop of up to 2 km above the surrounding sea floor is indicated by lineament F in Fig. 2. Its strike of 30° , based on three crossings, is exactly perpendicular to the mean azimuth of the observed magnetic lineations. Sea-floor depth is distinctly different on either side of this presumed fracture zone and is consistent with the expected older sea floor being on

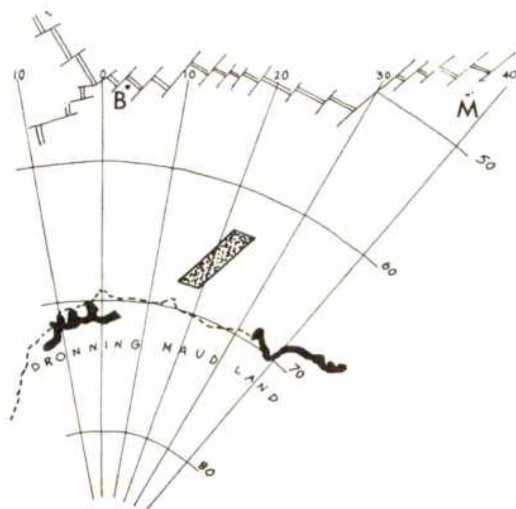


Fig. 1 Survey area (stippled) in Antarctic Basin. The South-west Indian Ocean Ridge and its junction with the Mid-Atlantic Ridge are shown schematically by double lines (sections of spreading ridge) and single lines (fracture zones and active transform faults). B, Bouvet Island; M, Marion Island.

the eastern side. A major west-facing fault scarp between 30° and 33°N in the Mozambique Basin, associated with Late Cretaceous spreading³ along the South-west Indian Ocean Ridge, is approximately co-polar (rotational) with fracture zone F, and has a similar depth contrast (500 m) across it. This is greatly in excess of calculations based on depth against age of ocean floor and could be related to a spreading style in which the two inward-facing walls of a deep transform fault crack move in opposite directions. The minor lineament f in Fig. 2 marks the eastward termination of identifiable magnetic anomalies, as indicated on profiles D and E in Fig. 3.

Magnetic anomaly and fracture zone trends reported here are consistent with their being initiated at the presently active ridge near Marion Island. More data will, of course, be required

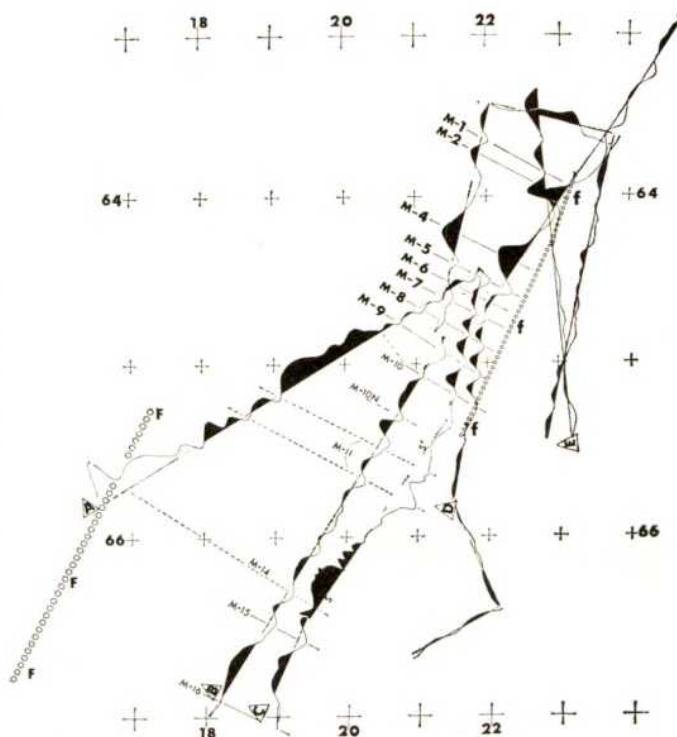
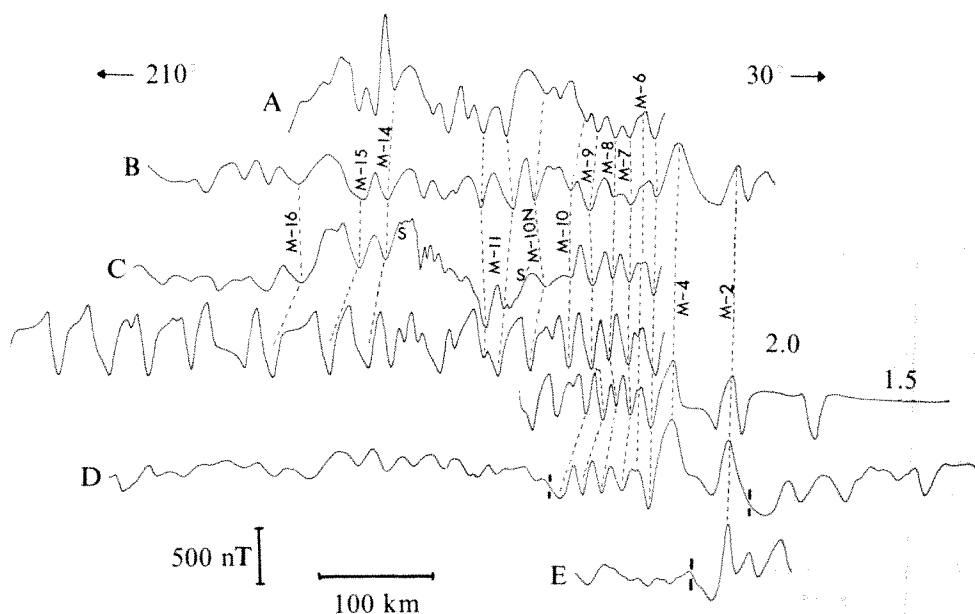


Fig. 2 Total field magnetic anomalies plotted perpendicular to ship's tracks after removal of International Geomagnetic Reference Field. Positive anomalies in black. Lineaments F and f were based respectively on a prominent basement ridge and the eastward termination of identifiable anomalies. Portion S-S' of profile C was surveyed during a magnetically disturbed period. Navigation by satellite and VLF Omega.

between the ridge and the M-sequence of anomalies before an unequivocal tie-in can be demonstrated. In Fig. 4 anomalies M-1 to M-9 as well as the lineaments F and f have been rotated by 20.5° about the present Africa-Antarctica rotation pole to bring anomaly M-1 close to the active accreting margin. Fault scarp F is nearly co-linear with the first fracture zone to the west of the Prince Edward fracture zone while lineament f coincides with an area of present dubious ridge signature which Bergh and Norton³ suggest might be an incipient transform fault. The ridge sections shown in Fig. 4 are based on data obtained subsequent to that included in their paper. The short section across 36°E is almost orthogonal to the Prince Edward fracture zone and is separated from the definitely oblique section ($37-38^{\circ}\text{E}$) by a nearly 50-km wide region where no central magnetic anomaly is apparent. The close correspondence between present and Mesozoic spreading might be fortuitous—it might also point to a persistent localised upper mantle property.

Fig. 3 Magnetic anomalies projected on to assumed spreading direction (Profiles A to E) and theoretical profiles for half spreading rates of 1.5 and 2.0 cm yr⁻¹. Simulations and anomaly numbering were based on the Larson and Hilde⁴ reversal time-scale with two-dimensional blocks of magnetised (1000 nT) sea floor trending 120° between depths 4.5 and 5.0 m. Vertical bars on profiles D and E mark the termination of identified anomalies and form the basis for the lineament f in Fig. 2.



As a working hypothesis, it is reasonable to assume that rifting along this segment of the ridge has proceeded in a remarkably stable way since the Early Cretaceous. Spreading rates have varied considerably and sections of ridge have changed orientation³ but the average pole describing the relative plate motion is very close to the present pole. Extrapolation of the Mesozoic spreading rate and direction back to the 2-km isobath off Dronning Maud Land dates the onset of spreading as roughly 150 Myr. or Late Jurassic. This slightly pre-dates estimates^{5,6} of the initial rifting of eastern Gondwanaland and is in very good agreement with the proposed early separation rate⁵ for that region.

This work was supported by the South African National Committee for Oceanographic Research. The M.V. RSA was

made available by the Department of Transport. I thank Captain E. Funk and his officers and crew for their cooperation and members of South African Antarctic teams and the Pretoria University Mammal Research Institute who assisted with watch-keeping.

HUGH W. BERGH

*Bernard Price Institute of Geophysical Research,
University of the Witwatersrand,
Johannesburg, South Africa*

Received 16 May; accepted 13 July 1977.

- ¹ Selater, J. G., Bowin, C., Hey, R., Hoskins, H., Pierce, J., Phillips, J. & Tapscott, C. *J. geophys. Res.* **81**, 1857–1869 (1976).
- ² Norton, I. O. *Earth planet. Sci. Lett.* **33**, 219–230 (1976).
- ³ Bergh, H. W. & Norton, I. O. *J. geophys. Res.* **81**, 5221–5239 (1976).
- ⁴ Larson, R. L. & Hilde, T. W. C. *J. geophys. Res.* **80**, 2586–2594 (1975).
- ⁵ Larson, R. L. *Geology* **5**, 57–60 (1977).
- ⁶ Johnson, B. D., Powell, C. McA. & Veivers, J. J. *Bull. geol. Soc. Am.* **87**, 1560–1566 (1976).

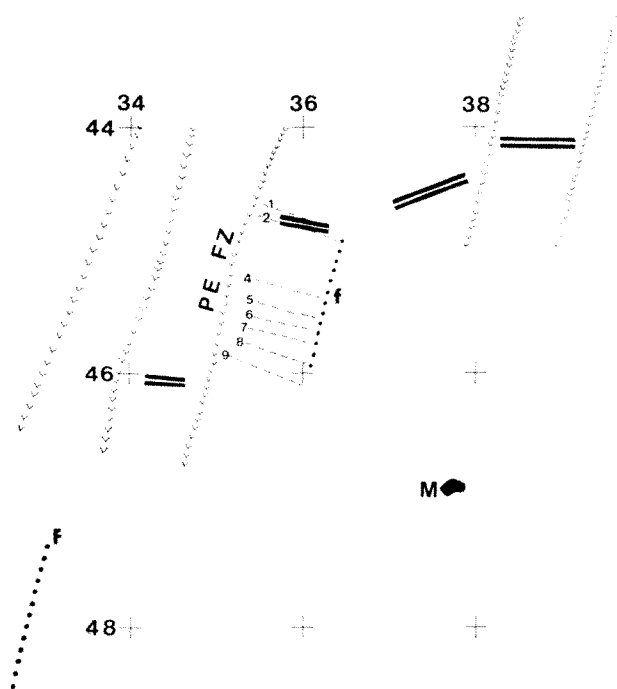


Fig. 4 Anomalies M-1 to M-9 and lineaments F, f rotated anti-clockwise by 20.5° about the present Africa–Antarctica relative rotation pole at 11°N, 41°W. Four sections of presently accreting plate margin are shown as thick double lines and the lines of vees represent fracture zones mapped by Bergh and Norton³. M, Marion Island.

Unusual diatom off the coast of south-west England and its effect on fishing

AN unfamiliar diatom was observed in the local townnettings taken by research vessels of the Plymouth Laboratory towards the end of January 1977. It was a large member of the genus *Coscinodiscus*, but a species not previously known in the English Channel. The mucilage produced by this diatom was so abundant as to restrict trawling in some areas off Plymouth.

The valve pattern of the diatom resembled that of some forms of *Coscinodiscus concinnus* Wm Smith¹ with the valve having a clear centre, but the diameter was greater than that recorded for *C. concinnus* in the English Channel. It was unlike any other species of *Coscinodiscus* recorded for the western English Channel in that the 50-μm deep valve mantle met the valve face at right angles. As the valve itself was almost flat the diatom had a markedly rectangular outline in girdle view. A search of the literature showed that this diatom was probably *Coscinodiscus nobilis* Grunow. Our material agrees essentially with the description and illustration of *C. nobilis* given by Simonsen² and it agrees with the material of Grunow designated as lectotype by Simonsen². Grunow³ described this species from the Java Sea and it has only otherwise been recorded from the

Indian and Pacific Oceans⁵, although there are two doubtful records from the east coast of England⁶ and one from Heligoland⁷.

As the diatom was apparently increasing in numbers near Plymouth sampling was initiated in which 10 l samples were poured through a tynet of 170- μ m mesh and the trapped diatoms counted. The diatom was successfully cultured in the laboratory and it divided every 2 d at 18 °C and every 3 d at 13 °C. In culture this diatom produced much more mucilage, which collected on the bottom of the culture vessel, than other species of *Coscinodiscus* we have cultured in similar conditions. Workers on our research ships complained that townets were becoming clogged with mucilage during sample collection.

During a hydrographic and biological cruise in the western English Channel on 19–21 April, the distribution of the diatom was plotted from tynet samples (Fig. 1). On 1 May 1977 Dr A. J. Southward took a sample from 48°13'N, 08°11'W which was rich in this diatom, showing that it was abundant further west than we had sampled.

By the end of April the Fishery Officer at Plymouth was receiving complaints from the Plymouth fishermen that their trawls were becoming clogged or broken by a very heavy grey slime. The slime made hauling difficult and prolonged washing or air drying did not completely remove it. The slime was most prevalent on the sea bed in areas where the unusual diatom was most abundant.

Light microscopy showed that the slime was heavily loaded with clay particles and the insoluble remains of plankton organisms, such as the skeletons of silicoflagellates. The slime and the mucilage from the diatom were shown to be of the same chemical structure.

The diatom had reached high numbers for a cell of this size and had produced copious mucilage which, in sinking, had accumulated the insoluble skeletons of plankton organisms and, on the sea bed, had collected mineral particles which increased its volume and density. From our cultures we estimated that for a given volume of cells, there was two to three times the volume of mucilage present. Counts from seawater samples

taken off Plymouth were about 100 cells per l for several weeks in April. As the water column was completely mixed at that time, we assume that the diatoms were distributed through the whole 50 m of the water column. With a mean cell volume of 0.01 mm³ per diatom, 150 ml of slime could accumulate on each square metre of seabed. The slime would be concentrated in certain areas of the seabed by tides and the act of trawling would also accumulate it until it caused inconvenience to the fishermen. If a trawl with a 16 m spread were towed at 3 knots for 1 h it could sweep up about 5 m³ of slime. Even if more than half the slime passed through the net the accumulation on a 3-h tow would be considerable. Furthermore, although slime would pass through the net initially, once it had started to adhere to the meshes the percentage retained would increase rapidly. The volumes given here are minimal as the calculations are based on the mucilage produced by the diatoms and the accumulation of large quantities of bottom material in the slime would considerably increase its volume and density.

There are two published records of exotic diatoms appearing in European waters and forming a major part of the phytoplankton. *Biddulphia sinensis* Grev. was described from the China Sea⁸ and its first arrival and subsequent spread was recorded⁹. This species is now a prominent contributor to the winter and spring phytoplankton of the western English Channel. The second species, *Pleurosigma planctonicum* Simonsen, was first described² from the Indian Ocean. Simonsen collected his material in 1964–65 and we found this species in the western English Channel in 1966 (ref. 10). *Pleurosigma planctonicum* became dominant in the Plymouth area in 1973 and is still present in the English Channel, although its occurrence is sporadic. In neither case were there any reported effects other than the displacement of native species by the new arrivals.

We cannot offer an explanation as to how *Coscinodiscus nobilis* reached the English Channel. By mid-May, numbers had declined, although the species had not completely disappeared, and, by the end of May, there were no reports of slime clogging nets. Whether this diatom together with the inconvenience to trawling caused by its mucilage will become a regular feature of the spring phytoplankton in the English Channel remains to be seen.

We thank Dr Elizabeth Percival of Royal Holloway College, London, for analysing the mucilage and slime, Mr C. Knott and the crew of R.L. Gammarus for taking samples, Dr A. J. Southward for samples from RRS Shackleton and Mr W. Williams, District Fishery Officer, Plymouth, for information on the occurrence of the slime on the fishing grounds.

G. T. BOALCH
D. S. HARBOUR

Marine Biological Association of the United Kingdom,
The Laboratory, Citadel Hill,
Plymouth, UK

Received 15 June; accepted 23 August 1977.

1. Boalch, G. T. *J. mar. biol. Ass. U.K.* **51**, 685–695 (1971).
2. Simonsen, R. *Meteor. Forsch. Ergebnisse, Reihe D*, No. 19, 1–107 (1974).
3. Grunow, A. *J. R. microsc. Soc.* **11**, 667–691 (1879).
4. Sornia, A. *Bull. mar. Sci.* **20**, 678–696 (1970).
5. Allen, W. E. & Cupp, E. E. *Ann. Jard. Bot. Buitenz.* **44**, 101–174 (1935).
6. Rattray, J. *Proc. R. Soc. Edinb.* **XVI**, 449–692 (1889).
7. Meunier, A. *Ducl'Orleans, Campagne Arctique de 1907*, Bulens, Bruxelles (1910).
8. Greville, R. K. *Trans. microsc. Soc. Lond.* **N.S.14**, 77–86 (1866).
9. Ostenfeld, C. H. *Meddr. Komm. Havunders.*, **S. Plankton**, 1, No. 6 (1908).
10. Boalch, G. T. & Harbour, D. S. *Nova Hedwigia Beih.* **54**, 275–280 (1977).

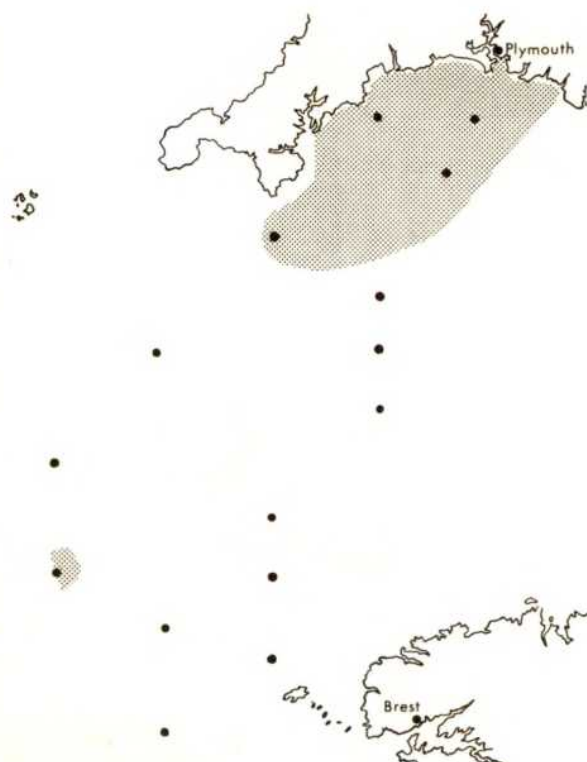


Fig. 1 Distribution of *Coscinodiscus nobilis* Grunow in the western English Channel, 19–21 April 1977. Shaded area, *C. nobilis* present in tynet samples. ●, Stations worked.

Pattern regulation and transdetermination in *Drosophila* imaginal leg disk reagggregates

WHEN *Drosophila* imaginal disk tissue is cultured *in vivo*¹, the proliferating cells can adopt new fates within the limits of a single disk ('pattern regulation'^{2,3}) or may even produce structures normally derived from a different disk ('trans-determination'⁴). Much of the pattern regulation occurring in

imaginal disk fragments may be controlled by interactions between cells that come together during wound healing^{3,5}. I report here further studies on the role of cell interactions in pattern regulation and transdetermination, by means of dissociation and reaggregation of specific disk parts. The data confirm the importance of cell interactions for pattern regulation, and suggest that interactions between cells with large positional disparities may also be crucial for the stimulation of transdetermination.

We have previously studied pattern regulation and transdetermination in non-dissociated male foreleg disk quadrants (ref. 6 and unpublished results), and in identical fragments which were dissociated together with an excess of heavily irradiated wing disks ('feeding layer') and then cultured as 'mixed reaggregates'⁸.

In intact fragments, distal transformation⁹ (as judged by the regeneration of claws, which represent the distal tip of the adult appendage; Fig. 1a) occurred only in UM^- and, with a lower frequency, in LM^- quadrants (Table 2; for abbreviations see Fig. 1), whereas in mixed reaggregates, cells from all four quadrants frequently underwent distal transformation. Furthermore, although among the intact fragments only the UM^- quadrants transdetermined, cells from UM^- and UL^- quadrants underwent extensive transdetermination in mixed reaggregates. Since LM^- and LL^- cells failed to transdetermine in either situation, the hypothesis was advanced that only certain cells in the upper half of the male foreleg disk are capable of transdetermination¹⁰ (Fig. 1b). The more frequent distal transformation and transdetermination in mixed reaggregates could not be explained, however. It seemed possible that the initial destruction of the tissue organisation had caused both events, but I show here that they were stimulated mainly by interactions of the leg disk cells with 'feeding layer' cells present in the mixed reaggregates.

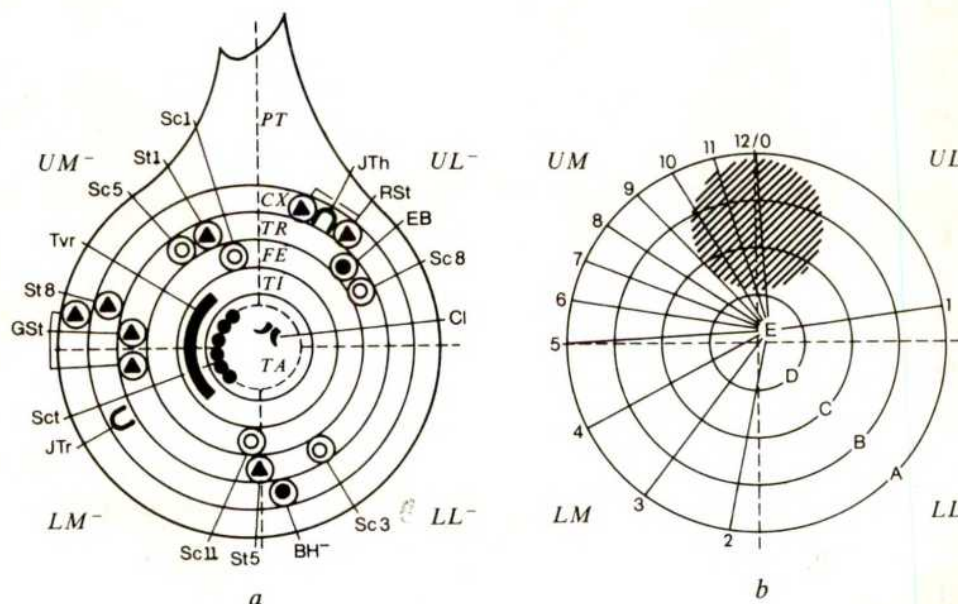
Drosophila male foreleg disk quadrants were dissociated, reaggregated, and cultured as 'pure reaggregates' (that is, without 'feeding layer'). In each experiment 120 quadrants (lacking most of the presumptive tarsal material; Fig. 1a) from third instar disks were dissociated with a microstirrer in a 0.05% trypsin solution⁷. The degree of dissociation varied as reported earlier⁸, clumps comprising up to about 20 cells occurring regularly. The reaggregated cell mass was divided and injected into fertilised adult females and cultured for 8 d at 25 °C. The implants were then transplanted into host larvae for

metamorphosis. Since most implants had to be cut into several pieces for this injection, the average number of larvae injected with material from one adult host represents a rough measure of the proliferative activity of the cells (Table 1). The death of injected larval hosts meant that a variable amount of material was lost in every experiment (Table 1). The differentiated implants consisted of a mass of vesicles, with each vesicle usually containing structures of a single leg segment. The number of vesicles typical for each leg segment was determined, and the cuticular landmarks (Fig. 1a) were scored. The implants were also examined for transdetermined structures. The data are presented in Table 1; Table 2 summarises the data available (refs 6, 8, 11, and this study) on pattern regulation and transdetermination in the male foreleg disk following different experimental treatments.

The main findings for pure reaggregates are (Table 1): (1) Distal transformation, as judged primarily by the regeneration of claws, but also by a comparison of the number of vesicles per leg segment (compared with control experiments, Table 1b), was very rare, even in UM^- reaggregates; (2) Regeneration of structures missing in a circumferential direction occurred rarely, if at all, in LM^- , LL^- and UL^- reaggregates, and even in UM^- reaggregates only a few cases were observed; (3) Transdetermination was only observed in UM^- reaggregates, where two small wing-blade spots were found; (4) The rate of proliferation of the LL^- cells was very low, confirming earlier findings^{6,8}.

The rarity of both distal transformation and transdetermination in pure reaggregates contrasts sharply with the behaviour of identical leg disk cells in mixed reaggregates (Table 2). The data indicate that the high frequency of both events in mixed reaggregates did not result from the dissociation procedure *per se*, but rather from interactions between leg disk cells and 'feeding layer' cells. A specific effect of the irradiation of the 'feeding layer' cells on leg disk cells was ruled out by experiments in which UM^- quadrants were dissociated together with unirradiated wing disks. Both distal transformation and transdetermination were frequent in such reaggregates (Table 1b, D). The disk type of the 'feeding layer' tissue was also irrelevant to the behaviour of the leg disk cells. In mixed UL^- reaggregates containing either antenna disk or male foreleg disk 'feeding layers' (Table 1b, B, C) distal transformation and transdetermination occurred frequently, and the transdetermined structures were almost exclusively of wing disk type, as with

Fig. 1. a, Simplified fate map of the *Drosophila* male foreleg disk (after Schubiger¹⁸). Leg segments: PT, prothorax; CX, coxa; TR, trochanter; FE, femur; TI, tibia; TA, tarsus. Cuticular landmarks: Δ , groups of sensilla trichodea (St1, St8, GST, St5, RSt); \circ , groups of sensilla campaniformia (Sc1, Sc5, Sc11, Sc3, Sc8); \bullet , single conspicuous bristles (BH⁻, EB), or sex comb teeth (Sct); JTr, JTh, joints; Tvr, transverse rows of bristles; Cl, claws. — — —, location of cuts. UM^- , LM^- , LL^- , UL^- , upper medial, lower medial, lower lateral and upper lateral quadrants, respectively, lacking their corresponding 'endknob' parts (which contain most of the tarsal material^{11,15}). UM^- , LM^- , LL^- , UL^- in the text indicate that experiments with quadrants both containing and lacking their 'endknob' portions had been carried out. b, Polar coordinate model specifying positions in the *Drosophila* male foreleg disk (modified⁶ after French *et al.*³), and approximate location of the cells capable of transdetermination in the disk¹⁰ (hatching). 1–12, Circumferential positional values; A–E, radial positional values.



reaggregates containing wing disk 'feeding layer'⁸. It thus seems that cells from different imaginal disks exert similar effects on pattern regulation and transdetermination in intermixed leg disk cells.

In pure reaggregates, the cells of all quadrants chiefly formed structures characteristic for their region of origin (Table 1). This indicates that dissociated cells and cell clumps retain their positional information⁷. The mixed reaggregates, however, clearly show that these cells or cell clumps can, in appropriate conditions, be stimulated to acquire specific new developmental programmes. What factors caused identical cells to behave differently in different experimental conditions? The polar coordinate model of French *et al.*³ postulates that pattern regulation in *Drosophila* imaginal disks is controlled by interactions among cells carrying disparate positional information², brought into contact by wound healing or mechanical intermixture. Such disparities are thought to induce proliferation which, in accordance with specific rules, leads to the intercalation of missing positional values. According to this model the regeneration of a complete field circumference, as well as distal transformation, are possible only if a blastema possesses the majority of the circumferential positional values. Since in mixed reaggregates cells from all quadrants underwent distal transformation, the possibility was considered that the pattern regulation rules might not apply in reaggregates⁸. The rarity of distal transformation in pure reaggregates (Table 1), however, indicates that this is not the case, and suggests that the rules are still followed by dissociated and reaggregated cells and cell clumps. (It should be noted, however, that the polar coordinate model cannot explain the rare cases of distal transformation in pure *LM*⁻ reaggregates, nor the observed instances of distal transformation in intact *LM*⁻ quadrants and lateral halves; see Table 2 and ref. 11.) A more likely explanation for the behaviour

of the leg cells in mixed reaggregates is that they interacted with 'feeding layer' cells possessing non-homologous positional values⁸. In this way, complete circumferential sequences of positional information could be generated and allow distal transformation to occur. Evidence for communication between non-dissociated leg and wing disk tissues, resulting in the stimulation of intercalary regeneration⁵ in intermixed fragments (highly irradiated fragments being as effective in stimulation as unirradiated ones¹²) has now been obtained (ref. 13, and unpublished results of P. Adler, M. Fain and S.S.). These findings thus support the concept of a common mechanism of positional specification in different imaginal disks^{2,3,14}.

The high regenerative potentials of intact *UM*⁻ fragments led to this disk region being ascribed the majority of the circumferential positional values³ (Fig. 1b). Pure *UM*⁻ reaggregates, however, showed only rare regeneration (Table 1), a disparity which can be explained in terms of the polar coordinate model³. In intact *UM*⁻ fragments, wound healing always juxtaposes the two radial cut surfaces^{3,15}, thus creating the conditions necessary for the intercalation of the missing circumferential values ('shortest intercalation rule'), followed then by distal transformation ('complete circle rule'). In pure *UM*⁻ reaggregates, however, the cells have to become confronted by chance with cells showing a positional disparity corresponding to about half of the circumferential values, in order to regenerate complete circles which could then permit distal transformation. Clearly, the probability of such an event is quite small, especially since the *UM* region probably possesses only slightly more than half of the circumferential values⁶. In mixed reaggregates, however, cells from all quadrants would frequently confront 'feeding layer' cells that have sufficiently disparate positional values to allow the formation of complete circles followed by distal transformation.

Table 1 Structures formed by dissociated and reaggregated cells of the male foreleg disk quadrants, without (a) or with (b) intermixed 'feeding layer' cells, after an 8-d culture period in adults

Quadrant tested*	'Feeding layer'†	Size of grown impl.‡	Diff. impl. (recov. rate)§	PT		CX		Leg structures¶				TI		TA		Transdetermination
				n	lm	n	lm	n	TR	n	FE	n	lm	n	lm	
(a) <i>UM</i> ⁻	-	4.2	26 (49%)	21	GSt(5)	27	St8(11) JTr(5) BH-(3) JTh(3) RSt(8)	9	Sc5(7) Stl(3) GSt(6) St5(2) Sc3(2) Sc8(3) EB(1)	12	Sc1(3) Sc11(1)	21	Tvr(13)	11	Sc1(24) Cl(11)††	< 1%
<i>LM</i> ⁻	-	4.5	17 (63%)	10	GSt(6)	20	St8(9) JTr(4) BH-(6) BH-(2)	16	GSt(2) St5(10) Sc3(9)	16	Sc1(1) Sc11(8)	32	Tvr(114)	36	Sc1(440) Cl(5)††	
<i>LL</i> ⁻	-	1.0	3 (50%)			2	BH-(2)			7		1				
<i>UL</i> ⁻	-	3.2	25 (78%)	12		24	JTh(3) RSt(6)	2	Sc8(2) EB(1)	44		53				
(b) A. <i>LM</i> ⁻	W**	5.3	14 (28%)	4		1		1	GSt(1)	7	Sc11(3)	11	Tvr(9)	41	Sc1(82) Cl(216)††	
B. <i>UL</i> ⁻	A**	4.0	10 (34%)	1		2				5		5		9	Cl(13)††	60%
C. <i>UL</i> ⁻	L**	4.2	24 (48%)			2	JTh(1) RSt(2)			2		6		24	Cl(51)††	85%
D. <i>UM</i> ⁻	W	16.4 ‡‡	56 (68%)							6		6		30	Sc1(95) Cl(41)††	10%

*Abbreviations as in Fig. 1a. Number of experiments performed: a, two experiments for each quadrant; b, A-D, one experiment each. Number of quadrants used per experiment: a, 120; bA and D, 50; bB and C, 10. Genotypes¹⁹ used: in a, bA and bD, equal numbers of *mwh e*¹¹ and *y v f mal*; bB and bC, *mwh e*¹¹.

†W, Wing disks; A, antenna disks; L, male foreleg disks. **Disks irradiated with 17.5 Kr (ref. 8). Number of disks added: bA and bD, 20; bB and bC, 70. Genotypes¹⁹ used: bA and bD, *y*; bB and bC, *y f*^{36 a}.

‡Value represents average number of larvae needed as hosts for the material recovered from one adult host.

§Recovery rate represents the percentage of injected implants recovered as differentiated implants.

¶Abbreviations as in Fig. 1a. n, Number of identified vesicles, printed in bold. In parentheses, number of times a certain landmark (lm) was encountered; underlining indicates that this landmark maps distinctly outside the quadrant tested.

||Percentage of total differentiated area occupied by transdetermined material (see ref. 8).

††Number of tarsal vesicles in which claws were present and (in parentheses) maximal number of claws observed in a single vesicle: 1, *UM*⁻ 4(4); *LM*⁻ 2(3); bA, 34(16); bB, 9(2); bC, 24(4); bD, 22(5).

‡‡Only the material from 5 out of 16 adult hosts was subjected to metamorphosis.

Table 2 Pattern regulation and transdetermination by cells of the *Drosophila* male foreleg disk quadrants using different experimental conditions

Type of cell fate alteration	Experimental condition*	Quadrants†,‡				Ref.
		UM ⁻	LM ⁻	LL ⁻	UL ⁻	
Regeneration in circumferential direction	F	+++	—§¶	—§	—§	6, 11 8
	R, mixed	+	—¶	—	—	
	R, pure	+	—¶	—	—	
Regeneration of claws (distal transformation)	F	++	+	—	—	11 8
	R, mixed	+++	++++	+	+++	
	R, pure	+	+	—	—	
Transdetermination	F	+++	—	—	—	6, 11 8
	R, mixed	++++	—	—	++++	
	R, pure	+	—	—	—	

*F, Intact fragments; R, reagggregates.

†Abbreviations of quadrants as in Fig. 1a.

‡The symbols —, +, ++, +++, ++++ give a rough comparison of the frequencies or extents of pattern regulation or transdetermination, graded from — (not observed) to ++++ (observed in very large amounts). For the exact data see the literature cited.

§LM⁻, LL⁻, and UL⁻ quadrants, instead of regenerating missing structures, produce mirror-image duplications: LM⁻, ++++; LL⁻, ++; UL⁻, ++ (unpublished results, and see ref. 6).¶LM cells might be able to regenerate certain structures of the LL quadrant⁶.||Fraction of cultured quadrants (lacking their corresponding 'endknob' part¹¹) with claw regeneration: UM⁻, 14/30 (ref. 11); LM⁻, 3/19; LL⁻, 0/10; UL⁻, 0/16 (my unpublished results).

A similar mechanism could also explain the differences in transdetermination frequencies using different treatments (Table 2). According to a recent hypothesis¹⁰, transdetermination occurs when cells capable of it (Fig. 1b), by juxtaposition with cells having sufficiently different positional values, are stimulated to a certain critical amount of proliferation. In intact UM⁽⁻⁾ fragments, the fusion of the radial cut surfaces regularly confronts the cells capable of transdetermination with cells having nearly maximal disparities in circumferential values. The amount of intercalary growth thus induced would often trigger transdetermination. In pure UM⁻ reagggregates, however, the chance for the transdetermination-competent cells to confront cells with positional disparities large enough for the stimulation of transdetermination is very small. In intact UL⁽⁻⁾ fragments and in pure UL⁻ reagggregates, the cells capable of transdetermination simply could never meet cells with a positional disparity sufficient for the stimulation of transdetermination, because of the low density of circumferential values in this disk region^{3,6}. In mixed UM⁽⁻⁾ and UL⁽⁻⁾ reagggregates, however, juxtaposition to 'feeding layer' cells with large disparities in positional values would often cause a sufficient amount of proliferation in the cells capable of transdetermination to stimulate them to undergo this change. Earlier reports of sharply increased transdetermination frequencies in imaginal disk reagggregates^{16,17} can similarly be explained by cell interactions of this kind, without having to assume a specific effect of the dissociation procedure *per se* ('wounding effect') on transdetermination.

I thank Suzanne Glenn and Becky Hsei for technical assistance. I was supported by a fellowship from the Swiss National Science Foundation and by a grant from the United States National Institutes of Health.

SIEGWARD STRUB

Center for Pathobiology,
University of California,
Irvine, California 92717

Received 18 June; accepted 22 August 1977.

- Nöthiger, R. in *The Biology of Imaginal Disks* (eds Ursprung, H. & Nöthiger, R.) 1–34 (Springer, Berlin-Heidelberg-New York, 1972).
- Wolpert, L. *Curr. Top. dev. Biol.* **6**, 183–224 (1971).
- French, V., Bryant, P. J. & Bryant, S. V. *Science* **193**, 969–981 (1976).
- Hadorn, E. in *Genetic Control of Differentiation. Brookhaven Symp. Biol.* **18**, 148–161 (1965).
- Haynie, J. L. & Bryant, P. J. *Nature* **259**, 659–662 (1976).
- Strub, S. *Wilhelm Roux Arch. EntwMech. Org.* **181**, 309–320 (1977).
- García-Bellido, A. & Nöthiger, R. *Wilhelm Roux Arch. EntwMech. Org.* **180**, 189–206 (1976).
- Strub, S. *Wilhelm Roux Arch. EntwMech. Org.* **182**, 75–92 (1977).
- Rose, S. M. *Symp. Soc. Stud. Dev. Growth* **20**, 153–176 (1962).
- Strub, S. *Wilhelm Roux Arch. EntwMech. Org.* **182**, 69–74 (1977).
- Schubiger, G. *Dev. Biol.* **26**, 277–295 (1971).

- Adler, P. N. & Bryant, P. J. *Dev. Biol.* (in the press).
- Wilcox, M. & Smith, R. J. *Dev. Biol.* (in the press).
- Postlethwait, J. H. & Schneiderman, H. A. *Dev. Biol.* **25**, 606–640 (1971).
- Reinhardt, C. A., Hodgkin, N. M. & Bryant, P. J. *Dev. Biol.* (in the press).
- García-Bellido, A. *Expl Cell Res.* **44**, 382–392 (1966).
- Tobler, H. J. *Embryol. exp. Morphol.* **16**, 609–633 (1966).
- Schubiger, G. *Wilhelm Roux Arch. EntwMech. Org.* **160**, 9–40 (1968).
- Lindsley, D. L. & Grell, E. H. *Carnege Inst. Wash. Publ.* **627** (1968).

Haemopoietic stem cells of rats but not of mice express Th-1.1 alloantigen

IN mice thymus-derived lymphocytes express θ -antigen specificities in two allelic forms, Th-1.1 (formerly called θ -AKR) and Th-1.2 (θ -C₃H), which are distributed reciprocally among the various mouse strains^{1,2}. Rats have also been tested for the occurrence of antigens of the Th-1 system, but so far all strains have been positive with anti-Th-1.1 only^{3,4}. Rats also differ from mice in the concentration of Th-1.1 carrying cells, which, in rats, is much higher in the bone marrow and lower in the peripheral lymphoid organs⁵. The stage at which haemopoietic stem cells differentiate towards thymocytes carrying the θ alloantigen is still ill-defined. We present here evidence that in rats Th-1.1 is already expressed on haemopoietic stem cells while murine haemopoietic stem cells have not yet developed this θ alloantigen.

Haemopoietic stem cells are defined operationally by their capacity to form cell colonies⁶, proliferate in diffusion chambers⁷ and, of course, repopulate a conditioned bone marrow recipient. The latter activity can be inhibited by sensitising the prospective bone-marrow recipient against antigens occurring on the donor's stem cells or by incubating the donor's marrow with antibodies against his stem cells before transferring it to the recipient. Both experimental models served as indicator systems for Th-1 antigens on stem cells in the following experiments.

Mouse strains CBA, C₃H, (C57BL/6 × CBA-T6)F₁, A and BALB/c, all Th-1.2 and AKR (Th-1.1) were obtained from The Jackson Laboratory, Bar Harbor, Maine and AKR/Cum (Th-1.2) mice from Cumberland View Farms, Clinton, Tennessee. The two AKR sublines are antigenically similar with respect to markers Ly-1, Ly-2, Ly-3, TL, H-2, G_{IX} and Gross cell surface antigen, while differing for Thy-1⁸. A/ θ AKR (Th-1.1) mice were produced from mice originally supplied by Dr E. A. Boyse of the Sloan-Kettering Institute, New York. They are congenic to A/J and differ only at the Th-1 locus. Inbred Lewis rats were produced in our laboratory: 90% of their thymocytes were lysed by anti-Th-1.1 but not by anti-Th-1.2 in the microcytotoxicity test. These antisera had a cytotoxic titre of 1:512 and more and had been produced by

Table 1 Transplantation of bone marrow from Th-1.1 mice or rats to Th-1.2 mice presensitised against Th-1.1

Expt no.	Recipient* Th-1.2	Immunisation†		Take (AKR/J) Th-1.1	8		Chimaerism on days‡		
		Th-1.1	Th-1.2		14				
1	(C57BL/6 × CBA-T6)F1	A/θAKR		+	50/0	18/2	48/2	50/0	20/0
2	(C57BL/6 × CBA-T6)F1	Lewis		+	15/5	18/2	98/2	50/0	0/20
				Take (Lewis)					
3	(C57BL/6 × CBA-T6)F1	AKR/J		—	20/0	15/2	13/0	20/0	20/0
4	(C57BL/6 × CBA-T6)F1		AKR/Cum	+	0/2	0/100	2/98	2/98	
5	CBA	AKR/J		—	10/0	66/45	14/0	50/0	8/2
6	CBA		C ₃ H	+	45/5	20/30	12/88	10/90	0/50
7	AKR/Cum	AKR/J		—			20/0	17/2	50/0
8	AKR/Cum		BALB/c	+			13/87	0/100	0/100

*Conditioned with 750–900 rad ¹³⁷Cs, 131 rad per min 24h before transplantation of 2 × 10⁷ AKR/J mouse or 10⁸ Lewis rat bone marrow cells.

†10⁸ thymocytes were injected i.p. three times 5d apart.

‡Allogeneic chimaeras: no. without/no. with the T6 marker chromosome; xenogeneic chimaeras: no. of alkaline negative mouse granulocytes/no. of alkaline positive rat granulocytes.

repeated immunisation of CBA, C₃H, AKR/J mice or Lewis rats with Th-1 incompatible thymocytes (10⁸ cells injected three times 5 d apart).

Failure of engraftment due to presensitisation against antigens on donor-type haemopoietic cells is a very sensitive indicator system. It reveals also minor antigen incompatibilities where serological *in vitro* tests fail⁹. To avoid any undesired cross-immunisation of the recipient from antigens on the donor's thymocytes other than Th-1.1, bone marrow recipients were immunised with thymocytes of third-party strains sharing the Th-1.1 antigen of the donor strain but no other tissue antigens which might prevent haemopoietic engraftment. Intraspecies sensitisation was therefore combined with interspecies bone marrow transplantation and vice versa. Interspecies xenogeneic bone marrow transplantation from rat to mouse (for conditions see Table 1 legend) regularly leads to complete chimaerism¹⁶. The following experimental design was used: prospective bone marrow Th-1.2 recipient mice were sensitised against Th-1.1 or Th-1.2 thymocytes of rats or different mouse strains. They were subsequently irradiated and transfused with third-party marrow from AKR/J or Lewis rats (both Th-1.1). Failure of engraftment was taken as evidence for sensitisation against alloantigens shared by sensitising cells and haemopoietic stem cells of the donor strain. Chimaerism was tested in the femora by chromosomal analysis in allogeneic chimaeras using the T6 marker chromosome and by selective alkaline phosphatase staining of rat granulocytes¹⁵ in xenogeneic chimaeras.

Experiments 1 and 2 in Table 1 show that immunisation with Th-1.1 thymocytes of A/θ AKR mice or Lewis rats did not interfere with a take of subsequently transferred AKR/J bone marrow. Control series of untransplanted mice of the recipients' strains receiving AKR/J or Lewis or A/θ-AKR thymocytes had regularly shown high titres (< 1:256). Rat bone marrow, in contrast, failed to grow in mice sensitised against the mouse Th-1.1 antigen. That this failure of engraftment is not inherent to the xenogeneic donor-recipient combination itself is shown in experiments 4, 6 and 8 where rat marrow grew easily in mice sensitised against thymocytes of congenic or allogeneic Th-1.2 strains.

Table 2 Chimaerism in mice after transfer of rat marrow pre-incubated with anti-Th-1.1 or anti-Th-1.2 serum

Incubation* with	Chimaerism on day 14†
Anti-Th-1.2 (AKR/J-anti-C ₃ H)	10/60, 5/95, 1/18, 0/10
Anti-Th-1.1 (C ₃ H-anti-AKR/J)	5/1, 4/0, 0/0, 0/0, 20/3, 20/0, 18/0
Normal mouse serum	0/100, 0/20, 3/0, 0/60, 0/100, 0/100, 2/98, 0/0
No serum	0/50, 0/3, 0/30, 3/17

*0.25 ml for 30 min at 37 °C.

†No. of alkaline negative mouse granulocytes per no. of alkaline positive rat granulocytes.

Chimaerism was an all-or-none phenomenon which occurred uniformly in all animals of an experimental group.

Group-specific stem cell toxicity of anti-Th-1.1 was also demonstrated by incubating rat marrow with anti-Th-1.2 (AKR/J-anti-C₃H) and anti-Th-1.1 (C₃H-anti-AKR/J) serum. Table 2 shows that only the latter antiserum prevented a take of the rat marrow, which was suggested by the absence of circulating rat granulocytes and proved by a completely aplastic bone marrow histology (Hoffmann-Fezer, unpublished) of the recipient mice. In contrast, transplantation of allogeneic bone marrow from AKR/J mice to (C57BL/6 × CBA-T₆)F₁ recipients was not inhibited by a pre-incubation of anti-Th-1.1 (CBA-anti-Lewis) serum (not shown in Table 2) though this antiserum reacted strongly with AKR/J (1:1024). The transfer experiments clearly show that sensitisation against Th-1.1 positive rat marrow either *in vivo* by immunisation of the marrow recipient against Th-1.1 mouse marrow or *in vitro* by incubation of the donor marrow with anti-Th-1.1, inhibits the repopulating capacity of haemopoietic rat stem cells. The immunogenetic specificity of this inhibition is evident because sensitisation against Th-1.2 in otherwise identical conditions did not inhibit haemopoietic repopulation of mice with rat marrow. It is difficult to preclude that a transplantation antigen closely linked to Th-1.1 rather than the θ antigen itself occurs on the rat's haemopoietic stem cells and prevents repopulation implying a tissue antigen occurring in rats and AKR/J but absent from AKR/Cum and the other Th-1.2 mouse strains tested: this seems unlikely, however.

On the other hand, the allogeneic cell transfer experiments also show unequivocally an absence of the Th-1.1 antigen on mouse haemopoietic stem cells. Stem cells of the Th-1.1 donors preserved the capacity to induce haemopoiesis in recipients in spite of an antiserum treatment inhibiting the Th-1.1 lymphocytes present together with the stem cells in the donor cell inoculum. That these stem cell-sparing anti-θ antibodies were active against the donor strain lymphocytes can be shown by their suppression of graft-versus-host reactions¹⁰.

The absence of the θ antigen from mouse stem cells is in agreement with reports on anti-mouse brain serum from rabbits, absorbed with B-cells, which spared haemopoietic stem cells and suppressed T cell-derived graft-versus-host disease when incubated with the donor's marrow¹¹. Anti-brain serum, however, does not lose its stem-cell toxicity after exhaustive absorption with T cells¹². Several other reports deal with antisera against haemopoietic or colony-forming stem cells. As a stem-cell label such antisera are, however, of little value because they usually react with species-specific antigens or H-2 alloantigens occurring on cells of various other organs.

Th-1.1 in rats is the first alloantigen restricted to stem cells and θ antigen-bearing cells. It is not only absent from B lymphocytes but, in contrast to mice, also from most peripheral T cells¹³. Again in contrast to mice where the concentration of Th-1.1 lymphocytes in the marrow is below 5%¹—Williams found 30–45% Th-1.1 positive cells in the rat bone marrow⁵. Considering the poor

immunocompetence of rat marrow Williams classifies Th-1.1 marrow cells as prothymic—having expressed their Th-1.1 molecule already in the bone marrow—rather than as mature post-thymic lymphocytes. Such a comparatively early expression of the Th-1.1 antigen in rat marrow may explain why Th-1.1, in the rat, can be detected also on haemopoietic stem cells. While this is speculative, the restrictive specificity of the Th-1.1 antigen, which is absent from myeloid cells and, in the rat, from over 90% of thoracic duct and lymph-node cells^{1,3}, should make it a useful marker of haemopoietic stem cells.

I thank Ms J. Molitor and B. Glöckner for technical assistance.

S. THIERFELDER

Abteilung Immunologie,
Institut für Hämatologie, GSF
Landwehrstrasse 61
Munich, FRG

Received 6 May; accepted 23 August 1977.

1. Reif, A. E. & Allen, J. M. *J. exp. Med.* **120**, 413 (1964).
2. Ralf, M. C. & Wortis, H. H. *Immunology* **18**, 931 (1970).
3. Douglas, T. C. *J. exp. Med.* **136**, 1054 (1972).
4. Micheel, B., Pasternak, G. & Steuden, J. *Nature new Biol.* **241**, 221 (1973).
5. Williams, A. F. *Eur. J. Immun.* **6**, 526 (1976).
6. Till, J. E. & McCulloch, E. A. *Rad. Rev.* **14**, 213 (1961).
7. Breivik, B., Benestad, H. B. & Boyum, A. *J. cell. Physiol.* **78**, 65 (1971).
8. Acton, R. T. *et al. Nature new Biol.* **245**, 8 (1973).
9. Storb, R., Epstein, R. B., Rudolph, R. H. & Thomas, E. D. *J. Immun.* **105**, 627 (1970).
10. Thierfelder, S. & Rodt, H. *Transplantation* **23**, 87 (1977).
11. Rodt, H., Thierfelder, S. & Eulitz, M. *Eur. J. Immun.* **4**, 25 (1974).
12. Golub, E. S. *J. exp. Med.* **136**, 569 (1972).
13. Acton, R. T., Morris, R. J. & Williams, A. F. *Eur. J. Immun.* **4**, 598 (1974).
14. Ford, C. E., Hamerton, J. L., Barnes, D. W. H. & Loutit, J. E. *Nature* **177**, 452 (1956).
15. Vos, O., Davids, J. A. G., Weyzen, W. W. H. & van Bekkum, D. W. *Acta physiol. pharmac.* **4**, 482 (1956).
16. Bau, J. & Thierfelder, S. *Transplantation* **15**, 564 (1973).

Catecholamine hormone receptors are reduced on chronic lymphocytic leukaemic lymphocytes

ADENYLATE cyclase (EC 4.6.1.1.) is a bifunctional enzyme that responds to biological signals such as hormones as well as catalysing the synthesis of cyclic 3',5'-AMP. Several reports indicate that the hormonal responsiveness of this enzyme in transformed or neoplastic cells is altered¹⁻³. This may explain the low cyclic AMP levels that have been associated with the unregulated cellular proliferation seen in transformed cells⁴⁻⁶. There is also evidence to implicate cyclic AMP in normal lymphocyte activation^{7,8}. Comparison of circulating lymphocytes from chronic lymphocytic leukaemia (CLL) patients with those from normal human controls indicates that cyclic AMP levels^{9,10}, cyclic nucleotide phosphodiesterase^{11,12} and adenylate cyclase¹³ activities are changed in the CLL lymphocyte. The membrane enzyme activity of 5' nucleotidase¹⁴ as well as complement¹⁵, antigen¹⁶ and lectin¹⁷ binding are also altered in the CLL plasma membrane. The observation that catecholamine hormone (β -adrenergic) responsiveness is depressed in CLL lymphocytes is further evidence for a functionally altered plasma membrane¹³. Until now, it has not been possible to identify the molecular lesion responsible for the reduced catecholamine sensitivity. But, with the availability¹⁸⁻²⁰ of radiolabelled molecules that bind efficiently and directly to specific β -adrenergic hormone receptor sites (for example (–)³H-dihydroalprenolol), we can now determine whether the CLL adenylate cyclase defect occurs in the β -adrenergic hormone-receptor complex, in the transducing mechanism that couples the receptor to the catalytic component, or in the intrinsic catalytic mechanism. We show here that the number of β -adrenergic hormone receptor sites is reduced on CLL lymphocyte membranes while the catalytic capacity of the cyclase enzyme is normal. The low density of catecholamine hormone receptors could account for the altered cyclic AMP metabolism and may

contribute to the unregulated growth of CLL lymphocytes.

Lymphocytes were isolated from heparinised blood from healthy human volunteers (100–400 ml) and from patients with chronic lymphocytic leukaemia (10–30 ml). The diagnosis of CLL was made after complete haematological evaluation including bone marrow biopsy. All steps in the isolation were done using sterile techniques. The lymphocytes were separated by centrifuging the blood on Ficoll-Hypaque density gradients using the method of Boyum²¹. Cells at the interphase were collected and diluted 1:1 with 199 medium plus 5% foetal calf serum, centrifuged at 450g for 10 min, and the resulting pellet was washed with 20 ml of the same medium. The washed cells were further purified immediately or resuspended in 40 ml 199 medium plus 10 ml calf serum, placed in a Falcon 75 cm² flask and stored in a 37 °C, 5% CO₂ humidified incubator (up to 10 h).

Subsequently, the non-adherent mononuclear cells were collected by centrifugation at 200g for 10 min. The cells were resuspended to a concentration of about 5×10^7 cells per ml and passed over nylon wool. Lymphocytes were collected by centrifugation (300g for 10 min) and washed twice (50 ml, 20 ml) with phosphate buffered saline pH 7.2 containing 0.1% gelatin. Greater than 90% of the cells were lymphocytes by microscopy. In selected experiments separation of B and T cells with recovery of both depleted and enriched fractions was performed as described by Kaplan *et al.*²². The final pellet was resuspended in 15 ml cold 50 mM Tris-HCl, 10 mM MgCl₂, pH 8.1 at 4 °C (incubation buffer), allowed to swell for 20 min, and homogenised by 25 up and down strokes in a pre-chilled (0 °C) Dounce homogeniser. The homogenate was centrifuged at 25,000g for 15 min, the pellet was rinsed once with cold incubation buffer (1 ml buffer per 5×10^7 cells). Adenylate cyclase activity of this membrane preparation was determined by the method of Brown²³. Protein was measured by the method of Lowry *et al.*²⁴.

The procedure for determining the density of hormone receptor sites on lymphocyte membranes was based on the method of Williams *et al.*²⁵. To tubes containing (–)³H-dihydroalprenolol (final concentration 1–80 nM) with and

Table 1 Adenylate cyclase activity of normal and CLL lymphocytes

Cell population	Adenylate cyclase activity (pmol per min per mg protein)		
	Basal	F [–] (10 mM)	Isoprenaline (10 μ M)
Normal lymphocytes	39	131	103
CLL lymphocytes	24	70	32

Adenylate cyclase activity was measured by analysis of the cyclic AMP²³ produced in a 10-min, 30 °C, incubation of the lymphocyte membranes in a buffer containing 40 mM Tris, 5 mM Mg²⁺, 5 μ g ml^{–1} R020-1724 (Hoffman-La Roche), 1 mM dithiothreitol, 2 mM ATP and additives at the concentrations indicated. The values are averages of duplicates from a representative experiment.

without (\pm)propranolol (10^{-3} M final concentration) was added 160 μ l of lymphocyte homogenate (40–130 μ g protein) to give a total volume of 200 μ l. Tubes were incubated for 13 min at 37 °C. Incubation was terminated by adding 4 ml of ice-cold incubation buffer to the tube and rapidly filtering contents through a Gelman-type A/E glass fibre filter under low vacuum. The filter was rinsed (ice-cold incubation buffer) with a 4 ml wash of the tube and a final (8 ml) rinse. Filters were placed in 10 ml of 10% BioSolv (Beckman), 90% toluene, 0.4% omnifluor, scintillation cocktail and allowed to stand overnight before counting.

In each experiment, 'nonspecific' binding was determined by parallel assay tubes which contained a large excess (10 μ M) of (\pm)propranolol. 'Specific' binding was defined

as the difference between the total and nonspecific binding.

The (–)³H-dihydroalprenolol was from New England Nuclear who prepared the radiolabelled product by catalytic reduction of (–)alprenolol using tritium gas with palladium as a catalyst. The molecular structure (determined by mass spectroscopy) is dihydroalprenolol and the tritium is probably found at the unsaturated bond in the aliphatic chain on position 2 of the aromatic ring.

The reactivity of the CLL lymphocyte adenylate cyclase to the β-adrenergic agonist, (–)isoprenaline (Sigma) is appreciably less than that of the enzyme prepared from normal lymphocytes (Table 1). However, fluoride stimulation of the CLL enzyme is comparable with that of normal controls (threefold stimulation). This response of the enzyme to fluoride indicates that the catalytic mechanism of the CLL enzyme is functioning properly.

Using (–)³H-dihydroalprenolol as a specific β-adrenergic ligand to bind directly and specifically to β-adrenergic hormone receptors, we compared crude membrane preparations from normal and CLL lymphocytes. Figure 1 illustrates the reduced binding of the radiolabelled ligand by CLL membranes. The normal cells showed considerable binding which corresponded to approximately 6,000 molecules bound per cell. Ligand binding to CLL membranes was too low to assess accurately, but it was less than 2,000 molecules per cell. The control dose-response curve exhibits saturation at 6×10^{-8} M, indicating an approximate K_d of 3×10^{-8} M for the dihydroalprenolol. Both control and CLL binding reactions showed about 70% specific binding of the (–)³H-dihydroalprenolol in the experimental conditions. In addition to the saturability and high affinity, the specificity of the dihydroalprenolol binding was indicated by its stereospecificity (1-stereoisomers of isoprenaline and propranolol were more efficient in displacing the bound (–)³H-dihydroalprenolol than the *d*-stereoisomers) and the binding

showed rapid kinetics for association reaching a steady-state within two minutes. Furthermore, the displacement of (–)³H-dihydroalprenolol by 10^{-6} M propranolol was approximately the same as 10^{-4} M isoprenaline which is consistent with the potency of these agents on other well characterised β-adrenergic receptors.

All of the CLL patients in this study had increased percentages of lymphocytes with monoclonal surface immunoglobulin (Ig) suggesting that the disease probably represents a proliferation of B lymphocytes¹⁶. To exclude the possibility that the depressed (–)³H-dihydroalprenolol binding was not simply a characteristic of cells with surface Ig, separation of normal lymphocytes into T and B populations was performed. Table 2 shows, in fact, that membranes isolated

Table 2 (–)³H-Dihydroalprenolol bound to human lymphocytes

Cell population	(–) ³ H-dihydroalprenolol bound† (fmol per mg membrane protein)
Normal lymphocytes (8)*	682 ± 114
'T' lymphocytes, normal (2)	532 ± 132
'B' lymphocytes, normal (2)	1,439 ± 83
CLL lymphocytes (10)	220 ± 69

Lymphocyte preparation and separation into 'B' and 'T' subpopulations were performed as described in the text. Of the cells in the 'B' fraction, 61% have surface immunoglobulin, while 89% of the 'T' rich portion formed red blood rosettes²². Binding was measured²⁵ at a concentration of 5×10^{-8} M (–)³H-dihydroalprenolol. The averages of triplicate determinations were done for each experiment and the averages used to determine the standard error of the differences between the normal and CLL lymphocytes. Normal and CLL values showed a significant statistical difference which is represented by $P < 0.001$.

*Value in parentheses is number of separate experiments.

† Mean ± s.e.m.

from non-T cells (surface Ig positive) bind more (–)³H-dihydroalprenolol than those isolated from T lymphocytes. This indicates that enrichment of B cells in the CLL lymphocyte population could not account for the observed hormone receptor differences between normal and CLL cells.

CLL lymphocyte adenylate cyclase is β-adrenergic unresponsive, although the homogenates exhibit fluoride-stimulated adenylate cyclase activity, showing that the catalytic component of the enzyme is behaving normally. We have shown that the density of the β-adrenergic hormone receptor site is decreased in CLL lymphocytes. This alteration in the number of β-adrenergic hormone receptor sites could explain the loss of CLL β-adrenergic adenylate cyclase responsiveness observed first by Polgar *et al.*¹³. It may also account for the depressed cellular levels of cyclic AMP found in CLL lymphocytes⁹. Depressed cyclic AMP levels are known to be correlated with unregulated cellular growth⁴, a characteristic of the neoplastic state.

A decreased capacity of cultured transformed cells to bind radiolabelled hormones has been recently observed. Differences in polypeptide hormone receptor sites^{26,27} as well as the catecholamine hormone receptors²⁸ were found in comparisons of virus-transformed cells with normal, growth regulated cells. Previous studies of 5' nucleotidase¹⁴ complement¹⁵ antigens¹⁶ and plant lectins¹⁷ have all indicated that the membrane of the CLL cells is structurally and functionally aberrant. Our observations show that the density of β-adrenergic hormone receptor sites is decreased on the CLL lymphocyte surface. Membrane alterations such as these that interfere with basic mechanisms of biological communication may be fundamental to the aetiology of diseases characterised by unregulated cellular proliferation.

We thank Elizabeth Anton for technical assistance. The separation of lymphocyte subpopulations was performed in the laboratory of Dr M. Kaplan. This research was supported by grants from the USPHS, the Veterans'

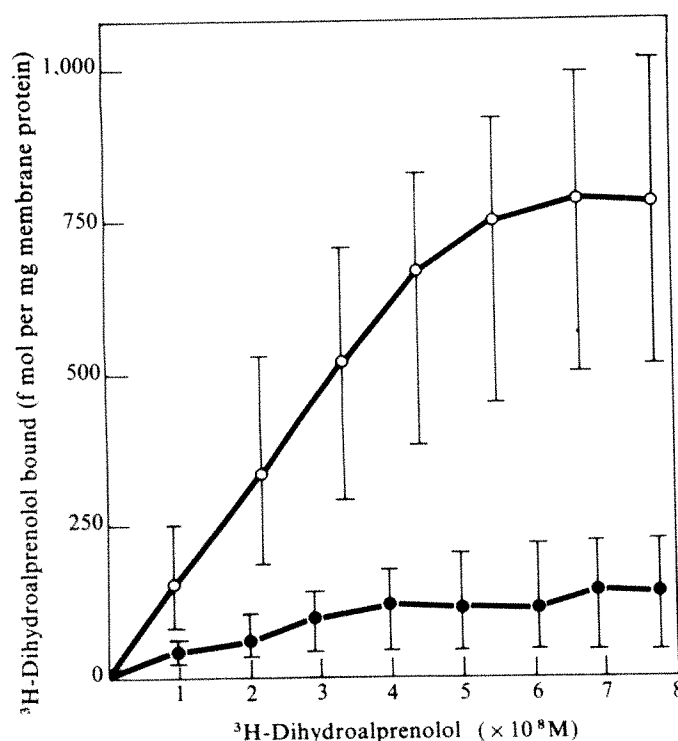


Fig. 1 (–)³H-dihydroalprenolol binding, as a function of concentration, was measured for lymphocyte membranes prepared as described in the text. This figure shows a single representative experiment with the range bars enclosing the area of all the experiments performed (12 normal and 10 CLL lymphocyte preparations). ○, Normal lymphocytes; ●, CLL lymphocytes.

Administration, the Minnesota Medical Foundation, the Graduate School of the University of Minnesota, and the Leukaemia Task Force.

J. R. SHEPPARD

Dight Institute of Human Genetics,
Department of Genetics and Cell Biology,

R. GORMUS

Veterans' Administration Hospital,
Minneapolis,

C. F. MOLDOW

Department of Medicine,
University of Minnesota Medical School,
Minneapolis, Minnesota 55455

Received 21 April; accepted 8 August 1977.

- Criss, W. E. & Morris, H. P. *Cancer Res.* **36**, 1740-1743 (1976).
- Christofferson, T. & Øye, I. *Endocrinology* **77**, 67-71 (1974).
- Sheppard, J. R., Cromwell, R. C., Meyers, R. & McLaughlin, W. in *The Role of Cyclic Nucleotides in Carcinogenesis* (eds Schultz, J. & Gratzner, H. G.), 19-37 (Academic, New York, 1973).
- Sheppard, J. R. *Nature new Biol.* **236**, 14-17 (1972).
- Pastan, I. H., Johnson, G. S. & Anderson, W. G. *A. Rev. Biochem.* **44**, 491-522 (1975).
- Ryan, W. L. & Heidrick, M. L. in *Adv. Cyclic Nucleotide Res.* **4**, 81-116 (1974).
- Abell, C. W. & Monahan, T. M. *J. Cell Biol.* **59**, 549-558 (1973).
- Cyclic AMP, Cell Growth and the Immune Response* (eds Braun, W., Lichtenstein, L. M. & Parker, C. W.) (Springer, New York, 1974).
- Monahan, T. M., Marchand, N. W., Fritz, R. R. & Abell, C. W. *Cancer Res.* **35**, 2540-2547 (1975).
- Smith, J. W., Steiner, A. L., Newberry, W. M. & Parker, C. W. *J. clin. Invest.* **50**, 423-441 (1971).
- Hait, W. N. & Weiss, B. *Nature* **259**, 321-323 (1976).
- Scher, N. S. *et al. Cancer Res.* **36**, 3958-3962 (1976).
- Polgar, P., Vera, J. C., Kelley, P. R. & Rutenburg, A. M. *Biochim. biophys. Acta* **297**, 378-383 (1973).
- Quagliata, F., Faig, D., Conklyn, M. & Silber, R. *Cancer Res.* **34**, 3197-3202 (1974).
- Pincus, S., Bianco, C. & Nussenzweig, V. *Blood* **40**, 303-310 (1972).
- Bentwich, A., Pollack, A. & Douglas, S. D. *Israel J. med. Sci.* **12**, 304-324 (1976).
- Novogrodsky, A., Binaminov, M., Ramot, B. & Katchalski, E. *Blood* **40**, 311-320 (1972).
- Maguire, M. E., Wirlund, R. A., Anderson, H. J. & Gilman, A. G. *J. biol. Chem.* **251**, 1221 (1976).
- Brown, E. M., Aurbach, G. D., Hauser, D. & Troxler, F. *J. biol. Chem.* **251**, 1232 (1976).
- Mukherjee, C., Coverstone, M., Caron, J. G. & Lefkowitz, R. J. *J. biol. Chem.* **250**, 4869 (1975).
- Boyum, A. *Scand. J. clin. Lab. Invest.* **21**, 77 (1968).
- Kaplan, M. E., Woodson, M. & Clark, C. in *In Vitro Methods in Cell Mediated and Tumor Immunity* (eds Bloom, B. & David, J. R.) 83-88 (Academic, New York, 1976).
- Brown, B. L. *Biochem. J.* **121**, 561 (1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193**, 265 (1951).
- Williams, L. T., Snyderman, R. & Lefkowitz, R. J. *J. clin. Invest.* **57**, 149 (1976).
- Todaro, G. J., Delarco, J. E. & Cohen, S. *Nature* **264**, 26-31 (1976).
- Thomopoulos, P., Roth, J., Lovelace, E. & Pastan, I. *Cell* **8**, 412-423 (1976).
- Sheppard, J. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1091-1094 (1977).

Actin co-caps with concanavalin A receptors

PREVIOUS studies have given mainly indirect^{1,2}, and some direct^{3,4} evidence that the mobility of surface membrane receptors may be controlled by cytoplasmic microfilaments and microtubules. It has been proposed^{1,2} that the receptor-cytoskeletal complex may have important roles in cell-cell recognition, cell motility and cell division. This study, using two fluorochromes to identify concanavalin A (con A) receptors and cytoplasmic actin in the same cell, shows that con A binding to neoplastic and embryonic cell surfaces results in dissolution of actin filaments and that con A receptors and actin co-cap and occur in closely related sites in untreated and cytochalasin B-treated cells.

Monolayer cultures of rat fibroblasts were prepared from normal adult kidney, dimethylnitrosamine (DMN)-induced renal mesenchymal tumours⁵, transformed kidney cell lines derived from DMN-treated rats⁶, 15-20 d foetal kidney or lung⁷ and 5-10 d whole embryos. The cells were tested either untreated, or after exposure for 20-60 min to 2×10^{-5} M cytochalasin B, 10^{-2} to 10^{-6} M colchicine, 10^{-5} M vinblastine, 2×10^{-3} M procaine or $10 \mu\text{g ml}^{-1}$ trypsin.

Marginal and/or random surface staining was seen in adult, tumour, transformed, embryonic and foetal fibroblasts incubated with fluorescein-isothiocyanate (FITC)-labelled con A at 4 °C, and in adult and foetal fibroblasts incubated at 37 °C (Fig. 1a, Table 1). In contrast, tumour, transformed and embryonic fibroblasts, incubated with FITC-con A at 37 °C for 20 min, showed staining in fine clusters (Fig. 2a), and at the cell margins and processes. Incubation of these cells for increasing time periods

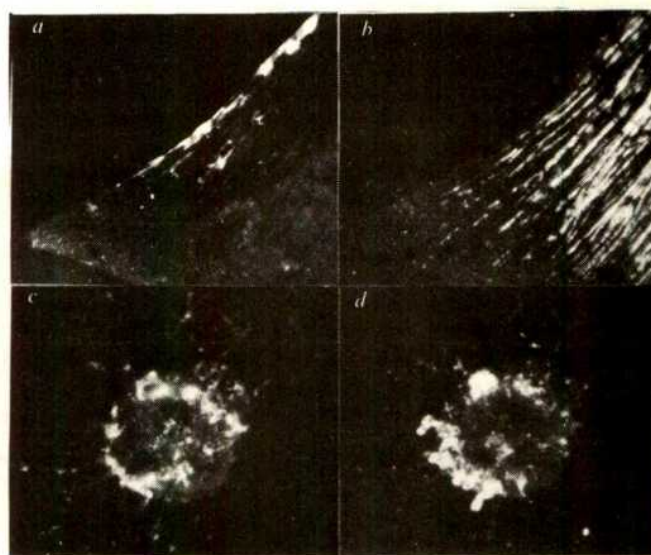


Fig. 1 FITC-con A followed by AAA/R-AHG staining of cultured adult kidney fibroblasts. Con A receptors were demonstrated by immunofluorescence reactivity¹⁵ of cell surfaces with FITC-labelled con A. Cytoplasmic actin was detected by AAA traced with R-AHG. The fluorescein: protein molar ratio and protein content of the conjugates were 4.9 and $300 \mu\text{g ml}^{-1}$ for FITC-con A, and 3.3 and 4 mg ml^{-1} for R-AHG. Tests were done at 4 °C or at 4 °C warmed to 37 °C for 20-360 min. Specificity of the FITC-con A staining was established by inhibition with unlabelled con A ($1,000 \mu\text{g ml}^{-1}$) or with 0.1 M α -methyl-D-mannopyranoside. The AAA specificity was established by immunoabsorption with muscle actin⁸. a, Adult kidney fibroblast, sub-1 stained with FITC-con A showing linear staining of the cell periphery ($\times 330$). b, Same cell as in (a) stained with AAA/R-AHG showing parallel actin filaments located in closely related sites as those seen in (a) ($\times 330$). c, Adult kidney fibroblast, sub-1, treated with cytochalasin B at 37 °C for 20 min and stained with FITC-con A at 37 °C for 20 min showing coarse clusters in the cell periphery. ($\times 330$). d, Same cell as in (c) stained with AAA/R-AHG showing coarse clusters in sites closely related to those seen in (c). ($\times 330$).

produced fine to coarse clusters in a central mass with clearing of the cell periphery at 40 min, a supranuclear cap of clusters at 60 min (Fig. 2c) and perinuclear globules at 120 min persisting until 360 min. Cells pretreated with 1% or 4% paraformaldehyde at 4 °C or 37 °C for 10 min showed staining restricted to cell margins both at 4 °C and 37 °C.

After 20-40 min of cytochalasin B (BL) treatment, adult and foetal fibroblasts tested with FITC-con A at 37 °C, showed fine clusters in cells with preserved cell shape and in contracted cells with cytoplasmic arborisations. After treatment for 40-60 min, the cells showed coarse, peripheral clusters in incompletely (Fig. 1c) or completely rounded up cells; supranuclear caps and perinuclear globules were not seen. Procaine treatment also produced fine to coarse clusters but colchicine and vinblastine did not alter the marginal and/or random surface staining seen in untreated cells. Trypsin induced a staining pattern of fine clusters. The cytoskeletal-disrupting drugs did not cause any changes in the initial staining pattern of fine clusters seen in tumour, transformed and embryonic cells (Table 1) but, with increasing incubation times, supranuclear caps and perinuclear globules were not seen.

Anti-actin antibody (AAA) (ref. 8) staining, traced with a rhodamine-labelled goat anti-human (γ) globulin (R-AHG), showed long parallel bundles of thick filaments in adult, embryonic and foetal fibroblasts (Fig. 1b) and short thin filaments with occasional fine aggregates in tumour and transformed cells⁹. Previous incubation of tumour, transformed, embryonic or foetal cells with FITC-labelled or unlabelled con A for 20 min and subsequent testing with AAA showed staining of fine aggregates, diffuse staining or no staining (Fig. 2b). The changes in staining patterns were most marked in neoplastic cells (> 90% cells), moderate in embryonic cells (70-90%) and minimal in foetal cells (< 10%). The change was more pronounced at 4 °C than at 37 °C. It was not seen in adult fibroblasts or in cells pre-treated with

Table 1 Immunofluorescence staining of monolayer cultures of adult, neoplastic, embryonic and foetal fibroblasts by FITC-con A or AAA

Experimental conditions	Adult, foetal kidney or lung fibroblasts		Tumour, transformed or whole embryo fibroblasts	
	FITC-con A	AAA	FITC-con A	AAA*
4 °C	Cell margins	Filaments	Cell margins and random	Filaments and fine aggregates
37 °C	Cell margins	Filaments	Fine clusters, supranuclear caps or perinuclear globules	Filaments and fine aggregates
Cytochalasin B†	Fine or coarse clusters	Fine or coarse aggregates	Fine clusters	Fine or coarse aggregates
Procaine	Fine or coarse clusters	Fine or coarse aggregates	Fine clusters	Fine or coarse aggregates
Colchicine or vinblastine	Cell margins	Filaments	Fine clusters	Filaments and fine aggregates
Trypsin	Fine clusters	Fine aggregates, diffuse or negative	Fine clusters	Fine aggregates, diffuse or negative

Viable monolayer cultures were treated for 20–360 min with $300 \mu\text{g ml}^{-1}$ FITC-con A in tissue culture medium 199. Drug-treated cells were pre-incubated with the reagent for 20–60 min followed by a further period of incubation, with or without the reagent, with FITC-con A for 20–120 min. AAA staining was carried out with cultures, fixed by air-drying and leaving at 4 °C for 2 h. Staining was still detectable with $20 \mu\text{g ml}^{-1}$ FITC-con A or 1:256 dilution AAA. The staining patterns were present in > 90% of cultured cells.

*Previous treatment of viable cultures with FITC-labelled or unlabelled con A resulted in dissolution of actin filaments.

†Treatment of control cultures with the cytochalasin B solvent, dimethylsulphoxide, had no effect on FITC-con A or AAA staining patterns.

FITC-labelled AHG at 4 °C or 37 °C, or pre-cooled at 4 °C for 10 min and tested at 4 °C with AAA.

Our data show that con A receptors may be structurally linked to actin since the two components occur in closely related sites in all the cell types examined (Figs 1, 2). This link may take the form of an actin sub-set, not stained by AAA, which restricts con A receptor mobility in adult and mature foetal cells and which is poorly expressed in neoplastic and embryonic cells. The suggestion is

supported by our finding that cluster formation may occur in cells containing actin filaments (Fig. 2a, b) and can be induced in adult or foetal cells by cytochalasin B treatment. The proposal is also consistent with the observed decrease in membrane-associated actin in tumour cells¹⁰. Because the linear marginal distribution of con A receptors is in close proximity to actin filaments (Fig. 1a, b) we suggest that the actin sub-set may anchor con A receptors to actin filaments. An actin sub-set is also thought to limit con A receptor mobility in normal alveolar macrophages¹¹. Lattice microfilaments¹² are a possible candidate for this actin sub-set.

Con A binding to surface receptors result in conversion of filamentous actin to aggregates or a diffusely stained form. These latter forms of actin seem essential for capping and endocytosis since FITC-con A and AAA-stained areas occur in close proximity (Fig. 2c, d) and the processes are inhibited by CB. Microtubules may also have a role in capping and endocytosis as these processes are inhibited by colchicine or vinblastine. Inhibition of capping by cytochalasin B, vinblastine, colchicine or procaine supports the results of previous workers^{13,14}. The cytochalasin B-induced 'caps' reported in a recent study³ may not represent true caps but may result from cytochalasin B-induced disruption of actin filaments.

This study was supported by grants from the Anti-Cancer Council of Victoria and the National Health and Medical Research Council. We thank Vivien Randell, Helen King and John Lee for technical assistance, Dr C. R. Lucas of the Fairfield Infectious Diseases Hospital for AAA serum and Dr H. A. Ward and staff for preparation of the fluorescent conjugates.

B. H. TOH

Department of Pathology and Immunology,
Monash University Medical School,
Melbourne, Victoria 3181, Australia

G. C. HARD

Department of Pathology,
Melbourne University,
Melbourne, Victoria 3052, Australia

Received 5 July; accepted 10 August 1977.

1. Nicolson, G. L. & Poste, G. N. *N. Engl. J. Med.* **295**, 197–203 (1976).
2. Edelman, G. M. *Science* **192**, 218–226 (1976).
3. Sundqvist, K.-G. & Ehrnst, A. *Nature* **264**, 226–231 (1976).
4. Ash, J. F. & Singer, S. J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4575–4579 (1976).

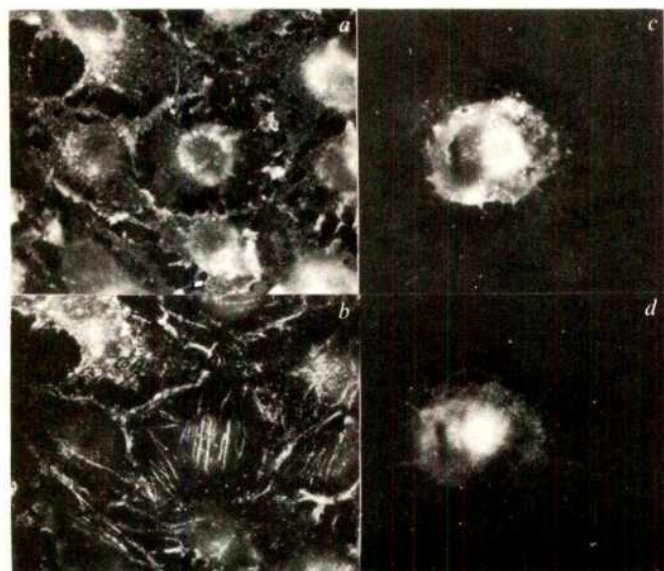


Fig. 2 FITC-con A followed by AAA/R-AHG staining of cultured embryonic or tumour fibroblasts. *a*, Tumour fibroblasts, GHF117BL, sub 88 reacted with FITC-con A at 37 °C for 20 min showing fine clusters. ($\times 330$) *b*, Identical field as (*a*) stained with AAA/R-AHG at 37 °C showing filamentous and diffuse or aggregate staining. Note that areas stained in an aggregated or diffuse pattern occur in areas similar to those of FITC-stained clusters shown in (*a*), but filaments bear no relationship to these clusters. ($\times 330$) *c*, Embryonic fibroblast, sub-1, reacted with FITC-con A at 37 °C for 60 min showing a diffusely stained supranuclear cap containing fine clusters. ($\times 330$) *d*, Same cell as in (*c*) reacted with AAA/R-AHG showing a cap located in a similar position and stained in a similar pattern as in (*c*). ($\times 330$)

5. Hard, G. C. & Borland, R. J. *nat. Cancer Inst.* **54**, 1085-1095 (1975).
6. Hard, G. C. & Borland, R. J. *nat. Cancer Inst.* **58**, 1377-1382 (1977).
7. Toh, B. H., Muller, H. K. & Erick, W. L. *Br. J. Cancer* **33**, 195-202 (1976).
8. Toh, B. H. *et al.* *Nature* **264**, 648-650 (1976).
9. Toh, B. H., Hard, G. C., Cauchi, M. N. & Muller, H. K. *Br. J. Cancer* **34**, 533-545 (1976).
10. Wickus, G., Gruenstein, E., Robbins, P. W. & Rich, A. *Proc. nat. Acad. Sci. U.S.A.* **72**, 746-749 (1975).
11. Williams, D. A., Boxer, L. A., Oliver, J. M. & Baehner, R. L. *Nature* **267**, 255-257 (1977).
12. Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43-49 (1975).
13. de Petris, S. *Nature* **250**, 54-56 (1974).
14. Ryan, G. B., Unanue, E. R. & Karnovsky, M. J. *Nature* **250**, 56-57 (1974).
15. Nairn, R. C. *Fluorescent Protein Tracing* 4th edn (Churchill Livingstone, Edinburgh, 1976).

Actin and tubulin co-cap with surface immunoglobulins in mouse B lymphocytes

CYTOSKELETAL control of cell surface receptor movements¹ has been suggested by several authors^{2,3} and is compatible with the fluid mosaic model of cell membranes⁴. Evidence for this hypothesis comes mostly from experiments using drugs which alter the activity of cytoskeletal components^{5,6}. We report here co-capping of actin and tubulin with immunoglobulins (Ig) in B lymphocytes from mouse spleen. These findings provide structural evidence for cytoskeletal control of receptors.

Purified anti-actin and anti-tubulin antibodies were obtained respectively from the serum from a patient with chronic aggressive hepatitis, which contained specific anti-actin antibodies^{7,8} and from the sera of rabbits repeatedly immunised with pig brain tubulin⁹ adsorbed to alum (A.Z. and G.G., in preparation). These sera were passed through columns of Sepharose covalently linked¹⁰ respectively with rabbit skeletal muscle actin¹¹ or with pig brain tubulin¹⁴, and the absorbed antibody was eluted at pH 2.7. The specificity of these antibodies was shown by immunodiffusion—a single precipitation line was observed against the antigen (Fig. 1)—by immunoelectrophoresis, and by immunofluorescence. In the immunofluorescence test the anti-actin antibody (AAA) was fixed on striated (I bands) and smooth muscle tissue sections and the anti-tubulin antibody (ATA) on mitotic spindles, cultivated fibroblasts and spermatozoa; cell staining was abolished by previous incubation of the antibody solution with actin and tubulin respectively.

Mouse spleen lymphocytes were incubated with rabbit antiserum against mouse Ig (RAMIG), either conjugated with rhodamine¹² or unconjugated, and then with sheep antiserum against rabbit Ig (SARIG) conjugated with rhodamine¹²; conditions were as described elsewhere¹³ and incubations were for 30 min at 0 or 37 °C. After this incubation the cells were suspended in 0.035 M citrate¹⁴, a drop of this suspension was applied on a slide and dried. For actin staining, cells

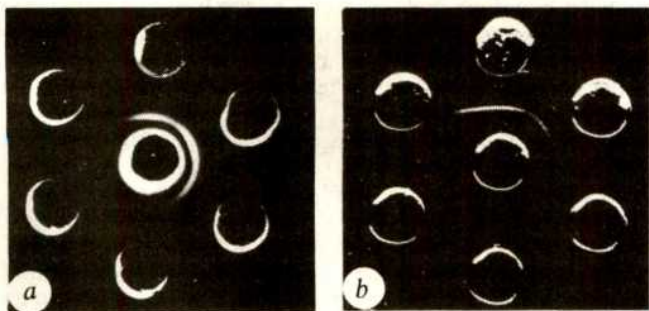


Fig. 1 Immunodiffusion experiments using the Ouchterlony technique. Readings were made between 24 and 48 h after a single filling of the wells. *a*, AAA serum was placed in the centre against decreasing concentrations of actin (3.0; 1.5; 0.75; 0.375; 0.19 mg ml⁻¹). To keep actin depolymerised, Agarose (Behring-Werke) was dissolved in 2 mM Tris-HCl buffer at pH 8.0 containing 0.2 mM ATP, 0.5 mM 2-mercaptoethanol and 0.2 mM CaCl₂ (ref. 10). *b*, ATA serum was placed in the centre against similarly decreasing concentrations of tubulin. Agarose was dissolved in 0.15 M NaCl containing 0.5 mM 2-mercaptoethanol. A single precipitation line is visible in both cases.

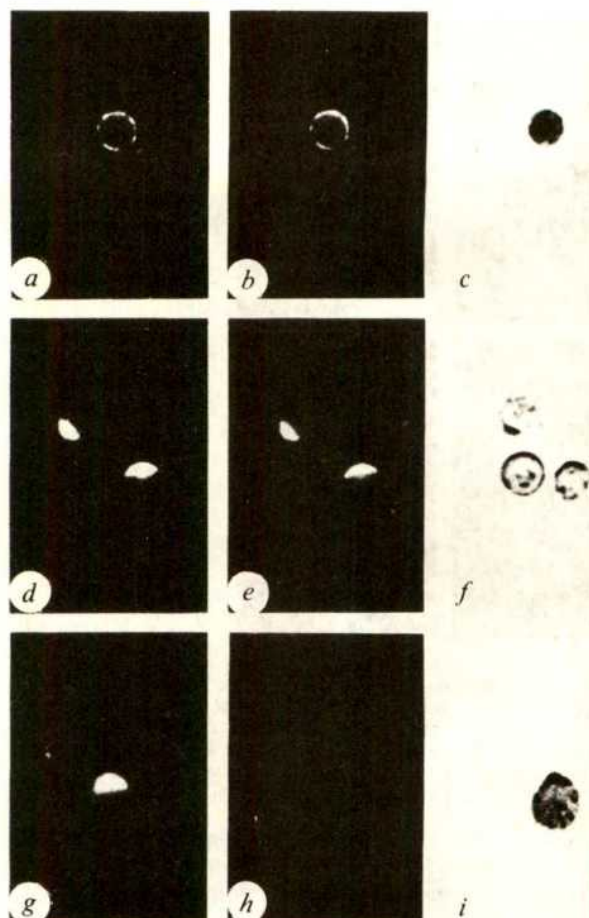


Fig. 2 Immunofluorescent staining of splenic B lymphocytes incubated in suspension with RAMIG and SARIG-rhodamine, then fixed with ethanol and incubated with AAA followed by anti-human IgG-fluorescein. All photographs were taken with a Zeiss ultraviolet photomicroscope equipped with epi-illumination and specific filters for rhodamine and fluorescein, using a Plan Apo-Chromate 63x/1.0 objective on HP5 Ilford black and white film. *a-c*, Incubation in suspension at 4 °C; *a*, rhodamine; *b*, fluorescein; *c*, phase contrast. *d-f*, Incubation in suspension at 37 °C; *d*, rhodamine; *e*, fluorescein; *f*, phase contrast. *g-i*, Incubation in suspension at 37 °C; as control, after ethanol fixation cells were incubated with human Ig instead of AAA and then with anti-human IgG. *g*, Rhodamine; *h*, fluorescein; *i*, phase contrast ($\times 2,000$).

were fixed for 30 s in absolute ethanol, incubated for 15 min with purified AAA and for a further 15 min with fluorescein conjugated Ig fraction of goat antiserum to human IgG (Miles Seravac). To avoid cross reactions between these antisera, the goat anti-Ig was passed on a solid immunoabsorbent made with glutaraldehyde-coupled mouse, rabbit and sheep Ig¹⁵. In control experiments the human purified AAA was replaced by a solution of normal human Ig with a similar concentration of proteins. For tubulin staining, cells incubated with RAMIG-rhodamine were fixed for 20 min in 3.7% formaldehyde and 5 min in acetone, and then incubated for 1 h with a solution of fluorescein conjugated¹² rabbit purified ATA.

After incubation in suspension with RAMIG-rhodamine or RAMIG followed by SARIG-rhodamine, 70% of splenic lymphocytes had a positive staining. Cells incubated at 0 °C had a generally diffused labelling along the cell membrane with some patching and very rare capping, whereas a high proportion of cells incubated at 37 °C showed typical cap formation. When this incubation was followed by fixation of the cells and immunofluorescent staining for cytoskeletal proteins, the pattern of actin and tubulin corresponded to that of Ig (Figs 2 and 3). In non-capped cells, the fluorescence was weak, particularly for actin, and difficult to detect on the nuclear area. By contrast, in cells showing capped immunoglobulins,

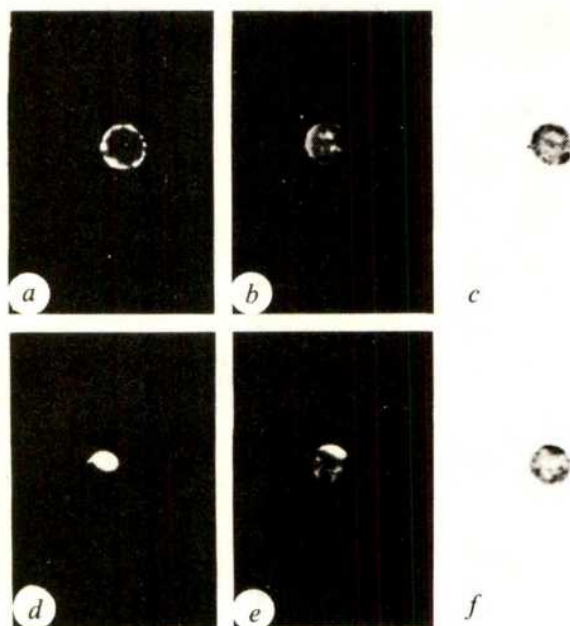


Fig. 3 Immunofluorescent staining of splenic B lymphocytes incubated in suspension with RAMIG-rhodamine then fixed with formaldehyde and acetone and incubated with ATA-fluorescein. *a-c*, Incubation in suspension at 4 °C; *a*, rhodamine; *b*, fluorescein; *c*, phase contrast. *d-f*, Incubation in suspension at 37 °C; *d*, rhodamine; *e*, fluorescein; *f*, phase contrast ($\times 2,000$).

the fluorescence for actin and tubulin was intense and restricted to the region of the cap. Among the cells which were rhodamine negative (that is, non-B cells) a high proportion (85%) were positive for actin and tubulin.

These findings show in a simple and well established model¹ that two cytoskeletal proteins, namely actin and tubulin, probably having different functions, move concurrently along the cell membrane with a single class of surface receptors—Ig. It has been reported that, in rabbit ovarian granulosa cells, concanavalin A-induced capping redistributes microtubules in the cytoplasmic region underlying the cap¹⁶. An analogous redistribution of surface antigens and actin has been described in Lu-106 cells after treatment with cytochalasin B¹⁷.

It is generally recognised that capping is dependent on cell metabolism^{1,18}. Several experiments have shown that cytochalasin B (a drug which alters microfilament morphology and function¹⁹) inhibits this phenomenon (though sometimes only partially)^{5,20} and hence it has been proposed that redistribution of cell surface molecules depends on microfilament activity. Microtubular integrity has also been shown to be linked with capping in B lymphocytes²¹. It has been proposed that microtubules and microfilaments exert skeletal and contractile actions respectively in regulating the movement of cell surface proteins³. Our findings are compatible with this hypothesis, and support the possibility that skeletal and contractile elements are linked to one another and/or to the same plasma membrane components.

This work was supported by the Swiss National Science Foundation (grant 3.692-0.76). We thank Miss M. Bouland for technical assistance and Mr J.-C. Rumbeli and Mr E. Denking for photographic work.

GIULIO GABBANI
CHRISTINE CHAPONNIER
ALBERT ZUMBE
PIERRE VASSALLI

Department of Pathology,
University of Geneva,
40 Boulevard de la Cluse,
1211 Geneva 4, Switzerland

Received 13 June; accepted 31 August 1977.

1. Taylor, R. B., Duffus, P. H., Raff, M. C. & Petris, S. *Nature new Biol.* 233, 225-229 (1971).
2. Yahara, I. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* 69, 608-612 (1972).
3. Poste, G., Papahadjopoulos, D. & Nicolson, G. L. *Proc. natn. Acad. Sci. U.S.A.* 72, 4430-4434 (1975).
4. Singer, S. J. & Nicolson, G. L. *Science* 175, 720-731 (1972).
5. Ehrnst, A. & Sundqvist, K. G. *Cell* 5, 351-359 (1975).
6. Poste, G., Papahadjopoulos, D., Jacobson, K. & Vail, W. *Biochim. biophys. Acta* 394, 520-539 (1975).
7. Gabbiani, G. *et al. Am. J. Path.* 72, 473-488 (1973).
8. Chaponnier, C., Kohler, L. & Gabbiani, G. *Clin. exp. Immun.* 27, 278-284 (1977).
9. Fuller, G. M., Brinkley, B. R. & Boughter, J. M. *Science* 187, 948-950 (1975).
10. Cuatrecasas, P., Wolchek, M. & Anfinsen, C. B. *Proc. natn. Acad. Sci. U.S.A.* 61, 636-643 (1968).
11. Spudich, J. A. & Watt, S. J. *biol. Chem.* 246, 4866-4871 (1971).
12. Cebra, J. J. & Goldstein, G. J. *Immun.* 95, 230-245 (1965).
13. Lamelin, J. P., Lisowska-Bernstein, B., Matter, A., Ryser, J. E. & Vassalli, P. *J. exp. Med.* 136, 984-1007 (1972).
14. Norberg, R., Lidman, K. & Fagraeus, A. *Cell* 6, 507-512 (1975).
15. Avrameas, S. & Pernynck, T. *Immunochimistry* 6, 53-66 (1969).
16. Albertini, D. F. & Clark, J. I. *Proc. natn. Acad. Sci. U.S.A.* 72, 4976-4980 (1975).
17. Sundqvist, K. G. & Ehrnst, A. *Nature* 264, 226-231 (1976).
18. Loo, F., Forni, L. & Pernis, B. *Eur. J. Immun.* 2, 203-212 (1972).
19. Wessels, N. K. *et al. Science* 171, 135-143 (1971).
20. De Petris, S. *Nature* 250, 54-57 (1974).
21. De Petris, S. *J. Cell Biol.* 65, 123-146 (1975).

Intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane

SPECIFIC high affinity binding sites for insulin have been shown to be located on the plasma membrane of target cells¹, and they are also present on purified intracellular organelles² including intact nuclei³, smooth and rough endoplasmic reticulum^{2,4}, and the Golgi apparatus⁵. Although these intracellular sites have certain binding characteristics similar to those on the plasma membrane²⁻⁵, it is not known whether these binding sites share other biochemical features. Using an antiserum directed at the cell surface binding sites, we have found that several types of intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane.

It has already been demonstrated that the sera of certain patients with extreme insulin resistance and acanthosis nigricans contain immunoglobulins directed against the plasma membrane binding site⁶⁻⁹. When any of human monocytes or cultured lymphocytes, avian erythrocytes, or highly purified rat liver plasma membranes are preincubated with these antisera, the subsequent binding of radiolabelled insulin is inhibited⁶⁻⁹. That these antibodies are specific for insulin binding sites on the plasma membrane is seen by their failure to inhibit both the binding of human growth hormone to intact human cultured lymphocytes⁶⁻⁹ and the binding of NSILA-s, an insulin-like peptide, to rat liver plasma membranes⁶⁻⁹. Furthermore, using conditions where there is nearly complete inhibition of the specific binding of insulin, there is no inhibition of the binding of glucagon (Table 1). Other studies indicate that the binding of purified radiolabelled antibodies to plasma membranes is inhibited by insulin and insulin analogues in direct proportion to the biological activity of those analogues⁶⁻⁹. These findings suggest that these antibodies bind either near to or at the portion of the plasma membrane that recognises and binds the insulin molecule.

When we pre-incubated rat liver plasma membranes with B2-1975 antiserum⁶⁻⁹ at a 1:100 dilution for 1 h at 4 °C, 85-90% of the subsequent binding of ¹²⁵I-insulin was inhibited (Table 1, Fig. 1). With progressive serial dilution, the inhibiting effect decreased; but even at a titre of 1:6,400, 25% inhibition of binding was demonstrable. In contrast to its ability to inhibit insulin binding to the plasma membrane, this antiserum had no significant effects on the binding of insulin to purified nuclei (Table 2). We have previously shown that the nuclear membrane is the major site of insulin binding to the cell nucleus¹². In contrast to the marked inhibition of the binding of insulin to plasma membranes, pre-incubation of nuclear membranes with B2-1975 antiserum had only small effects on insulin binding (Table 2, Fig. 1). Similar results were seen with the smooth endoplasmic reticulum (Table 2, Fig. 1). At a dilution of 1:100, specific binding of insulin to both nuclear membranes and smooth endoplasmic reticulum was inhibited by only 10-20%;

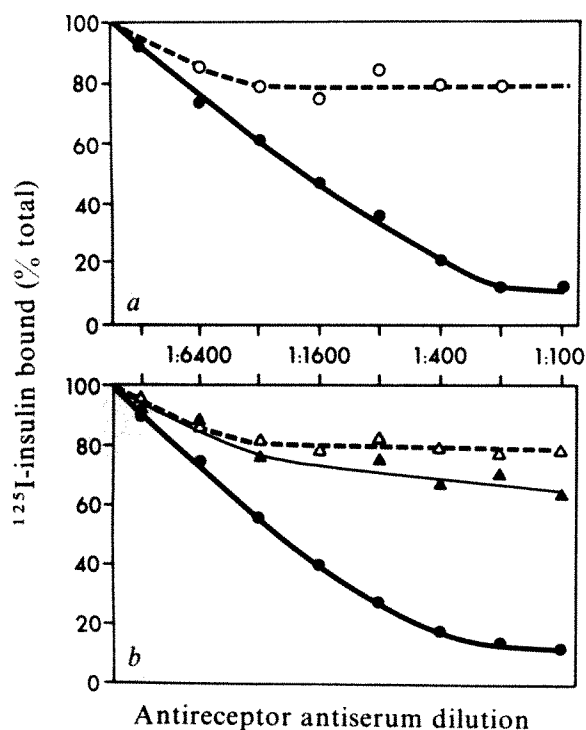


Fig. 1 Effects of serial dilutions of an anti-plasma membrane antiserum on ^{125}I -insulin binding to several types of cellular membranes. Plasma membranes¹⁰ (●) at 0.087 mg protein ml^{-1} (a) and 0.125 mg protein ml^{-1} (b), nuclear membranes¹³ (○) at 0.087 mg protein ml^{-1} , and smooth (Δ) and rough (▲) endoplasmic reticulum¹⁴ at 0.47 mg protein ml^{-1} were pre-incubated for 1 h with either normal human serum or antiserum at various dilutions as described in Table 1. ^{125}I -Insulin (0.5 ng ml^{-1}) and albumin (5 mg ml^{-1}) were then added and the incubation was continued for 2 h. Insulin bound to nuclear and plasma membranes was separated from free insulin by centrifugation in a microcentrifuge. Insulin bound to the rough and smooth endoplasmic reticulum was separated by a filtration through Oxoid filters (Amersham)^{3,11}. Each point is the mean of triplicate determinations. Binding is expressed as a percentage of that seen in the presence of pooled human serum. In the absence of antiserum specifically bound ^{125}I -insulin (% of total) was 3.95 ± 0.07 (a) and 5.75 ± 0.14 (b) for plasma membranes, 0.85 ± 0.008 for nuclear membranes, 3.4 ± 0.14 for smooth endoplasmic reticulum, and 1.9 ± 0.04 for rough endoplasmic reticulum. Values are the mean \pm standard deviation.

the degree of inhibition, moreover, did not change with dilution of the antiserum up to 1:3,200.

The ability of this antibody to inhibit insulin binding to the rough endoplasmic reticulum was also much less than that seen with plasma membranes (Table 2, Fig. 1). At a dilution of 1:100, approximately one-third of the specific binding of insulin was inhibited. In contrast to the results obtained with smooth endoplasmic reticulum and nuclear membranes, the inhibition of insulin binding to the rough endoplasmic reticulum progressively diminished on further dilution of the antiserum.

There are two possible reasons why this antiserum partially inhibits insulin binding to intracellular membranes. In the case of the rough endoplasmic reticulum, it is possible that the inhibition observed represents the interaction of the antibody to plasma membrane insulin binding sites with nascent plasma membrane binding sites present on ribosomes. Since this antiserum (B2-1975) also contains antibodies directed against a variety of cell proteins⁹, it is possible that the non-progressive inhibition observed with nuclear membranes or smooth endoplasmic reticulum is caused by a second antibody present in the antiserum that only weakly inhibits binding. In either case, the present studies suggest that the insulin binding sites on these intracellular membranes from rat liver are

Table 1 Effect of an anti-plasma membrane antiserum on the binding of ^{125}I -insulin and ^{125}I -glucagon to rat liver plasma membranes

	^{125}I -hormone bound (% per 0.1 mg protein per ml)	
	Insulin	Glucagon
Control serum	4.0 ± 0.21	10.2 ± 0.13
Antiserum	0.5 ± 0.12	9.9 ± 0.40

Rat liver plasma membranes¹⁰ at 0.057 mg protein ml^{-1} were preincubated for 1 h at 4 °C with a 1:100 dilution of antiserum (B2-1975) (refs 6-9) or control serum in buffer containing 0.25 M sucrose, 10 mM MgCl_2 , 2 mM EDTA, 20 mM Tris (pH 7.85). After this pre-incubation, either ^{125}I -insulin³ at 0.75 ng ml^{-1} in the presence or absence of 200 $\mu\text{g ml}^{-1}$ of unlabelled insulin (to determine nonspecific binding) or ^{125}I -glucagon¹¹ at 0.5 ng ml^{-1} in the presence or absence of 50 $\mu\text{g ml}^{-1}$ of unlabelled glucagon was added with bovine serum albumin at 5 mg ml^{-1} . The incubation was continued at 24 °C for 2 h for insulin or 1 h for glucagon. Bound and free hormones were separated by centrifugation in a microcentrifuge^{3,11}. Each value is the mean \pm the standard deviation for triplicate determinations. Nonspecific binding has been subtracted.

immunologically distinct from those found on the plasma membrane.

It is evident that, per unit of protein, plasma membranes bind more insulin than do the intracellular membranes (Table 2). Our preliminary studies suggest that this increased binding of insulin to plasma membranes is due to the higher affinity of its insulin binding sites. In contrast, the total number of insulin binding sites per unit of protein (binding capacity), is similar in the various cellular membrane fractions. Since it is well known that the liver cell has far more intracellular membranes than plasma membranes, this observation suggests that there may be more binding sites for insulin in the cell interior than there are on the cell surface.

The biological significance of these intracellular binding sites for insulin is unknown. It has been postulated that certain of these intracellular binding sites, especially those on Golgi membranes, are precursors of those on plasma membranes⁸; this argument may also pertain to those sites on the rough endoplasmic reticulum. Conversely since it is unlikely that the insulin binding sites on the nuclear membrane and on the smooth endoplasmic reticulum are precursors of those on the plasma membrane, and since the present studies indicate that these intracellular binding sites for insulin are immunologically distinct from those on the plasma membrane, an alternative hypothesis can be made. We and others have either deduced or observed that both insulin and other polypeptide hormones may enter the intact cell¹⁵⁻²⁰. On the basis of these data, we have speculated that these intracellular binding sites for insulin may be involved in the regulation of several long term effects of insulin, such as RNA and DNA synthesis^{15,16}. Since the

Table 2 Effect of an anti-plasma membrane antiserum on ^{125}I -insulin binding to various cellular fractions

Fraction	^{125}I -insulin bound (% of total)		Inhibition of binding (%)
	No antiserum	Antiserum 1:200	
Plasma membranes (0.035)	1.14 ± 0.02	0.16 ± 0.006	86
Nuclei (1.03)	1.34 ± 0.15	1.36 ± 0.16	0
Nuclear membranes (0.12)	1.04 ± 0.01	0.86 ± 0.02	17
Smooth ER (0.23)	1.70 ± 0.08	1.50 ± 0.10	12
Rough ER (0.47)	1.91 ± 0.04	1.32 ± 0.04	31

For each fraction the protein concentration (shown in parentheses as mg ml^{-1}) was adjusted so that 1-2% of the ^{125}I -insulin was specifically bound. Incubation conditions were as described in Table 1 and Fig. 1. Binding is expressed as the mean \pm standard deviation for triplicate determinations. Inhibition of insulin binding by the antiserum was significant in all fractions ($P < 0.05$) except nuclei.

relationship between the intracellular binding sites for insulin and the biological functions of insulin is unknown, further studies will be necessary to clarify the role, if any, of these intracellular binding sites.

We thank Drs Marvin D. Siperstein and Basil Rapoport for advice on this manuscript. These studies were supported by the Medical Research Service of the Veterans Administration, by a grant from Northern California Affiliate, Inc. of the American Diabetes Association, and by a grant (AM 19415 01A1) from the National Institutes of Health. R.V. was a recipient of a Fulbright International Fellowship.

IRA D. GOLDFINE
RICCARDO VIGNERI
DAVID COHEN
NATHAN B. PLIAM

Department of Medicine,
University of California Hospitals,
San Francisco, and
Metabolism Section, Medical Service,
Veterans Administration Hospital,
4150 Clement Street (111F),
San Francisco, California 94121

C. RONALD KAHN

Diabetes Branch, National Institute of Arthritis,
Metabolism, and Digestive Diseases,
National Institutes of Health,
Bethesda, Maryland 20014

Received 31 May; accepted 22 August 1977.

1. Roth, J. *Metabolism* **22**, 1059-1073 (1973).
2. Horvat, A., Li, E. & Katsoyannis, P. G. *Biochim. biophys. Acta* **382**, 609-620 (1975).
3. Goldfine, I. D. & Smith, G. J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1427-1431 (1976).
4. Kahn, C. R. J. *Cell Biol.* **70**, 261-286 (1976).
5. Bergeron, J. J. M., Evans, W. H. & Geschwind, I. I. *J. Cell Biol.* **59**, 771-776 (1973).
6. Flier, J. S., Kahn, C. R., Roth, J. & Bar, R. S. *Science* **190**, 63-65 (1975).
7. Flier, J. S., Kahn, C. R., Jarrett, D. B. & Roth, J. *J. clin. Invest.* **58**, 1442-1449 (1976).
8. Jarrett, D. B., Roth, J., Kahn, C. R. & Flier, J. S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4115-4119 (1976).
9. Kahn, C. R. *et al.* *New Engl. J. Med.* **294**, 739-745 (1976).
10. Ray, T. K. *Biochim. biophys. Acta* **196**, 1-9 (1970).
11. Goldfine, I. D., Roth, J. & Birnbaumer, L. *J. biol. Chem.* **247**, 1121-1218 (1972).
12. Vigneri, R., Wong, K. Y. & Goldfine, I. D. *Clin. Res.* **25**, 500A (1977).
13. Kashnig, D. M. & Kasper, C. B. *J. biol. Chem.* **244**, 3786-3792 (1969).
14. Dallner, G. & Ernster, L. *J. Histochem. Cytochem.* **10**, 611-632 (1968).
15. Goldfine, I. D. *Diabetes* **26**, 148-155 (1976).
16. Goldfine, I. D., Smith, G. J., Wong, K. Y. & Jones, A. L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1368-1372 (1977).
17. Nordquist, R. E. & Palmieri, G. M. A. *Endocrinology* **95**, 229-237 (1974).
18. Carpenter, G. & Cohen, S. J. *Cell Biol.* **71**, 159-171 (1976).
19. Nolin, J. M. & Witorsch, R. J. *Endocrinology* **99**, 949-958 (1976).
20. Steiner, D. F. *Diabetes* **26**, 322-340 (1977).

Inhibition of high-affinity choline transport in peripheral cholinergic endings by presynaptic snake venom neurotoxins

Use of purified snake venom neurotoxins with potent curarimimetic action on peripheral cholinergic transmission (for example, α -bungarotoxin¹) in the study of the biochemical properties of the nicotinic cholinergic receptor has met with considerable success (see ref. 2 for review). Some of the crude venoms from which such toxins have been isolated also contain toxins which act presynaptically, such as β -bungarotoxin¹, notexin³ and taipoxin⁴. These three toxins inhibit the release of acetylcholine by a mechanism which is independent of the release process *per se*³⁻⁷. We show here that in an *in vitro* membrane preparation⁸ derived from the cholinergic endings in *Torpedo* electric organ these and related toxins are potent inhibitors of the high-affinity choline transport system. These observations provide a biochemical explanation for the inhibitory action of these toxins on transmitter release as they effectively block re-synthesis of acetylcholine from choline.

Nerve terminal sacs (T sacs, originally termed synaptosomes⁸) were prepared from the electric tissue of *Torpedo marmorata* essentially as described previously⁸ (for a detailed account see

ref. 9). After a brief period of pre-homogenisation of this tough tissue in 0.8 M glycine using a Waring blender, homogenisation in a Potter-Elvehjem glass and Perspex homogeniser was possible. Uptake of ³H-choline into partially purified T sacs (fraction *P*₂) was measured using a Millipore filtration technique as described recently¹⁰. The effects of the various toxins and related proteins on choline uptake were tested in several ways. The concentration dependency of their inhibitory action was tested by simultaneous exposure of T sacs to ³H-choline (1 μ M) and toxins (varying concentrations) for 10-15 min before filtration. In other experiments T sacs were exposed to toxins before the addition of ³H-choline, and vice versa. In experiments designed to test the calcium dependence of the toxin action the 4.4 mM CaCl₂ of the normal *Torpedo* Ringer (for composition see ref. 10) was replaced by 4.4 mM SrCl₂.

Figure 1 shows that all three toxins (β -bungarotoxin, notexin, and taipoxin) that are known to have presynaptic effects are potent inhibitors of high-affinity choline transport. By contrast those with a postsynaptic action (α -bungarotoxin and *siamensis* 3)¹⁸ in the same concentration range were without effect (data not shown). Two other toxins (*Notechis* II-5 and *Enhydryna schistosoma* myotoxin) which are structurally homologous to notexin and the taipoxin subunits^{7,11-13} are also very potent inhibitors. Experiments in which T sacs were exposed to taipoxin or β -bungarotoxin for 10-15 min at room temperature before the addition of ³H-choline show that this potentiates the inhibitory action of taipoxin 10-fold whereas that of β -bungarotoxin is not significantly altered. The increased potency of taipoxin is not due to increased binding of the toxin to the T-sac membrane but rather to a potentiating factor released from the tissue during incubation (M.J.D. and J.P.F., in preparation).

The shapes of the inhibition curves suggested that all of the inhibitory toxins except β -bungarotoxin were capable of producing full blockade. For notexin and *Notechis* II-5 it was not possible to demonstrate complete inhibition because of the increased nonspecific binding of ³H-choline to the Millipore filters at high toxin concentrations. In contrast to the retention of ³H-choline at lower toxin concentrations this binding was insensitive to the presence of hemicholinium-3 and thus probably represents a strong interaction between ³H-choline and filter-bound toxin. With β -bungarotoxin, inhibition was never more than 60% even at concentrations above 30 μ g ml⁻¹. In the presence of 100 and 200 μ g ml⁻¹ β -bungarotoxin nonspecific binding of ³H-choline to the filters was also observed and this obscured the real inhibition pattern of this toxin at these elevated concentrations. By extrapolation this effect was not sufficient to explain the partial blockade caused by β -bungarotoxin, however. The most potent uptake-blocker, notexin, had an IC₅₀ value (concentration for 50% inhibition) of about 4×10^{-11} M. In cases where comparison is possible (β -bungarotoxin, notexin and taipoxin) the range of concentrations in which inhibition was observed is similar to or lower than that previously reported for *in vitro* presynaptic inhibition based on physiological findings^{1,3,4,7}. The latency of the presynaptic toxins *in vivo* is well established. If the failure of transmission is due to termination of acetylcholine synthesis caused by inhibition of choline uptake one would not expect transmission to cease until the pre-existing pool of acetylcholine was exhausted. Comparisons of IC₅₀ values for choline transport in T sacs with the LD₅₀ values observed on intravenous administration to mice shows a high degree of correlation—particularly for the single-chain neurotoxins. The multiple-chain toxins, β -bungarotoxin and taipoxin, are more toxic to whole animals than might be predicted from their effects on choline transport.

All of the presynaptically-active neurotoxins shown in Fig. 1 are Ca²⁺-dependent phospholipases, and all of those which have been totally^{11,12} or partially^{7,13} sequenced are highly homologous to phospholipases A₂ from other snake venoms and from mammalian pancreas. We have therefore tested (Table 1) whether calcium is required for their inhibitory action on choline transport by performing some experiments in

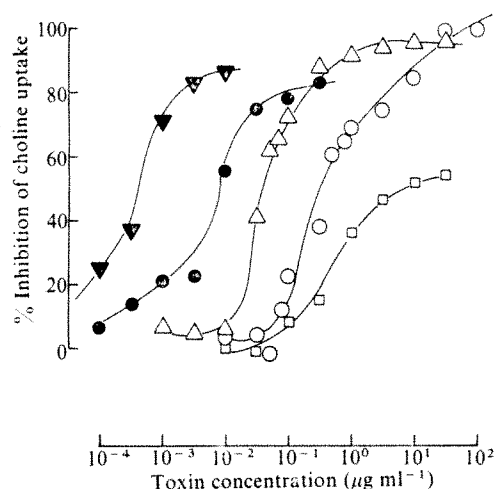


Fig. 1 Inhibitory action of presynaptic snake neurotoxins on high-affinity choline transport. ^3H -choline uptake into partially purified T sacs (fraction P_2) from *Torpedo* electric organ was measured for 10–15 min at room temperature with 1 μM choline exactly as described previously¹⁰. The effect of increasing concentration of the various toxins added at the onset of incubation with ^3H -choline is expressed as % inhibition of controls to which no toxin was added. Each point represents the mean of two or more independent experiments with duplicate or triplicate determinations in each case. Replication of individual points was good both within or between experiments. For any one point the standard deviation from the mean was $< 10\%$ for $n = (4-13)$ observations. The identities of the various toxins, assumed molecular weights (MW) and references to the details of their purification are as follows: \blacktriangledown , notexin¹¹, MW 13,500; \bullet , *Notechis* II-5¹², MW 13,500; \triangle , *Enhydryna schistosa* myotoxin¹³, MW 13,500; \circ , taipoxin⁷, MW 45,600; \square , β -bungarotoxin (a gift from Boehringer prepared as in ref. 17), MW 22,000. Toxin concentrations causing 50% inhibition in ng ml^{-1} were: notexin, 0.5 (3.7×10^{-11} M) (2 expts); *Notechis* II-5, 9 (6.7×10^{-10} M) (2 expts); *En. schistosa* myotoxin, 36 (2.7×10^{-9} M) (2 expts); taipoxin, 310 (6.8×10^{-9} M) (4 expts); β -bungarotoxin, 5,600 (2.5×10^{-7} M) or for 50% of maximum inhibition, 630 (2.8×10^{-8} M) (5 expts).

Ca^{2+} -free Sr^{2+} -*Torpedo* Ringer. Although substitution of Ca^{2+} by Sr^{2+} has little effect on choline transport in the absence of the toxins none of the toxins exhibited either inhibitory activity or phospholipase A activity in the Sr^{2+} medium, which suggests that the integrity of the catalytic site is required for the toxic action.

Experiments of the type shown in Fig. 2 indicate that these toxins do not cause gross lysis of the presynaptic nerve-terminal membrane in the conditions used. Furthermore, the influx of ^3H -choline is immediately and totally inhibited by the addition of taipoxin, but continued incubation does not lead to loss of ^3H -choline. In the same conditions, 80% of the

Table 1 Ca-dependence of toxin action on choline uptake

Toxin ($\mu\text{g ml}^{-1}$)	Choline uptake at 1 μM (% of controls)	
	Sr^{2+} -Ringer	Ca^{2+} -Ringer
Notexin (0.01)	91 \pm 37	23 \pm 6
<i>Notechis</i> II-5 (0.1)	111 \pm 9	23 \pm 3
<i>E. schistosa</i> myotoxin (0.2)	127 \pm 3	33 \pm 4
Taipoxin (3)	89 \pm 15	9 \pm 2
β -bungarotoxin (10)	111 \pm 12	45 \pm 6

Fraction P_2 was prepared from *Torpedo* electric organ and resuspended in 'normal' *Torpedo*-Ringer (Ca^{2+} -Ringer) or one in which CaCl_2 was replaced by SrCl_2 with a final concentration of 4.4 mM (Sr^{2+} -Ringer). ^3H -choline uptake (10 min) was then measured (20 μg protein per assay) in the presence and absence of toxins in both Ringer solutions as described in the legend to Fig. 1. Toxin concentrations were chosen so as to produce about 80% of their maximum inhibition (see Fig. 1). Results are expressed as % of the appropriate controls and are means \pm s.d. of three individual determinations. The Sr -Ringer control was 93% of the Ca -Ringer control.

choline taken up remains as free choline in the cytosol¹⁴ and is readily released by agents which rupture the external membrane (see effect of crude bee venom phospholipase A in Fig. 2).

β -Bungarotoxin behaves like taipoxin except that the blockade is only about 50%, as mentioned above (Fig. 1). Another reason for rejecting gross lysis as an explanation for the inhibitory action of these toxins on choline transport is the very poor correlation between phospholipase activity and inhibitory potency. A more subtle mechanism of action involving factors other than or, at least, in addition to phospholipase A activity is therefore indicated.

Taipoxin is a ternary complex of three separate, but homologous peptide chains⁷ which differ strikingly in their charge properties. The α component is very basic, the β component is neutral, and the γ component is very acidic⁷. Previous studies at the neuromuscular junction have shown that the α component is the only one of the three which has appreciable blocking action by itself^{6,7}. The same applies for the effect of the individual chains on choline transport (Table 2). In fact, all of the presynaptic snake venom neurotoxins for which data are available are either very basic polypeptide chains or contain such a chain as an essential subunit. These observations indicate that a positive charge might be important for toxicity and that ionic binding to the external membrane, which presumably has a negative charge, might thus be a prerequisite for the action of these toxins. That a highly charged molecule like protamine can also block choline uptake (Table 2) supports this view, but whereas the inhibition by protamine is reversible the toxin-induced block is not (M.J.D. and J.P.F., in preparation).

We do not yet know how specific the action of these presynaptic toxins is. Recent observations on the inhibition of high-affinity choline uptake in rat brain synaptosomes by β -bungarotoxin¹⁵ agree well with our results and suggest that some part of the high-affinity uptake mechanism might constitute the 'target' for these toxins. Alternatively, the toxins might interact with sites elsewhere on the presynaptic membrane, but thereby cause some general perturbation which

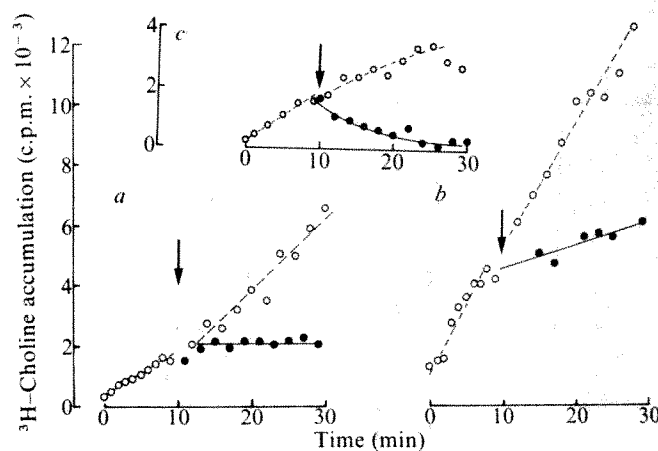


Fig. 2 Effect of toxin addition on ^3H -choline accumulated by T sacs. Fraction P_2 (about 2 mg protein in each case) from *Torpedo* electric organ was incubated in 32 ml of *Torpedo* Ringer containing ^3H -choline 1 $\mu\text{Ci ml}^{-1}$ (specific activity 1 Ci mmol⁻¹) at room temperature. Samples of 1 ml were filtered at the times indicated and the accumulated ^3H determined. After incubation for 10 min the remaining T-sac suspension (22 ml) was transferred (two equal portions) to separate vessels containing either a small vol of Ringer (control) or the same vol of Ringer containing toxin. Samples (1 ml) were filtered alternately from each of these suspensions at 1-min intervals over the next 20 min; \circ , controls; \bullet , toxin treated. *a*, Taipoxin, 0.1 $\mu\text{g ml}^{-1}$ final concentration. *b*, β -bungarotoxin, 1 $\mu\text{g ml}^{-1}$. *c*, To demonstrate that gross lysis of T sacs does lead to loss of accumulated ^3H -choline, bee venom phospholipase A (Sigma) was added at a total concentration of 3 $\mu\text{g ml}^{-1}$. Each plot represents the result with different T-sac preparations. Arrows represent times of toxin addition.

Table 2 Importance of positive charge for inhibition of choline uptake

Agent tested	Concentration range studied ($\mu\text{g ml}^{-1}$)	Concentration ($\mu\text{g ml}^{-1}$) causing 50% inhibition of choline uptake
α -chain Taipoxin (basic)	0.01–10	0.3
β -chain Taipoxin (neutral)	0.01–10	> 10 (90% of control at $10 \mu\text{g ml}^{-1}$)
γ -chain Taipoxin (acidic)	0.01–10	> 10 (120% of control at $10 \mu\text{g ml}^{-1}$)
Protamine (very basic)	0.1–100	0.4

Concentration-dependent inhibition of choline uptake into T sacs was measured as described in the legend to Fig. 1 using simultaneous exposure of T sacs to ^3H -choline and toxins (or protamine) for 10 min at room temperature. Protamine was used to test whether or not the binding of a positively charged macromolecule to the T-sac external membrane alone could cause inhibition of choline transport, which it does. Other experiments have shown that, unlike the toxins, the inhibitory influence of protamine is fully reversible. The molecular weights of the agents are as follows: α subunit, 13,800; β subunit, 13,500, γ subunit, 18,400 and protamine, $\approx 8,000$.

upsets membrane-associated processes including choline transport. That taipoxin and notexin can also interfere with vesicle recycling at the neuromuscular junction⁶ supports the idea of a generic attack on presynaptic membrane-dependent processes. It seems unlikely that blockade of choline uptake is a secondary effect of inhibition of vesicle recycling since for taipoxin the blockade is instantaneous (Fig. 2). A strong case for general membrane perturbation has been presented recently for β -bungarotoxin¹⁶. We are currently attempting to localise and identify the 'target' site by using radiolabelled toxins.

This work was supported in part by an EMBO Fellowship to J.P.F. We thank Dr E. Karlsson for *siamensis* 3 neurotoxin.

MIKE J. DOWDALL

Abteilung Neurochemie, Max-Planck-Institut für
biophysikalische Chemie, D-3400 Göttingen, FRG

JAN P. FOHLMAN
DAVID EAKER

Institute of Biochemistry,
Box 576, S-751 23 Uppsala, Sweden

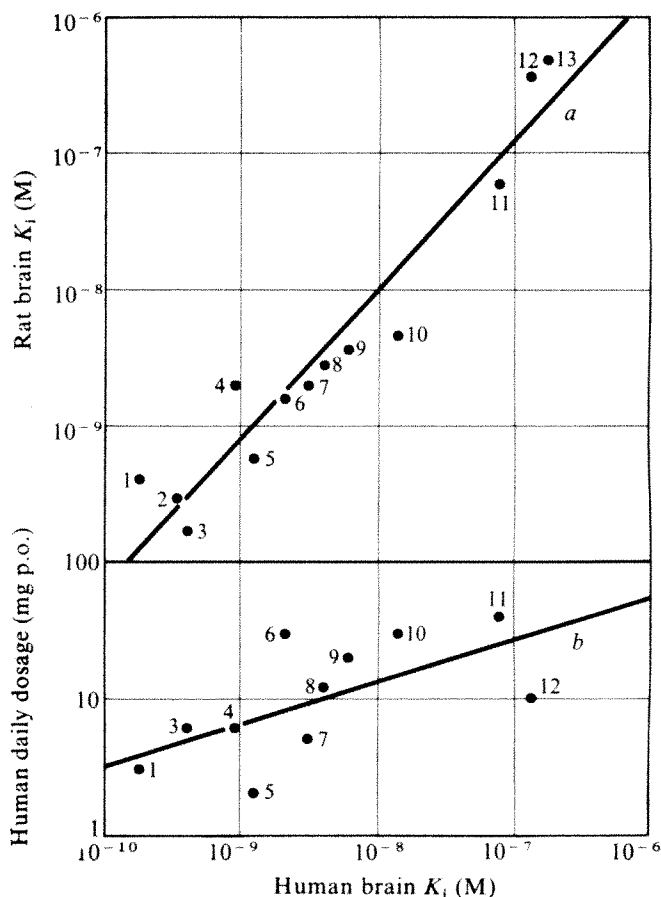
Received 5 May; accepted 24 August 1977.

- Chang, C. C. & Lee, C. Y. *Archs Int. Pharmacodyn.* **144**, 241–257 (1963).
- Rang, H. P. Q. *Rev. Biophys.* **7**, 283–399 (1975).
- Harris, J. B., Karlsson, E. & Thesleff, S. *Br. J. Pharmac.* **47**, 141–146 (1973).
- Kamenskaya, M. A. & Thesleff, S. *Acta. physiol. Scand.* **90**, 716–724 (1974).
- Lee, C. Y. & Chang, C. C. *Mem. Inst. Butanton*, **33**, 555–572 (1966).
- Cull-Candy, S. G. *et al. Neuroscience* **1**, 175–180 (1976).
- Fohlman, J., Eaker, D., Karlsson, E. & Thesleff, S. *Eur. J. Biochem.* **68**, 457–469 (1976).
- Dowdall, M. J., Fox, G. F., Wächter, K., Whittaker, V. P. & Zimmermann, H. *Cold Spring Harb. Symp. quant. Biol.* **40**, 65–81 (1976).
- Dowdall, M. J. & Zimmermann, H. *Neuroscience* **2**, 405–421 (1977).
- Dowdall, M. J., Barrantes, F. J., Stender, W. & Jovin, T. M. *J. Neurochem.* **27**, 1253–1255 (1976).
- Halpert, J. & Eaker, D. *J. biol. Chem.* **250**, 6990–6997 (1975).
- Halpert, J. & Eaker, D. *J. biol. Chem.* **251**, 7343–7347 (1976).
- Fohlman, J. & Eaker, D. *Toxicol* **15**, 385–393 (1977).
- Dowdall, M. J. in *The Biochemistry of Characterized Neurons* (ed. Osborne, N. N.) 177–216 (Pergamon, Oxford, 1977).
- Sen, I., Grantham, P. A. & Cooper, J. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2664–2668 (1976).
- Kelly, R. B., Oberg, S. G., Strong, P. N. & Wagner, G. M. *Cold Spring Harb. Symp. quant. Biol.* **40**, 117–125 (1976).
- Kelly, R. B. & Brown, F. R. *J. Neurobiol.* **5**, 135–150 (1974).
- Karlsson, E., Arnberg, H. & Eaker, D. *Eur. J. Biochem.* **21**, 1–16 (1971).

vitro is related to a physiologically relevant receptor for benzodiazepines *in vivo*. We report here that benzodiazepine receptors are also present in the human brain, that the cerebral and cerebellar cortical regions contain the highest densities of binding sites and that the receptors in human brain are very similar to those in rat brain.

The brains of four humans, two males and two females, aged 18, 32, 54, and 72 yr and dying from malformatio congenita cordis, ulcus duodeni, stenosis valvulae mitralis and arterio sclerosis were investigated. No patients had a recorded history of benzodiazepine treatment within 14 d of death. Autopsies were carried out 17–72 h after death and 0.4–1.8 g of brain tissue was excised and frozen at -70°C until analysis within 14 d (experiments with rats showed that the affinity constants and density of binding sites can be safely determined 1–3 d after cessation of lorazepam or diazepam treatment, and that brains, left for 6 h at room temperature and then for 3 d at 4°C , have normal receptors). White matter contamination of grey matter was kept to a minimum, and grey matter contamination of white was avoided. The regional distribution of specific ^3H -diazepam binding sites was similar in all the brains. Table 1 therefore presents the average values from two to four brains. The receptor binding assay² consisted of an osmotically shocked crude synaptosomal preparation which was incubated for 40 min at 0°C with 0.57 – $11.3 \text{ nM } ^3\text{H}$ -diazepam (*N*-methyl- ^3H), $14.4 \text{ Ci mmol}^{-1}$ (kindly donated by Willy Haefely of F. Hoffman-La Roche). After incubation, the receptor–ligand complex was separated by filtration through glass fibre filters and measured by scintillation counting. All binding

Fig. 1 Correlation between K_i values for ^3H -diazepam displacement of 13 clinically active benzodiazepines (the average for frontal and cerebellar cortex, from Table 2) and clinically recommended dosages (Danish Physicians desk reference to pharmaceutical specialities¹⁰). The K_i -values for rat brain benzodiazepine receptors¹⁸ are also shown. The best curve fit was calculated by linear regression analysis. Numbers on graph indicate drug number in Table 2. *a*, $r=0.974$; $P<0.001$. *b*, $r=0.631$; $P<0.05$.



High densities of benzodiazepine receptors in human cortical areas

THE presence of brain-specific benzodiazepine receptors in membranes from rat brain is now established^{1–4}. Highly significant correlations between the affinities of various benzodiazepines for the benzodiazepine receptor site in rat brain on the one hand and clinically predictive pharmacological activities in several species on the other, strongly suggest that the benzodiazepine receptor *in*

Table 1 Benzodiazepine receptors in various regions of the human brain

Brain region ²⁰	Specific ³ H-diazepam binding (c.p.m. per assay)	Density of ³ H-diazepam receptors (pmol per g protein)	K_D (nM)	IC ₅₀ (nM)	
				Diazepam	Clonazepam
Frontal lobe cortex (gyrus frontalis inf.)	960 ± 200 (3)	860 ± 130 (3)	3.5 ± 0.3 (3)	22 ± 8 (3)	6.6 ± 0.7 (3)
Frontal lobe cortex (gyrus centralis inf.)	730 ± 130 (4)	760 ± 90 (4)	6.8 ± 2.0 (4)	16 ± 4 (3)	5.6 ± 0.4 (3)
Occipital lobe cortex (gyrus occipitalis lat.)	1,280 (2)	840 ± 110 (3)	4.8 ± 0.8 (3)	11 (2)	5.9 (2)
Temporal lobe cortex (gyrus temporalis inf.)	870 ± 260 (3)	670 ± 150 (3)	4.9 ± 1.3 (3)	20 (2)	6.5 (2)
Cerebellar hem. cortex	580 ± 20 (3)	580 ± 40 (3)	4.2 ± 0.5 (4)	15 ± 3 (3)	5.2 ± 1.3 (2)
Vermis	350 (2)	760 (2)	5.7 (2)	24 (2)	3.7 (2)
Hippocampus	615 ± 190 (3)	670 ± 120 (3)	4.2 ± 0.6 (3)	14 (2)	5.2 (2)
Amygdala	165 (1)	510 (1)	9 (1)	13 (1)	2.3 (1)
Hypothalamus	250 ± 75 (3)	450 ± 120 (3)	7.4 ± 2.3 (4)	15 ± 4 (3)	8.4 ± 0.5 (3)
Thalamus	560 (2)	330 ± 55 (3)	4.6 (2)	20 (2)	4.0 (2)
Nucleus caudatus	450 ± 90 (3)	440 ± 90 (3)	4.3 ± 1.4 (3)	15 (2)	6.4 ± 0.9 (3)
Putamen + globus pallidus	455 ± 65 (3)	360 ± 60 (3)	4.1 ± 0.6 (4)	11 ± 0.3 (3)	5.2 ± 1.1 (3)
Nucleus dentatus	80 (2)	160 (2)	7 (2)	18 (2)	4.1 (1)
Corpus callosum	85 ± 20 (3)	110 (2)	20 ± 7 (4)	70 (2)	5.8 (2)
Pons	110 ± 5 (3)	160 ± 20 (3)	5 ± (3)	40 ± 24 (3)	
Medulla oblongata	130 (2)	200 (2)	12 ± 6 (3)	30 ± 20 (3)	9.5 (2)
Medulla spinalis	90 (2)	210 (2)	7 ± 2 (3)	50 (2)	9.1 (2)

The value for 'specific binding' represents the control binding (c.p.m. per assay, corresponding to 10 mg original tissue) at 1.9 nM ³H-diazepam used for IC₅₀-value determinations (see Table 2). The receptor densities were determined by Scatchard analysis (0.57, 1.6, 4.5, 11.3 nM ³H-diazepam, each in triplicate). In all regions Ro 5-4864 (300 nM) displaced less than 50% of specific ³H-diazepam binding. Values are the mean ± s.e.m. of (*n*) different brains, each assayed once. Nonspecific binding of ³H-diazepam amounted to 9–60% (120–200 c.p.m. per 10 mg wet tissue, at 1.9 nM ³H-diazepam) of total binding, depending on the region.

values were calculated as specific binding, defined as binding displaceable by 3×10^{-6} M unlabelled diazepam.

Table 1 shows the regional distribution of specific benzodiazepine receptors in the human brain. The densities of binding sites (determined by Scatchard analysis) show marked regional variations. The frontal cortex, occipital cortex, cerebellar cortex, temporal cortex and hippocampus contained the highest densities of specific binding sites (~700–900 pmol per g protein); intermediate densities were noted in corpus striatum, globus pallidus and hypothalamus (inclusive mammillary body) while corpus callosum, nucleus dentatus and pons showed low densities. In independent assays, the amount of specific binding was determined using a fixed concentration of ³H-diazepam (1.9 nM). These values paralleled the receptor densities determined by Scatchard analysis. The high levels of specific ³H-diazepam binding in cortical areas and the low levels in pons and medulla are very similar to the distribution in the rat brain^{2,3}.

Neuropharmacological data suggest that the hippocampus is a significant site of benzodiazepine action although several other brain areas have also been implicated¹⁷. Our results suggest that in addition to hippocampus, cortical areas may be important for the action of benzodiazepines.

Table 2 Inhibition of specific ³H-diazepam binding (1.9 nM) to human brain membranes by benzodiazepines

No.	Compound	K_i (nM)	
		Frontal cortex	Cerebellar cortex
1	Lorazepam	1.5	1.9
2	Flunitrazepam	3.5	3.2
3	Clonazepam	4.3	3.4
4	Diazepam	11	7.1
5	Estazolam	11	13
6	Flurazepam	21	21
7	Nitrazepam	36	25
8	Bromazepam	37	43
9	Tranxene	71	56
10	Oxazepam	150	120
11	Chlordiazepoxide	980	590
12	Medazepam	1,780	840
13	Ro 5-3636	1,910	1,410
14	Ro 5-4864	24,500	13,200

Serial dilution of benzodiazepines in duplicate were added to the high-affinity binding assays from either frontal cortex or cerebellar cortex. K_i values were calculated using the equation: $K_i = IC_{50}/(1 + (C/K_D))$; IC_{50} is the concentration causing 50% inhibition of specific ³H-diazepam binding; C = ³H-diazepam concentration (1.9 nM) and K_D = affinity constant (3.5 and 4.2 nM, Table 1). Each value is the mean of two or three determinations with a range less than $\times 2.5$.

The apparent affinity constants (K_D) were about equal in most areas (3–7 nM) and similar to the K_D values for the rat brain receptor (2.6–3.6)^{2,4}. Only two areas, the corpus callosum and the medulla oblongata, exhibited K_D values appreciably above 7 nM indicating that the specific binding of ³H-diazepam in these areas, which also exhibited low binding, is different in nature compared with other brain areas. The Scatchard analysis of the different regions showed no tendency for binding to resolve into more than one component showing the presence of only one receptor type. A single receptor is also indicated by the similar 50% displacement values (IC₅₀) for clonazepam and diazepam in several brain regions, as well as parallel K_i values for 13 benzodiazepines in frontal cortex and cerebellar cortex (Table 2).

Brain-specific ³H-diazepam binding in the rat is characterised by high affinities for ³H-diazepam ($K_D \sim 3$ nM) and clonazepam, but low affinity for the pharmacologically inactive benzodiazepine Ro 5-4864 (ref. 2). In contrast, a specific ³H-diazepam binding site in homogenates of rat kidney and liver exhibits low affinity for ³H-diazepam ($K_D \sim 40$ nM) and clonazepam and very high affinity for Ro 5-4864 (ref. 2). The findings that clonazepam and diazepam have high, and Ro 5-4864 low affinities for the ³H-diazepam binding site in all human brain regions suggest that this site is analogous to the brain specific site in rat and different from the site in rat kidney and liver.

There were no clear correlations between the regional distribution of ³H-diazepam binding site and the regional distribution of dopamine⁵, dopamine receptors^{6,7}, noradrenaline⁵, noradrenaline receptors^{6,8}, dopamine- β -hydroxylase (in the rat)⁹, 5-hydroxytryptamine (5-HT)⁵, 5-HT receptors⁶, acetylcholine (in the cat)⁵, acetylcholine receptors⁶, choline acetylase^{5,6}, γ aminobutyric acid (GABA)^{5,6}, GABA-receptors^{6,10}, glutamic acid decarboxylase^{6,11}, endorphins (unspecified¹² or rat¹³), opiate receptors^{6,14} and substance P (refs 15, 19). Only GABA, bicuculline binding sites (in the rat¹⁰), etorphine binding sites¹⁴ and the benzodiazepine receptors exhibit the rather unusual pattern of high levels in cerebral cortex and appreciable amounts in the cerebellar cortex.

The ability of several clinically active benzodiazepines to displace ³H-diazepam from the receptors (K_i values) from both frontal cortex and cerebellar cortex correlate with the recommended daily clinical doses (Fig. 1), with an *in vivo* test for human anti-anxiety¹⁷ ($r = 0.736$; $P < 0.025$) and with the K_i values for the rat brain receptor (Fig. 1). These results further demonstrate the similarity between the benzodiazepine receptors in human brain and those in the rat brain, and suggest that they are involved in the clinical effects of benzodiazepines.

This work was supported by a grant from Statens Laegevidenskabelige Forskningsråd, Copenhagen. We thank A. M. Buhl and A. Stenberg Knudsen for technical assistance.

C. BRAESTRUP

Psychopharmacological Research Laboratory,
Dept. E. DK-4000 Roskilde

R. ALBRECHTSEN

University Institute of Pathological Anatomy, Copenhagen

R. F. SQUIRES

Research Laboratories, A/S Ferrosan, Sydmarken 1-5, 2860
Soeborg, Denmark

Received 12 July; accepted 19 August 1977.

1. Squires, R. F. & Braestrup, C. *Nature* **266**, 732-734 (1977).
2. Braestrup, C. & Squires, R. F. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
3. Möhler, H. & Okada, T. *Science* (in the press).
4. Möhler, H. & Okada, T. *Life Sci.* **20**, 2101-2110 (1977).
5. Fahn, S. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. & Tower, D. B.), 169-186 (Raven, New York, 1976).
6. Enna, S. J. *et al. J. Neurochem.* **28**, 233-236 (1977).
7. Seeman, P., Lee, T., Chau-Wong, M., Tedesco, J. & Wong, K. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4354-4358 (1976).
8. Greenberg, D. A., U'Prichard, D. C. & Snyder, S. H. *Life Sci.* **19**, 69-76 (1976).
9. Hartman, B. K., Zide, D. & Udenfriend, S. *Proc. natn. Acad. Sci. U.S.A.* **69**, 2722-2726 (1972).
10. Möhler, H. & Okada, T. *Nature* **267**, 65-67 (1977).
11. Fahn, S. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. & Tower, D. B.), 169-186 (Raven, New York, 1976).
12. Hughes, J. *Brain Res.* **88**, 295-308 (1975).
13. Pasternak, G. W., Goodman, R. & Snyder, S. H. *Life Sci.* **16**, 1765-1769 (1975).
14. Hiller, J. M., Pearson, J. & Simon, E. J. *Rev. Comm. chem. Path. Pharmac.* **6**, 1052-1062 (1973).
15. Powell, D., Leeman, S., Tregear, G. W., Niall, H. D. & Potts, J. T. Jr *Nature new Biol.* **241**, 252-254 (1973).
16. *Laegemiddelkataloget* (ed. Jacobsen, E.) (Copenhagen, 1976).
17. Randall, L. O., Schallek, W., Sternbach, L. H. & Ning, R. Y. in *Psychopharmacological Agents* (ed. Gordon, M.), 175-281 (Academic, New York, 1974).
18. Braestrup, C. & Squires, R. F. *Eur. J. Pharmac.* (in the press).
19. Duffly, M. J., Wong, J. & Powell, D. *Biochem. Soc. Trans.* **2**, 1262-1264 (1974).
20. Ranson, S. W. & Clark, S. L. in *The Anatomy of the Nervous System, Its Development and Function* 29-70 (Saunders, Philadelphia & London, 1959).

Altered exploratory behaviour after 6-OHDA lesion to the dorsal noradrenergic bundle

IN spite of the considerable evidence about the behavioural functions of brain dopamine (DA), and especially the nigrostriatal tract¹⁻⁹, relatively little is known about the functions of noradrenergic (NA) systems. Although they were initially suggested to be involved in learning^{10,11} and the coding of reinforcement¹², this is now clearly no longer tenable¹³⁻¹⁷. Perhaps the best documented function of the dorsal noradrenergic bundle^{18,19} is in extinction situations¹³. The actual behavioural mechanism involved in these situations, and how it is altered by the lesion, remains complex although some progress has been made in its elucidation²⁰⁻²². We report here what may prove to be a new class of behavioural deficit produced by selective destruction of NA systems. It was found that the response to novelty was altered in two different test situations after the lesion.

Ten male albino Woodlyn rats weighing about 300 g, received 4 µg of 6-hydroxydopamine (6-OHDA) injected stereotactically into the fibres of the dorsal bundle. Ten control animals received sham operations. The animals were allowed to recover for two weeks before behavioural testing started. After testing the ten treated and four of the control animals were killed and their brains assayed for NA²³. This confirmed that the injection of 6-OHDA had produced severe depletion of forebrain NA (cortex-hippocampus to 3.9% of control values and hypothalamus to 25.5% of control). It is known from previous work using this same technique that no alteration occurs in the concentration of brain DA¹⁵. Behavioural testing consisted first of an examination of the exploratory behaviour in a complex, structured situation comprising a series of parallel runways²⁴, and second of the investigatory response of the animal to the introduction of a novel object into a familiar cage. Nine treated and nine control animals were deprived of food for 24 h, placed in the start box of the maze apparatus and a few seconds later the door leading to the alleyways was raised. Measures taken included the time to emerge from the start box into the alleyways, the number

of alleyways entered in successive 3-min periods for a total of 21 min and the total time spent in the goal box before consumption of the first of four food pellets present there in a small elevated food cup. The 21 min of alleyway exploration started as each animal emerged from the start box and so was not contaminated by any possible difference in the emergence time between the two groups.

The number of alleyways entered per 3-min period is shown in Fig. 1; treated animals showed considerably more exploration than controls. (Alleys entered in the total 21-min period; treated mean = 96.3, control mean = 65.6, Mann-Whitney²⁵ $U = 9$, $P < 0.01$.) The time course (Fig. 1) revealed that the difference lay not in the initial rate of exploration in the first 3 min in the apparatus, but in the habituation to the novel situation with continued exposure. The treated rats also took longer to emerge from the start box (treated mean = 104 s, control mean = 46 s, Mann-Whitney $U = 22$, $P < 0.05$) and took longer to consume the first food pellet following entry to the goal box (treated mean = 91.5 s, control mean = 20.6 s, Mann-Whitney $U = 15$, $P < 0.05$). Thus, it seems from this experiment that exploration may be increased by the 6-OHDA lesion, possibly by a failure to habituate to the novel environment; to test this further, a second experiment was carried out.

The same animals (ten treated and ten controls) that had been used previously were placed on an *ad libitum* diet for 2 d and then habituated to a wooden cage for 15 min. During this period the number of times the animals crossed the line dividing the cage in two along the long axis was recorded as an index of spontaneous locomotor activity. At the end of a 15-min period the animal was removed

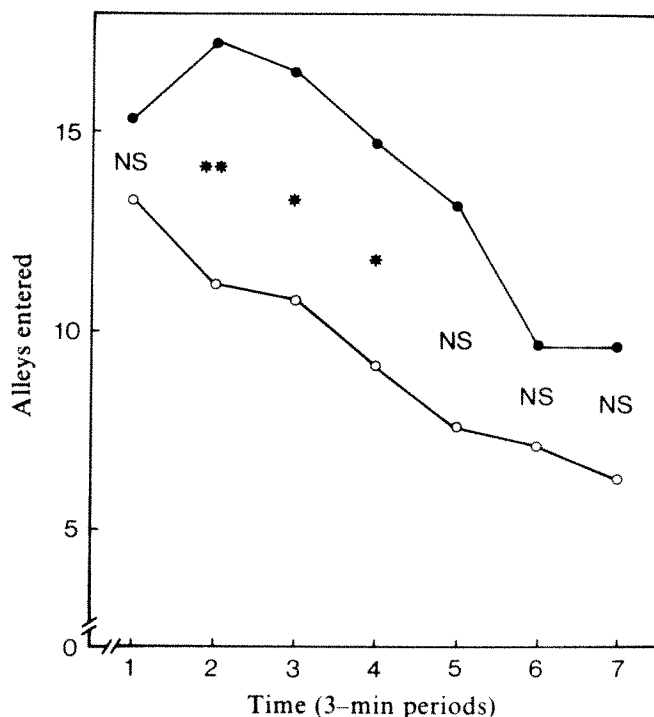


Fig. 1 The mean number of alleyways entered in a 3-min period plotted against successive periods for a total of 21 min in the 'parallel runway' apparatus for control and 6-OHDA-treated animals. Treated rats received stereotaxic injections of 4 µg of 6-OHDA base (obtained as 6-OHDA HBr.) dissolved in 2 µl of 0.9% saline with 0.3 mg ml⁻¹ ascorbic acid antioxidant infused at the rate of 0.2 µl min⁻¹ over 10 min at the coordinates AP2.6, ML1.1 and DV3.7 according to König and Klippel. Controls were anaesthetised, positioned in the stereotaxic and burr holes drilled at these coordinates. The stars indicate that the two groups differed significantly at that time point. One star at the 5% level, and two stars at the 1% level. NS, No significant difference between the groups. ○, Control animals ($n = 9$); ●, treated animals ($n = 9$).

briefly and a novel object (cylinder 4.5 inch by 2.5 inch in diameter) was placed in one side of the apparatus and the rat replaced in the opposite side. The latency of the first approach to the novel object was noted, as was the total time spent in contact with the object over the next 15 min. Although no alteration in the latency to approach the novel object was found (treated mean = 7.4 s, control mean = 7.9 s), the time in contact was significantly higher for the treated animals (treated mean = 79.8 s, control mean = 40.0 s, Mann-Whitney $U = 20$, $P < 0.05$). This suggests that, in this simple situation as well, the lesion increased the amount of exploratory behaviour shown towards a novel object. The number of times that the rat initiated a bout of exploration aimed at the novel object was also recorded and this did not differ from controls (treated mean = 30.3, control mean = 22.2, $U = 39$, not significant) suggesting that the lesion had not altered the frequency of initiation of an exploratory bout, but since the total time in contact with the object was increased in the treated rats, had rather interfered with the ability to habituate to that object and stop exploration. The locomotor activity counts during the habituation phase before introduction of the novel object rule out either hyper- or hypoactivity as the cause of the increased exploration, since no difference was found between treated and control animals (treated mean = 29.7, control mean = 23.7, $U = 43$, not significant), and this has also been shown several times in other situations³⁰. A second locomotor activity measure taken on these same rats in photocell cages confirmed this (treated mean = 790 beam interruptions in 21 min, control = 976, t statistic = 1.61, not significant). We have since replicated these two exploration experiments with identical results in another group of animals depleted of brain NA.

The results reported here add to the few functions already demonstrated^{13,14,17} for NA systems. Since our lesions resulted in severe depletion of hypothalamic NA as well as cortical-hippocampal NA we cannot as yet say whether this effect is due to the dorsal NA projection from the locus coeruleus or the so-called ventral bundle arising in more posterior cell groups and innervating the hypothalamus^{18,19}. The behavioural mechanism involved must also remain indeterminate at this time. It is of interest, however, that animals with similar lesions have been shown to be resistant to extinction in a number of situations^{13,14,17}. Whether the elevated exploratory behaviour observed in the dorsal bundle lesioned animals similarly reflects impaired extinction of the exploratory response is not known. It is possible that the present observations may be yet another measure of the deficit which has been observed in other situations^{13,14,17}, or they may be due to damage to independent mechanisms possibly reflecting noradrenergic deafferentation in different terminal regions of the brain. Another possibility is that, as has been reported after locus coeruleus lesions^{26,27}, these animals may be less fearful and so tend to 'freeze' less and thus explore more. This seems less likely since the initial rate of exploration in the first 3 min in the maze apparatus did not differ from controls, suggesting that the immediate fear reaction was not altered. The subsequent habituation was retarded, however, suggesting perhaps that the treated animals might be unable to learn to overcome their fear. It has been suggested^{28,29} that the central emotional state produced by fear has similarities to that produced by frustration (caused by omission of an expected reward) and that the dorsal bundle lesioned rat may be impaired in learning about or remembering frustration (S. T. M. and S. D. Iversen, unpublished), although its general learning capacity in situations not involving frustration or similar states is unaltered¹³⁻¹⁷. An increase in the immediate baseline fear reaction is also counter-indicated inasmuch as no increase in the latency to approach the novel object immediately after its introduction was observed. Certain additional mechanisms may be tentatively

excluded since it has been shown that no deficit in internal inhibition (ref. 30 and S. T. M. and S. D. Iversen, unpublished), some forms of attention (S. T. M. and T. W. Robbins, unpublished), perseverance^{13,17,30} or general learning ability¹³⁻¹⁷ is present in these animals. The data reported here also rule out simple locomotor activity mechanisms. Further research will be required, however, to elucidate the basis of this alteration in exploratory behaviour found after depletion of forebrain NA.

S. T. M. is an MRC Fellow and the work was supported by the Medical Research Council. The technical assistance of Betty Richter is acknowledged. D. C. S. Roberts performed the 6-OHDA lesions.

STEPHEN T. MASON
HANS C. FIBIGER

*Division of Neurological Sciences,
Department of Psychiatry,
University of British Columbia,
Vancouver V6T 1W5, Canada*

Received 27 June; accepted 15 August 1977.

1. Fibiger, H. C., Phillips, A. G. & Zis, A. P. *Pharm. Biochem. Behav.* **2**, 87-96 (1974).
2. Price, M. T. C. & Fibiger, H. C. *Eur. J. Pharmac.* **29**, 249-252 (1974).
3. Price, M. T. C. & Fibiger, H. C. *Pharm. Biochem. Behav.* **3**, 285-290 (1975).
4. Kelly, P. H. *Brain Res.* **100**, 163-169 (1975).
5. Kelly, P. H. & Iversen, S. D. *Eur. J. Pharmac.* **40**, 45-56 (1976).
6. Kelly, P. H., Seviour, P. W. & Iversen, S. D. *Brain Res.* **94**, 507-522 (1975).
7. Creese, I. & Iversen, S. D. *Brain Res.* **55**, 369-382 (1973).
8. Creese, I. & Iversen, S. D. *Nature new Biol.* **238**, 24-28 (1972).
9. Creese, I. & Iversen, S. D. *Brain Res.* **83**, 419-436 (1975).
10. Kety, S. S. *Res. Publ. Res. Nerv. Ment. Dis.* **50**, 376-389 (1972).
11. Crow, T. J. *Psychol. Med.* **2**, 414-417 (1972).
12. Crow, T. J., Spear, P. J. & Arbuthnot, G. W. *Brain Res.* **36**, 275-287 (1972).
13. Mason, S. T. & Iversen, S. D. *Nature* **258**, 422-424 (1975).
14. Mason, S. T. & Iversen, S. D. *J. comp. Physiol. Psychol.* **91**, 165-173 (1977).
15. Roberts, D. C. S., Price, M. T. C. & Fibiger, H. C. *J. comp. Physiol. Psychol.* **90**, 363-372 (1976).
16. Sessions, G. R., Kant, G. J. & Koob, G. F. *Physiol. Behav.* **17**, 853-859 (1977).
17. Mason, S. T. & Iversen, S. D. *Brain Res.* (in the press).
18. Ungerstedt, U. *Acta Physiol. scand. Suppl.* **367**, 1-49 (1971).
19. Lindvall, O. & Bjorklund, A. *Acta Physiol. scand. Suppl.* **412**, 1-48 (1974).
20. Mason, S. T. & Iversen, S. D. *8th Ann. mtg Eur. Brain Behav. Soc., Copenhagen.* (1976).
21. Iversen, S. D. & Mason, S. T. *Br. J. Pharmac.* **55**, 239P (1976).
22. Tremmel, F., Morris, M. D. & Gebhart, G. F. *Brain Res.* **126**, 185-188 (1977).
23. McGeer, E. G. & McGeer, P. L. *Can. J. Biochem. Physiol.* **40**, 1141-1146 (1962).
24. Whishaw, I. Q. & Cooper, R. M. *Physiol. Behav.* **5**, 647-649 (1970).
25. Siegel, S. *Non-parametric Statistics* (McGraw-Hill, 1956).
26. Huang, Y. H., Redmond, D. E., Snyder, D. R. & Maas, J. W. *6th Ann. Mtg Soc. Neurosci., Toronto* (1976).
27. Redmond, D. W., Huang, Y. H., Snyder, D. R. & Maas, J. W. *Brain Res.* **116**, 502-509 (1976).
28. Gray, J. A. *The Psychology of Fear and Stress*. (Weidenfeld & Nicholson, London, 1969).
29. Wagner, A. R. in: *Punishment and Aversive Behaviour* (Eds Campbell, B. A. & Church, R. M.) (Appleton-Century-Crofts, 1969).
30. Mason, S. T. & Iversen, S. D. *Pharm. Biochem. Behav.* (in the press).

Cerebral lateralisation effects in visual half-field experiments

THE systematic study of behavioural differences related to differential functioning of the cerebral hemispheres has increased markedly in recent years. The studies of Sperry and his collaborators¹ on cerebral commissurotomy patients have stimulated many investigations into the hemisphere specialisations of normally functioning subjects. There are now many reports^{2,3} of statistically significant differences in hemisphere performance. Although hemisphere differences are consistent across independent experiments, they are small, occasionally inconsistent, and found within a limited experimental context, and so the generality of hemisphere differences remains to be established. We present here brief accounts of six experiments on cerebral lateralisation, four of which failed to find any differences and two which produced highly significant hemisphere differences. On the basis of this and published work, we believe that we can offer both a possible explanation for both the presence and absence of cerebral lateralisation effects in visual half-field experiments and also procedural guidelines that will produce cerebral lateralisation effects.

Much of the method for our six experiments is identical. Adult right-handed subjects fixated on a reference point in a three-field tachistoscope and indicated readiness to the experimenter. Stimuli were then shown for a period not exceeding 150 ms to the light visual field projecting to the left hemisphere (RVF-LH), or the left visual field projecting to the right hemisphere (LVF-RH), or with one member of a stimulus pair projected to each visual field (EVF). In all experiments, stimuli were located 2.5° of visual angle from the fixation point. Illumination level was 15 ft L.

Experiment 1: Stimulus materials were 96 words selected from a list of common Chinese terms using the norms developed by Liu and Chuang⁴ and their English equivalents (most English words used have A or AA Thorndike-Lodge ratings). The Chinese stimuli were all single characters which were drawn with a felt-tip pen and spanned 0.84° of visual angle. The English words (ranged from three to six letters) were done with Letraset 20 point Helvetica and spanned horizontally 0.84°–1.30° of visual angle at maximum. Forty-eight of the pairs were direct unambiguous translations and 48 were dissimilar terms. Presentation to RVF-LH, LVF-RH, and EVF were equal and randomised. Eight subjects fluent in reading Chinese and English responded by saying 'yes' if a stimulus pair had the same meaning and 'no' if they did not. Each of the 96 stimulus pairs was shown once. No term occurred more than once.

An analysis of variance of reaction times for making correct decisions revealed a statistically significant effect ($F(1, 7) = 19.90, P < 0.01$) for same-different judgments, indicating that decisions that two terms did not have the same meaning required more time than judgments of identity. Differences between visual fields and interactions did not differ appreciably from chance levels. Mean reaction times for experiments 1–4 are given in Table 1.

Experiment 2: At random, six of the stimulus pairs used in experiment 1 were discarded. The remaining 90 were then arranged as 30 English-English pairs, 30 Chinese-Chinese pairs, and 30 Chinese-English pairs (selected from the word pairs of experiment 1). Within each 30-pair set,

15 terms were identical and 15 different. Presentation to visual fields was as in experiment 1. Each stimulus pair was shown once and no terms were repeated. Subjects were 20 Chinese-English bilinguals, fluent at reading Chinese and English, and this time a manual (instead of oral) response was used.

Data analysis was identical to that in experiment 1. Only one comparison—that for judgments of same-different—reached an acceptable level of statistical significance ($F(1, 18) = 19.60, P < 0.001$). As was found in experiment 1, judgments of sameness take less time than judgments of difference. No visual field-hemisphere differences were present. The Chinese-English EVF data from experiments 1 and 2 were further partitioned into trials in which the Chinese was on the left and the English was on the right and vice versa. No reliable effect was obtained from this contrast either.

Experiment 3: Eight monolingual English subjects made judgments of same-different to the 30 English-English pairs and 30 Chinese-Chinese pairs used in experiment 2. Stimuli were shown once, with no repetition. Manual response reaction time was taken as dependent measure. Analysis revealed no statistically significant effects for any condition. No visual field-hemisphere differences were present and differences in response time to language (Chinese against English) and type of judgments (same against different) were not significant.

Experiment 4: Eight monolingual English subjects made judgments of same-different on stimulus pairs made up of only four Chinese characters and four English words. Within each language, every word was paired with every other word for 'different' stimulus pairs and with itself for 'same'. Presentation of either the Chinese-Chinese or English-English pairs was made only to RVF-LH and LVF-RH fields for 200 trials, which were distributed over five blocks with 40 trials per block. An analysis of variance of reaction times revealed a significant interaction effect of language by visual field by trial ($F(4, 28) = 4.00, P < 0.01$). Inspection of mean values indicated that reaction time by hemisphere effects increases systematically over trials and the data in the last block show a faster reaction time to English-English stimuli in the RVF-LH and a similar, though smaller, effect for Chinese-Chinese stimuli in the LVF-RH.

Experiment 5: Seventy-five subjects made judgments of same-different on pairs of figures selected from the spatial ability subtest of the Thurstone primary mental abilities test⁵. Stimulus pairs occupied about 2.5° of visual angle, were immediately adjacent, and at either 45° or 180° of rotational difference from each other. Stimuli were defined as 'same' when rotation in a clockwise or counter-clockwise direction allowed one stimulus to completely cover the other. When stimuli were mirror images, they were defined as 'different'. Seventy-two stimulus pairs were used, each pair shown once. The types of figures used each appeared twice, although no combination was ever repeated exactly. Reaction time was not measured in this experiment, the accuracy of response being the only parameter. Analysis of variance indicated no significant effects for visual field-hemisphere, type of judgment, or degree of rotation.

Experiment 6: Six pairs of stimuli were selected at random from the stimuli used in experiment 5. Three stimuli were 'same' and three were 'different', as defined in experiment 5. Eight subjects were shown the stimulus pairs, randomly presented to RVF-LH or LVF-RH only for 200 trials. An analysis of response accuracy showed that stimuli shown in the LVF-RH are judged more accurately than the same stimuli shown in the RVF-LH and that more errors were made in the 'same' pairs than in the 'different' pairs. Mean accuracy judgments for experiments 5 and 6 are given in Table 2.

Table 1 Mean reaction times for experiments 1–4

Condition			Experiments			
			1	2	3	4
			X	X	X	X
English-English	LVF-RH	Same		1,185	1,220	767
		Different		1,310	1,017	806
	RVF-LH	Same		1,176	1,264	715
		Different		1,320	1,035	740
	EVF	Same		1,084	1,048	
		Different		1,367	1,066	
Chinese-Chinese		Same		1,093	1,087	854
		Different		1,141	1,084	796
		Same		1,188	1,085	834
		Different		1,098	1,127	794
		Same		1,057	1,202	
		Different		1,132	1,107	
Chinese-English	LVF-RH	Same	1,341	1,175		
		Different	1,976	1,722		
	RVF-LH	Same	1,413	1,355		
		Different	1,955	1,606		
	EVF	Same	1,240	1,229		
		Different	2,099	1,584		

In experiment 1, response time was taken with an electronic voice key stopping an ms-timer activated when the stimulus was shown. In experiments 2–4, it was measured by pressing one of two buttons on a horizontal panel in front of the subject, so arranged as to allow comfortable arm placement. Subjects were instructed to press one button if the members of the pair shown were the same and the other button if they were different. The index finger of each hand was used to respond, with hand used for 'same' or 'different' responses counterbalanced across subjects. The timing mechanism was that used in experiment 1.

Table 2 Mean accuracy scores for experiments 5-6

LVF-RH	Same	6.43	29.23
	Different	7.24	36.49
RVF-LH	Same	6.06	18.12
	Different	7.73	33.12
EVF	Same	7.28	
	Different	9.15	

The accuracy scores were obtained by counting the number of correct responses made by subjects across all experimental trials. The highest score possible in experiment 5 is 12 and in experiment 6 is 50.

In summary, experiments 1, 2, 3 and 5 failed to detect any sign of hemisphere differences in judgment processes. These null effects should not be attributed to the insensitivity of the experimental design, since by merely changing the ratio of trials to experimental stimuli experiments 4 and 6 both found significant hemisphere function differences, similar to those reported by other investigators⁸.

In most interpretive models of cortical processing, an individual listening to the spoken word or engaged in reading, is hypothesised as having language information sent to the left hemisphere for processing, semantic analysis and understanding. An appropriate language response is hypothetically generated within the left hemisphere. Similar formulations are proposed for visuospatial processes within the right hemisphere. Our results cannot be explained within such interpretative models.

An activation model, such as that proposed by Kinsbourne⁷, also seems unable to account for our results. Given that all judgments will be semantic comparisons or pattern matches, the expectation from an activation model is that the hemisphere specialised for such processing will be activated and will show superior performance. No such process is evident in our experiments.

An alternative explanation is that lateralisation effects often reported in visual half-field experiments are not a function of immediate cognitive processing in specialised cortical areas, but do reflect hemisphere difference in memory storage location. The experiments reported here are consistent with such a formulation. If a subject has to evaluate new information on each trial, his reaction times or his response accuracy do not differ systematically with visual field presentation. Given such a reinterpretation of lateralisation phenomena, it is important to know if others have obtained similar results using the conditions described. A review of experiments reporting significant hemisphere specialisation effects indicates the number of stimuli used to range from 4 to 64, with the number of trials from 40 to 1,072. By contrast, published accounts which report no hemisphere specialisation differences⁸⁻¹⁰ have, in general, equal numbers of stimuli and trials. It would be of interest to reanalyse data from studies reporting lateralisation effects to see if the trends found in our experiments 4 and 6 are present there. Bryden¹¹ has reported such trends but to our knowledge they have not otherwise been investigated systematically.

The interpretation of these results seems straightforward: to obtain cerebral lateralisation effects, produce a condition that allows judgments to be made from memory. Experimentally, where a new evaluation and decision must be made on every trial, there is little evidence for any lateralisation of function. When the number of stimuli to be judged are so few that the subject may adopt a new strategy and carry out simple matching from a memory set of known size, then cerebral lateralisation effects are found.

These results suggest a number of interesting possibilities for the study of specific kinds of memory storage. Decreases in response time in relation to visual field presentations also suggest an interesting way of assessing when an item is firmly committed to memory. The most important

aspect of these findings, however, is the strong implication that (1) active cognitive processes are not lateralised, and (2) interpretative models which view active cognitive processing as being lateralised are not correct. The present work gives no support to the theory that, dealing with events which require active, immediate, continuous processing of a constantly changing flow of information is limited to one hemisphere at a time.

CURTIS HARDYCK

*Institute of Human Learning,
University of California, Berkeley*

OVID J. L. TZENG

*Department of Psychology,
University of California, Riverside,
Riverside, California 92521*

WILLIAM S-Y. WANG

*Department of Linguistics,
University of California, Berkeley,
Berkeley, California 94720*

Received 3 June; accepted 22 August 1977.

1. Sperry, R. W. *Am. Psychol.* **23**, 723-733 (1968).
2. Hardyck, C. in *Studies in Neurolinguistics*, 3 (eds Avakian-Whitaker, H. & Whitaker, H. A.) (Academic, New York, in the press).
3. Levy, J. in *Hemisphere Function in the Human Brain* (eds Diamond, S. J. & Beaumont, J. G.) (Paul Elek, London, 1974).
4. Liu, I. M. & Chuang, C. J. *Acta Psychol. Taiwan*, **12**, 33-53 (1970).
5. *Primary Mental Abilities Test, Adult Form* (Science Research Associates, Chicago, 1962).
6. Hardyck, C., Tzeng, O. J. L. & Wang, W. S-Y. *Brain Lang.* (in the press).
7. Kinsbourne, M. *Acta Psychol.* **33**, 193-201 (1970).
8. Diamond, S. J., Gibson, A. R. & Gazzaniga, M. S. *Neuropsychologia* **10**, 379-381 (1972).
9. Greffen, A., Bradshaw, J. L. & Wallace, G. J. *exp. Psychol.* **87**, 415-422 (1971).
10. Patterson, K. & Bradshaw, J. L. *J. exp. Psychol. Hum. Percept. Perf.* **1**, 246-252 (1975).
11. Bryden, M. D. *Neuropsychologia* **8**, 443-450 (1970).

Calcium-dependent regenerative responses in rods

NEURONES are thought to contain ion-selective channels whose opening and closing are regulated by the potential difference across the plasma membrane. It is thought that the sodium, potassium, and calcium currents which generate action potentials and modulate synaptic transmitter release pass through these channels. Investigations of voltage-dependent currents in various species have revealed remarkable uniformities in the ion selectivity and pharmacology of presumed membrane channels¹⁻³. The use of agents which block specific currents has provided a tool for the identification of voltage-dependent conductances in nerve membrane, which we have used to investigate vertebrate photoreceptors. We show here that when the retina of the toad *Bufo marinus* is superfused with Ringer containing 6-12 mM tetraethyl ammonium (TEA), rods generate oscillations and action potentials resembling the calcium spikes which have been described in various vertebrate and invertebrate preparations³. These experiments demonstrate that the rod membrane is not passive, as is often assumed, but contains at least one and probably two voltage-dependent conductances.

Retinas were removed from the eyes of dark-adapted toads under dim red illumination and were secured with receptors upwards in a perfusion chamber. Oxygenated Ringer flowed from a gravity-controlled perfusion system into a 0.03-ml pool above the retina at a rate of 0.5-1.0 ml min⁻¹. Silver-silver chloride electrodes were fixed in the chamber below the retina and above it in the perfusion pool to record the electroretinogram (ERG), and simultaneous recordings were made of intracellular responses with fine micropipettes with resistances of 200-400 M Ω . When the b-wave of the ERG reached a stable, dark-adapted threshold, a micropipette was lowered through the perfusion pool with an hydraulic microdrive. Just after reaching the retina, the pipette penetrated cells with response waveform, absolute

sensitivity, spectral sensitivity, and light adaptation characteristic of 'red' rods recorded in previous studies on toad eyecup¹. Rods were stimulated with large-field, 516 nm light from a conventional, dual-beam photostimulator. Normal Ringer had the following composition: 96 or 106 mM NaCl, 1.8 mM Na₂SO₄, 0.13 mM NaHCO₃, 2.5 mM KCl, 1.2 mM MgCl₂, 1.8 mM CaCl₂, 5.6 mM glucose, and 3.0 mM HEPES, adjusted to pH 7.8 with approximately 2 mM NaOH (Ringer modified from ref. 5). All experiments were performed at approximately 22 °C.

Since TEA has been shown to block potassium currents in nerve membrane¹, we examined its effect on the rod photoresponse. We found that 6–12 mM TEA, either added to normal Ringer or substituted for NaCl, depolarises the rod membrane by 3–5 mV. In addition, the receptor potential returns more quickly to the baseline and is followed by 3–4 Hz, regenerative oscillations (Fig. 1a). The peak-to-peak amplitude of the oscillations is initially as large as 35 mV but decays to zero within 4–5 s. After extended perfusion in TEA-Ringer, the amplitudes of the oscillations decay to zero following the light flash more

slowly than shown in Fig. 1, and eventually the oscillations become spontaneous in the dark. Similar oscillations are produced when the retina is superfused with Ringer containing 3 mM of the potassium channel blocking agent 4-aminopyridine⁶, or when the Ca²⁺ in the Ringer is replaced by Sr²⁺ or Ba²⁺. The oscillations can be recorded in the b-wave of the ERG, suggesting that the rods and second-order cells are all oscillating in phase. This is not unexpected as we stimulate the retina with a large, uniform field, and as the responses of the rods are tightly coupled by an extensive network of gap junctions⁷.

The effect of TEA on toad rods resembles that described for crustacean muscle fibres^{3,8,9} and for the presynaptic terminal of the squid giant synapse¹⁰. In these cells the membrane contains a voltage-dependent calcium conductance which, in the absence of TEA, is thought to be shunted by the development of a much larger, voltage-dependent potassium conductance. TEA seems to block a part of the potassium conductance, permitting the calcium current to become regenerative. Although we have no direct evidence that TEA is blocking a potassium conductance in rods, we have tested to see if a calcium current contributes to the oscillations in TEA Ringer by examining the effect of changes of external calcium concentration on the amplitude and duration of the oscillations. We found that as the calcium concentration is reduced, either by substituting Mg²⁺ for Ca²⁺ or by removing Ca²⁺ from the Ringer, the membrane potential depolarises, probably as the result of an increase in sodium conductance^{11,12}, and the oscillations become smaller and decay more quickly. This is shown in Fig. 1b. In 0.5 mM Ca²⁺ (right trace) the oscillations become quite small and, with prolonged perfusion, may vanish altogether. When all of the calcium is removed from the Ringer, the oscillations rapidly disappear. These effects are completely reversible if exposure to low calcium Ringer is not prolonged. In contrast, the oscillations are unaffected by the removal of Mg²⁺ from the 12 mM TEA Ringer.

Increases in [Ca²⁺] produce somewhat more complicated effects. We found that small increases in extracellular calcium cause small membrane hyperpolarisations, probably by decreasing the sodium conductance^{11,12}. Concomitant with this change in potential, there is a dramatic increase in the oscillation amplitude. In Ringer containing TEA and 2.7 mM (1.5 times normal) or 3.6 mM (twice normal) Ca²⁺, the oscillations of most cells become action potentials, up to 45 mV in amplitude and 60–100 ms in duration (Fig. 2). The action potentials are spontaneous in the dark but are interrupted by stimulation with light. The depolarising components of the action potentials are followed by 5 mV hyperpolarising undershoots, which decay to the baseline in 0.5–1 s. If the concentration of Ca²⁺ is increased to 5–16 mM, the rod is further hyperpolarised, and both the action potentials and oscillations are suppressed. We believe that the suppression of the spikes in high Ca²⁺ concentration may simply be the result of the hyperpolarisation of the membrane potential below the spike threshold. Our reason for thinking this is that large spontaneous action potentials can be recorded when high concentrations of Sr²⁺ are added to TEA Ringer containing normal Ca²⁺ (1.8 mM). For the rods, as for many other nerve and muscle cells³, Sr²⁺ seems to be able to substitute for Ca²⁺ as a current carrier in calcium action potentials. Unlike Ca²⁺, however, Sr²⁺ has little effect on the rod membrane potential. As the Sr²⁺ concentration is increased, the amplitude of the action potential increases continuously, and in 28 mM Sr²⁺ the spikes are nearly 60 mV.

To provide further evidence for the identification of the mechanism producing the oscillations, we studied the effects on the TEA-induced responses of agents known to block calcium channels^{2,3}. We found that the oscillations were reversibly blocked by the addition of low concentra-

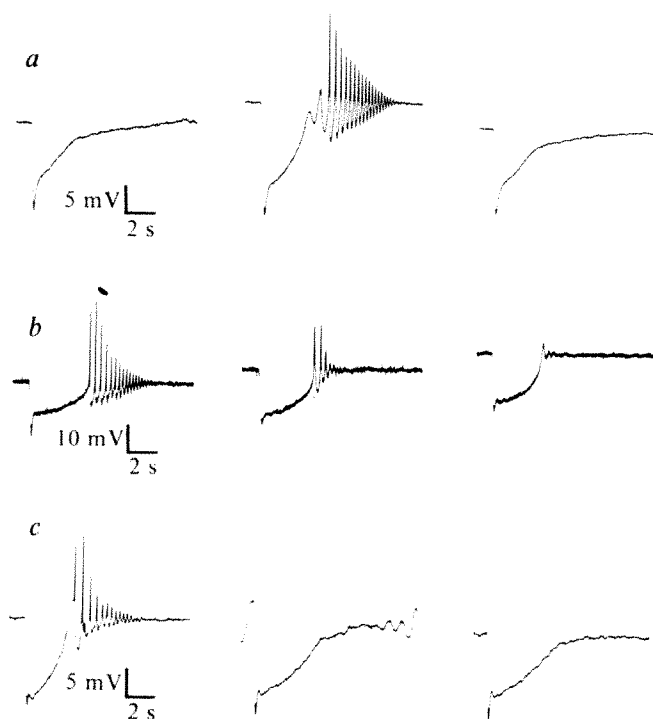


Fig. 1 TEA-induced oscillations in toad rods. *a*, *b*, and *c*, from different retinas. Each shows representative records taken from one continuous recording from a single rod in a dark-adapted retina. All responses are to 109-ms flashes of 516 nm, full-field illumination. *a*, Effect of TEA on rod responses. Left trace, response in normal Ringer (see text). Middle trace, after changing to Ringer containing 12 mM TEA chloride substituted for NaCl. Note 3.5 mV depolarisation of the dark membrane potential, faster decay of receptor potential toward baseline, and oscillations. Right trace, after return to normal Ringer. Membrane potential has repolarised, and the receptor potential waveform has returned to that observed initially. Stimulus intensity for each response was 9.3 log quanta per cm² per flash. *b*, Ca²⁺ dependence of rod oscillations. Left, response in Ringer containing 12 mM TEA and normal Ca²⁺ concentration (1.8 mM). Middle, in 12 mM TEA Ringer containing 1.0 mM Ca²⁺. Note 5 mV depolarisation of membrane potential and decreased amplitude and duration of oscillations. Right, in 12 mM TEA and 0.5 mM Ca²⁺. Membrane potential has depolarised 11 mV from that observed in TEA and normal Ca²⁺. Oscillations have nearly disappeared. Stimulus intensity: 9.8 log quanta per cm² per flash. *c*, Oscillations blocked by Co²⁺. Left, response in Ringer containing 12 mM TEA. Middle, in Ringer containing 12 mM TEA and 50 μM Co²⁺. Note slower decay of response to baseline and decreased amplitude of oscillations. Right, in Ringer containing 12 mM TEA and 100 μM Co²⁺. Oscillations have disappeared. Stimulus intensity: 9.8 log quanta per cm² per flash.

tions of cobalt chloride (Fig. 1c). In $50 \mu\text{M}$ Co^{2+} (Fig. 1c, middle trace), the effect of TEA on the decay of the response is partially reversed (that is, the decay to the base line becomes much slower than in normal TEA Ringer), and the oscillations are smaller and begin several seconds after the decay of the photoresponse. In $100 \mu\text{M}$ Co^{2+} (Fig. 1c, right trace), the oscillations disappear. The oscillations are also reversibly blocked by Mg^{2+} , but the required concentration is much higher (2.5–5 mM). A greater sensitivity to Co^{2+} than to Mg^{2+} has been previously observed for calcium currents in crustacean muscle and for transmitter release^{3,13}. The drug D-600, which has been shown to block the late phase of calcium entry in squid axon¹⁴, also produces a reversible block of rod oscillations at a concentration of 10^{-4} M.

In contrast to Co^{2+} , Mg^{2+} and D-600, tetrodotoxin at $2 \mu\text{M}$, a concentration more than sufficient to block sodium currents in axons¹, has no effect on the rod oscillations. The substitution of choline chloride for NaCl in the Ringer eliminates the oscillations, but this procedure also produces a large hyperpolarisation of the rod membrane potential⁵. In choline chloride Ringer containing 28 mM SrCl_2 , rods generate 35–40 mV, spontaneous action potentials. This experiment shows that sodium is not necessary for the generation of regenerative responses in rods but does not exclude the possibility that sodium currents contribute to the oscillations in normal Ringer.

The results described above are not incompatible with the possibility that the oscillations are caused by an interaction between receptors and second-order cells, since changes in Ca^{2+} concentration or the addition of Co^{2+} or Mg^{2+} to the Ringer could also affect synaptic transmission. In fact, Normann and Pochobradsky¹⁵ have reported spontaneous oscillations in the membrane potential of toad rods which they believe to be caused by such interactions. We have not been able to examine the possible relation between the spontaneous oscillations of Normann and

Pochobradsky and the TEA-induced regenerative responses we report, as we have never observed spontaneous oscillations in rods superfused with normal Ringer. It is unlikely, however, that the regenerative responses we have described are caused by synaptic interactions. The addition to the Ringer of 2 mM sodium aspartate, which has been shown to block the responses of second-order horizontal and bipolar cells in the retina^{5,15–17}, has no effect on the amplitude or frequency of TEA-induced oscillations, even though in our experiments sodium aspartate blocks the simultaneously recorded b-wave of the ERG.

Our results suggest that the oscillations and action potentials produced by the rods in the presence of extracellular TEA are caused by regenerative calcium currents. We cannot as yet exclude a contribution to the oscillations from an inward-going sodium current; however, the dependence of oscillation amplitude on external calcium concentration, the extreme sensitivity of the oscillations to external Co^{2+} and D-600, and their insensitivity to tetrodotoxin all argue against a major role for sodium. Furthermore, the spikes in high Ca^{2+} TEA Ringer have a time course resembling that of calcium spikes in other systems³, being much slower in rise time and longer in duration than that of any sodium spike so far described.

Although we believe that calcium carries the inward current which produces the rising phase of the spikes and oscillations, we are uncertain of the mechanism for their falling phase. It seems unlikely that the repolarisation is caused by inactivation of the calcium conductance, as calcium inactivation in other systems seems to be too slow^{3,18,19}. We have tested for a possible role for a chloride conductance by replacing all the NaCl in the TEA Ringer with sodium isethionate, but we found no effect on the oscillations. We suggest that, in addition to a calcium conductance, the rod membrane contains a voltage-dependent or calcium-activated potassium conductance, which is only partially blocked by external TEA.

We have no evidence as to the location of the presumed calcium channels in the rod membrane or their possible functional significance, though we suppose that at least part of the calcium conductance is involved in synaptic transmitter release. Our recordings are probably made from the outer segment of the rod^{20–22}, but it is possible that the oscillations are generated near the synaptic terminal and spread passively to the distal end of the receptor.

We thank Mr Bernard Lacaille for constructing the perfusion chamber and photostimulator and Dr S. Hagiwara for reading a draft of the manuscript. This investigation began in Paris with the assistance of an exchange fellowship to G.L.F. from Harvard University and The Institut National de la Santé et de la Recherche Médicale, France. The work was supported by grants from the DGRST (74-7-0385) and CNRS (ATP 1288) to H.M.G.; from the Norton Simon Foundation, Fight For Sight, Inc. and the NIH (EY 01844) to G.L.F.; and by a postdoctoral traineeship (EY 7026) to F.N.Q.

Note added in proof: Measurements of the current-voltage curve of rods in normal Ringer show a region of outward-going rectification positive to the dark membrane potential which is markedly reduced in 12 mM TEA. Calcium-dependent regenerative responses have also been recorded from the presynaptic terminal of barnacle photoreceptors²³.

GORDON L. FAIN
FRED N. QUANDT

Jules Stein Eye Institute,
UCLA School of Medicine,
Los Angeles, California 90024

HERSCH M. GERSCHENFELD

Laboratoire de Neurobiologie,
Ecole Normale Supérieure,
Paris, France 75005

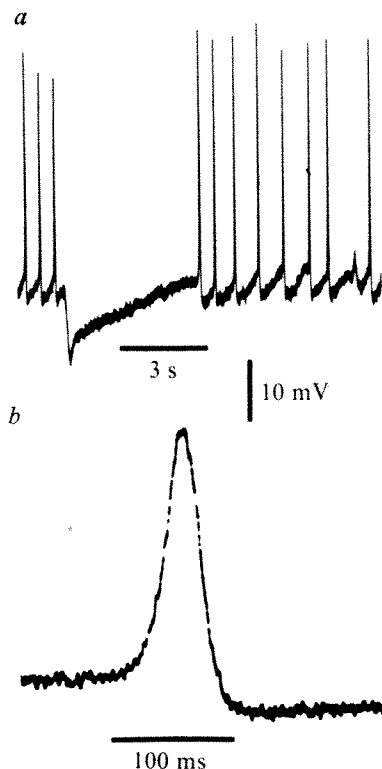


Fig. 2 *a*, Action potentials recorded from a toad rod during superfusion with Ringer containing 3.6 mM Ca^{2+} (twice normal concentration), no Mg^{2+} , and 12 mM TEA. Spikes are spontaneous in the dark but cease during the response to a 109-ms 516-nm, full-field flash of intensity $9.8 \log$ quanta per cm^2 per flash. *b*, Action potential from cell of Fig. 2a on an expanded timescale.

Received 31 May; accepted 8 August 1977.

1. Narahashi, T. *Physiol. Rev.* **54**, 813–889 (1974).
2. Baker, P. F. & Glitsch, H. G. *Phil. Trans. R. Soc. B* **270**, 389–409 (1975).
3. Hagiwara, S. *Adv. Biophys.* **4**, 71–102 (1973); in *Membranes—A Series of Advances* 3 (ed. Eisenman, G.) 359–381 (Marcel Dekker, New York, 1974).
4. Fain, G. L. *Science* **187**, 838–841 (1975); *J. Physiol., Lond.* **261**, 71–101 (1976).
5. Brown, J. E. & Pinto, L. H. *J. Physiol., Lond.* **236**, 575–591 (1974).
6. Pelhate, H. & Pichon, Y. *J. Physiol., Lond.* **242**, 90 P (1974).
7. Fain, G. L., Gold, G. H. & Dowling, J. E. *Cold Spring Harb. Symp. quant. Biol.* **40**, 547–561 (1976).
8. Fatt, P. & Katz, B. *J. Physiol., Lond.* **120**, 171–204 (1953).
9. Fatt, P. & Ginsborg, B. L. *J. Physiol., Lond.* **142**, 516–543 (1958).
10. Katz, B. & Miledi, R. *J. Physiol., Lond.* **203**, 459–487 (1969).
11. Yoshikami, S. & Hagins, W. A. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 245–255 (Springer, Berlin, 1973).
12. Hagins, W. A. & Yoshikami, S. *Expl Eye Res.* **18**, 299–305 (1974).
13. Weakly, J. N. *J. Physiol., Lond.* **234**, 597–612 (1973).
14. Baker, P. F., Meves, H. & Ridgway, E. B. *J. Physiol., Lond.* **231**, 511–526 (1973).
15. Normann, R. A. & Pochobradsky, J. *J. Physiol., Lond.* **261**, 15–29 (1976).
16. Cervetto, L. & MacNichol, E. F. Jr *Science* **178**, 767–768 (1972).
17. Murakami, M., Ohtsuka, T. & Shimazaki, H. *Vision Res.* **15**, 456–458 (1975).
18. Katz, B. & Miledi, R. *J. Physiol., Lond.* **216**, 503–512 (1971).
19. Keynes, R. D., Rojas, E., Taylor, R. E. & Vergara, J. *J. Physiol., Lond.* **229**, 409–455 (1973).
20. Toyoda, J.-I., Nosaki, H. & Tomita, T. *Vision Res.* **9**, 453–463 (1969).
21. Murakami, M. & Pak, W. L. *Vision Res.* **10**, 965–975 (1970).
22. Toyoda, J., Hashimoto, H., Anno, H. & Tomita, T. *Vision Res.* **10**, 1093–1100 (1970).
23. Ross, W. N. & Stuart, A. E. *J. Physiol., Lond.* (in the press).

Ionic interactions in the potassium channel of squid giant axons

ONE of the simplest questions that can be asked about how ions cross biological membranes is whether or not they do so independently of one another. That is, is the probability of movement of an ion unaffected by the presence of other ions? In particular, with independent movement, the efflux of an ion should not be a function of the external concentration of that ion. Using long-lasting depolarisations, Hodgkin and Keynes demonstrated¹ non-independent movement of K^+ ions across the membrane of *Septa* axons. Since the Na channel responsible for initiation of the action potential inactivates very rapidly on depolarisation, potassium ions were probably not moving through this pathway—even though the channel is permeable to them as well as to Na^+ ions. But since the K channels (partly responsible for termination of the action potential) also inactivate on prolonged depolarisation², the K efflux measured by Hodgkin and Keynes was presumably mainly through inactivated K channels and the so-called 'leakage pathway'. We report here on experiments that demonstrate non-independent ion movement through the activated K channel of the giant axon of *Loligo pealei*, using tetrodotoxin to block the Na channels, and short-step depolarisations to minimise K-channel inactivation.

Techniques for membrane voltage measurement and control have been described previously³. The axons were perfused internally with a solution containing 400 mM potassium labelled with ^{42}K . The artificial seawater (ASW) bathing the axons contained various K concentrations up to 75 mM. All experiments were done with a holding potential of -78 mV, and the data obtained with 20–40-mV depolarisations lasting for 20–30 ms. These pulses opened a sufficient number of K channels to produce measurable flux rates, yet were small enough to minimise K loading of the periaxonal space. Even so, some K loading into the 5 or 20 mM K-ASW solutions was detected from the reversal potential at the end of the depolarising pulse. To obtain a 'mean' value of external K during the pulse, we averaged the nominal K concentration in the ASW and the value computed from the reversal potential. Very little loading was observed for the 'high K' solutions. More refined averaging procedures would not alter the conclusions of the study.

Figure 1 shows ^{42}K efflux into ASW containing either 20 or 5 mM K. As expected, resting ^{42}K efflux from this hyperpolarised axon ($2\text{--}4\text{ pmol cm}^{-2}\text{ s}^{-1}$) was lower than that reported for axons at rest^{4,5}. For a period of 7.5 min the membrane voltage was clamped once every 300 ms to a potential of -53 mV for 30 ms. The isotope efflux quickly increased during this period to a constant value, and rapidly returned to the low values following cessation of the pulses. With the membrane potential still maintained at -78 mV, the external solution was then changed to one containing only 5 mM K. After a 7.5-min control period, the same stimulation procedure was repeated. The efflux during repetitive

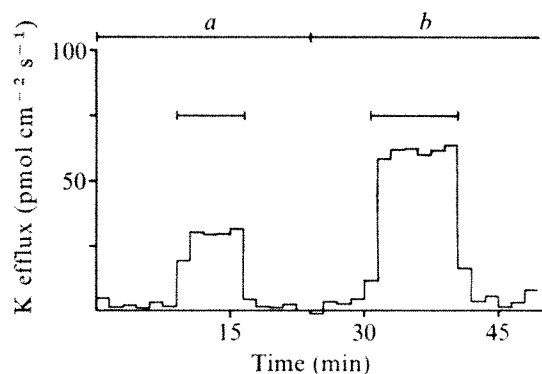


Fig. 1 Potassium efflux from an axon perfused internally with a solution containing 400 mM K^+ (labelled with ^{42}K) into artificial seawater (ASW) containing 20 (a) or 5 (b) mM K^+ . The perfusion solution contained (in mM): K glutamate, 320; KF, 50; sucrose 260; K phosphate, 30; pH 7.3. Perfusion pressure was produced by a motor-driven syringe delivering about $2\text{ }\mu\text{l min}^{-1}$. ASW composition was as follows (in mM): NaCl + KCl, 430; $MgCl_2$, 25; $MgSO_4$, 25; $CaCl_2$, 10; EDTA, 0.2; Tris-HEPES (pH 7.8), 5. ASW was delivered, at 2.7 ml min^{-1} , to the centre of the experimental chamber (volume ~ 0.5 ml) by a peristaltic pump, removed by two pumps (0.9 ml min^{-1} each) through outlets located 3 mm on either side of the inlet, and collected in 1.5-min aliquots. The remainder of the flow was removed by suction through outlets located at the outermost edges of the chamber, and discarded; this was done in order to prevent collection of ^{42}K originating from imperfectly clamped regions of the axon ('end-effects'). The axon membrane voltage was held at -78 mV throughout the experiment, except during the periods indicated by the horizontal bars, when the axon was depolarised by a 25-mV, 30-ms rectangular pulse every 300 ms.

depolarisation was about twice that in the 20-mM solution; any spontaneous deterioration of the axon could only have caused an underestimation of this difference. Our voltage-clamp records showed little or no effect of external potassium on the kinetics of opening or closing of the K channels.

Figure 2 shows a similar record for another fibre, first in 50 mM K and then in 20 mM K-ASW, depolarised once every 200 ms by 40 mV for 30 ms. Since many more K channels are opened at -38 mV than at -53 mV, extra efflux here was much larger than that in the experiment of Fig. 1. Again, the efflux was greater into the solution containing the lower K concentration. After about 45 min, 2 mM 4-aminopyridine was added to the ASW. This compound is known to block the 'late' (that is, potassium) channels of giant axons^{6,7}. In the present experiment, the late outward current was reduced to less than 10% of control; extra ^{42}K efflux was also reduced to very low levels by this treatment, suggesting that a very large proportion of the measured flux in our experiments took place through K channels rather than 'leakage' pathways.

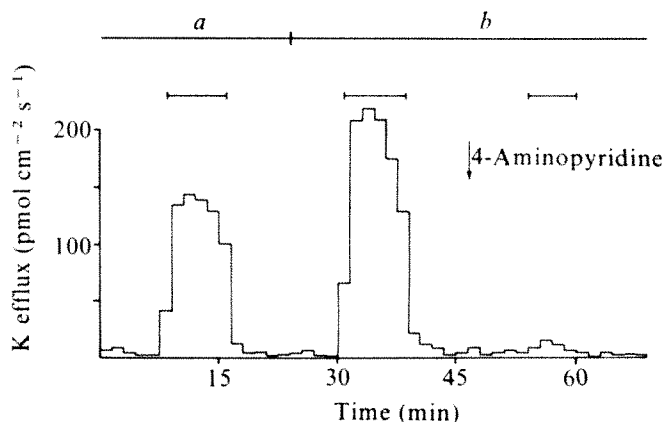


Fig. 2 Effect of external potassium ions and 4-aminopyridine on ^{42}K efflux. Experimental procedures were as in Fig. 1. Holding potential was -78 mV throughout the experiment. During the periods indicated, the axon was depolarised by a 40-mV, 30-ms rectangular pulse every 200 ms. a, 50 mM K^+ ; b, 20 mM K^+ .

The results of our experiments are summarised in Fig. 3. The ^{42}K efflux (normalised to the value for 20 mM K-ASW) is plotted against external K concentration for two values of the depolarising pulse (30 and 40 mV). In both cases ^{42}K efflux decreases with increasing external [K], in violation of the independence principle. The relative inhibitory effect of external K on ^{42}K efflux, however, seems stronger for smaller depolarising pulses. (In additional experiments with 20-mV pulses, this trend was continued.)

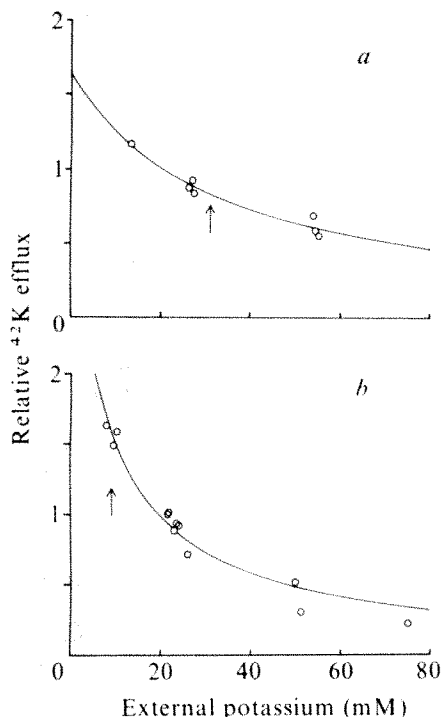


Fig. 3 Relative inhibition, by potassium in the seawater, of ^{42}K efflux through K channels of squid giant axon, for two different clamping pulse sizes. (See Fig. 1 for experimental procedures.) For each of the two pulse sizes: 40 mV (a), 30 mV (b), the fluxes were scaled to unity at 20 mM external K. Both sets of data were adequately fitted by simple adsorption isotherms of the form

$$\text{Flux} = \text{Flux}_{\text{max}} K_i / (K_i + [\text{K}]_o)$$

where K_i is the dissociation constant of a monomolecular reaction between external potassium and some site in the K channel, which results in blockage of ^{42}K efflux. The respective K_i values are indicated by arrows on the graphs. The apparent affinity of the channel site for external potassium is higher at the more negative clamping potential. This trend was continued in a few experiments (not shown) with 20-mV pulses (at -58 mV clamping potential).

The nature of the interaction of external K^+ ions with the K channel of squid axon membrane is unknown. Our data are consistent with the single-file model described by Hodgkin and Keynes for K^+ flux across steadily depolarised *Sepia* nerve membrane, but more work is needed to test detailed models. The most economical description of our data is to say that the activated K channel contains a site which, when occupied by external K, prevents efflux of internal ^{42}K . The dissociation constant of the potassium-site complex is of the order of 10^{-2} M, and decreases with increasing membrane potential.

This work was supported by the US NIH.

TED BEGENISICH

Department of Physiology,
University of Rochester,
School of Medicine and Dentistry,
Rochester, New York 14642

PAUL DE WEER

Department of Physiology and Biophysics,
Washington University,
School of Medicine,
St Louis, Missouri 63110

Received 20 June; accepted 8 August 1977.

1. Hodgkin, A. L. & Keynes, R. D. *J. Physiol., Lond.* **128**, 61–88 (1955).
2. Ehrenstein, G. & Gilbert, D. L. *Biophys. J.* **6**, 533–566 (1966).
3. Begenisich, T. & Lynch, C. J. *gen. Physiol.* **63**, 675–689 (1974).
4. Caldwell, P. C. & Keynes, R. D. *J. Physiol., Lond.* **154**, 177–189 (1960).
5. Brinley, F. J., Jr & Mullins, L. J. *J. Neurophysiol.* **28**, 526–544 (1965).
6. Meves, H. & Pichon, Y. *J. Physiol., Lond.* **251**, 60–62P (1975).
7. Yeh, J. Z., Oxford, G. S., Wu, C. H. & Narahashi, T. *gen. Physiol.* **68**, 517–536 (1976).

Permeant cations alter endplate channel characteristics

At amphibian neuromuscular junctions, the binding of acetylcholine to postsynaptic receptors opens endplate channels. An endplate current (e.p.c.) is generated as sodium and potassium ions flow through these channels according to their electrochemical gradients¹. In normal conditions the e.p.c. is a net inward current of predominantly sodium ions. It has been shown that the lifetime of endplate channels is affected by temperature^{2,3} and membrane potential³, depends on the nature of the cholinergic agonist^{4,5} and can be influenced by various chemicals and drugs^{6,7}. But, it is not known what normally determines the lifetime and conductance of an open channel. We report here that the nature of the permeating cations that generate the e.p.c. affects the lifetime and conductance of endplate channels. These observations indicate that these cations influence the mechanisms that determine channel behaviour.

Endplate channel characteristics were compared for three different permeating cations—sodium, lithium and caesium. Normal extracellular solution (sodium solution) contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 and 3 mM phosphate buffer. Lithium and caesium solutions were made by replacement of NaCl with 115 mM LiCl or 115 mM CsCl respectively. The pH of all solutions was 7.2 and experiments were performed at 20 °C.

Spontaneous miniature end-plate currents (m.e.p.cs) and current fluctuations (noise) generated by iontophoresis of acetylcholine were recorded in voltage-clamped fibres in sartorius muscles of toads (*Bufo marinus*) using techniques described previously^{8,9}. In most cases muscles were glycerol-treated to prevent contraction when fibres were depolarised. For analysis of acetylcholine noise, 8,192 or 16,384 data points, filtered by two low-pass, cascaded Butterworth filters in series (40 dB per decade roll-off) with cut-off at 800 Hz were sampled at 2 kHz. A fast Fourier transform method was used to analyse the frequency components of the noise and determination of single channel conductance and average lifetime was based on the model and theory described by Anderson and Stevens³. Lorentzian curves were fitted to data using a least squares fitting method.

In the solution containing lithium (lithium solution), the time constant of decay m.e.p.cs was significantly greater than the normal extracellular solution. This is illustrated in Fig. 1. Averages of 20 m.e.p.cs in normal (sodium) solution and 20 m.e.p.cs in lithium solution are shown in Fig. 1a. These m.e.p.cs were recorded in a fibre voltage-clamped at a membrane potential (V_m) of -70 mV. The decay phase remained exponential in lithium solution as is clear from the semilog plots of the decay phases of the same currents shown below (Fig. 1b). In this experiment the average decay time constant (τ_d) was 2.5 ms in sodium solution and 3.3 ms in lithium solution. The lengthening of m.e.p.cs seen in lithium solution could be reversed by returning to sodium solution. In addition to the increase

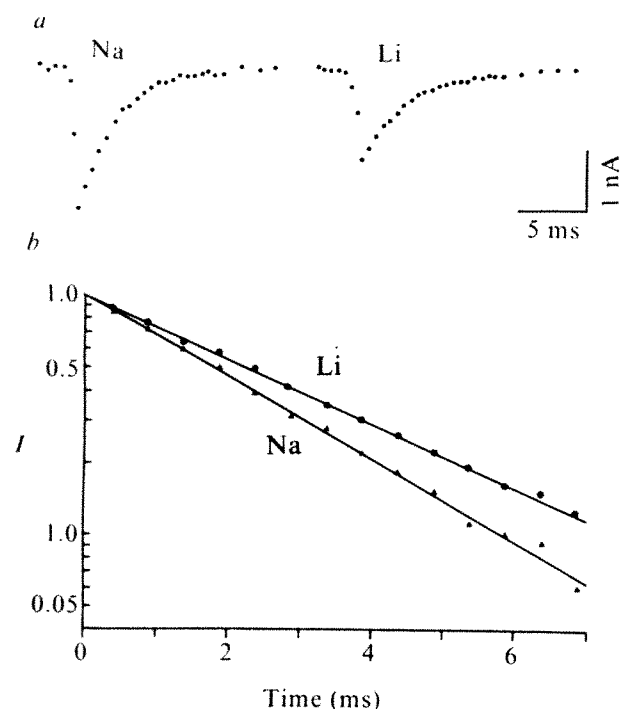


Fig. 1 Miniature endplate currents recorded in a muscle fibre voltage-clamped at -70 mV in sodium (Na) and lithium (Li) solutions. *a*, Averages of 20 m.e.p.cs in the two solutions. *b*, Semilogarithmic plots of the decay of the averaged m.e.p.cs (note normalised peak amplitudes) in sodium (Δ) and lithium (\bullet) solutions. The fitted lines are regression lines (correlation coefficients 0.999 and 0.998) and gave time constants of 2.5 ms and 3.3 ms in the sodium and lithium solutions respectively. (Temperature 20°C .)

in decay time constant, there was a decrease in the average amplitude of m.e.p.cs in this experiment from 2.3 ± 0.09 nA in the sodium solution to 1.46 ± 0.06 nA in lithium solution (mean \pm s.e.m.). This depression of m.e.p.c. amplitude was also reversible. The reduction in m.e.p.c. amplitude could not be explained by changes in acetylcholine null (reversal) potential (ϵ_s). ϵ_s was directly measured in each of the solutions by determining the potential at which there was no increase in current when acetylcholine was applied iontophoretically to an endplate. In seven fibres in lithium solution, the null potential was -6.1 ± 1.1 mV and in 15 fibres in sodium solution the null potential was -2.9 ± 0.5 mV. The increase in the time constant of decay and decrease in amplitude of m.e.p.cs caused by lithium were consistent with the increased lifetime and decreased conductance of individual endplate channels as determined by analysis of acetylcholine current noise. Logarithmic plots of spectral density against frequency obtained at the same endplate are shown in Fig. 2 for sodium (Na) and after exposure to lithium solution (Li). In this fibre, voltage-clamped at -70 mV, the average lifetime of endplate channels (τ_n), calculated from $\tau_n = 1/2\pi f_c$ (f_c = half power frequency) was 1.5 ms in sodium solution and 2.5 ms in lithium solution. The current flow through individual channels (γ_i), determined both from the current spectral density at low frequencies and from the variance of current noise, was found to be reduced in lithium solution. Using the measured null potential, γ_c was calculated from $\gamma_c = \gamma_i / (V_m - \epsilon_s)$. Results obtained in several experiments are shown in Table 1. It can be seen that lithium caused a significant reduction in γ_c from 26.7 pS to 17.7 pS and this probably explains the reduction in m.e.p.c. amplitude caused by lithium (Fig. 1).

In experiments in which sodium chloride was replaced with caesium chloride in the extracellular solution, m.e.p.cs were increased in amplitude and their time constant of

decay was reduced. The acetylcholine null potential in caesium solution was 0.1 ± 0.9 mV (measured in five fibres). From analysis of acetylcholine noise it could be shown that the increase in amplitude and reduction in the time constant of m.e.p.cs in caesium were associated with changes in endplate channel characteristics. In one fibre exposed first to sodium, then to caesium solution the average lifetime of endplate channels was reduced from 1.21 ms to 0.89 ms while endplate channel conductance increased from 28.2 to 35.0 pS (clamp potential -50 mV). In four fibres voltage-clamped at -50 mV in caesium solution, average endplate channel lifetime was 0.98 ± 0.05 ms and endplate channel conductance was 34.7 ± 2.1 pS from power spectra and 33.3 ± 2.6 pS from noise variance.

The results indicate that these cations which must be passing through open endplate channels to generate the endplate current, change the conductance and lifetime of the channels. The sequence of average channel conductances was $\text{Li} < \text{Na} < \text{Cs}$ and the conductance ratios were $0.7 : 1 : 1.3$. Null potentials also became more positive in accord with this sequence. It is interesting and perhaps significant that the sequence of mobilities of the ions in free solution is also $\text{Li} < \text{Na} < \text{Cs}$ with free solution mobility ratios¹⁰ of $0.77 : 1 : 1.56$ at 20°C . If the conductance of an endplate channel is determined by free-solution mobility of ions flowing through it, the channel may well be a relatively large, water filled pore. Of course, the similarity between the channel conductance, null potentials and mobility sequences may be only a coincidence. It could be that ions alter endplate channel conductance by directly affecting the structure that forms the channel and this explanation might apply also for the effects of the ions on endplate channel lifetime. The sequence of channel lifetimes was $\text{Li} > \text{Na} > \text{Cs}$ and the lifetime ratios were $1.4 : 1.0 : 0.7$. Interestingly, there is a reciprocal relationship between channel lifetime and conductance (at least with the three ions tested). This may also be coincidental and the changes in channel lifetime may reflect separate and independent effects of these ions on the mechanism that determines channel lifetime. Whatever the mechanism and

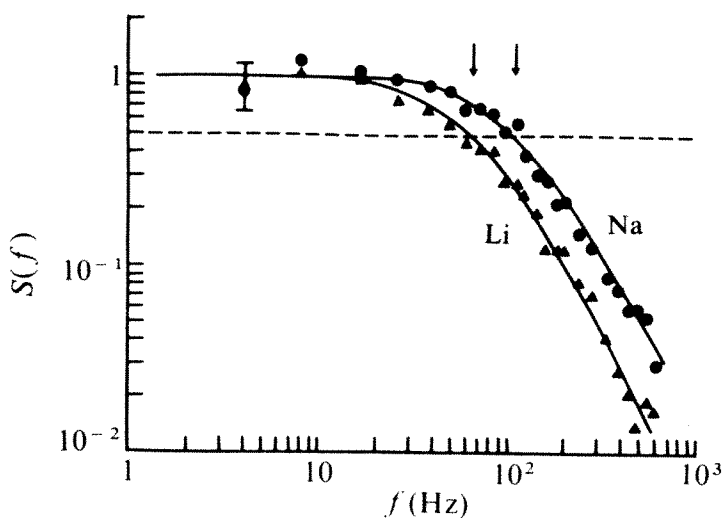


Fig. 2 Plots of normalised current spectral density $S(f)$ against frequency f (Hz) obtained from a fibre voltage-clamped at -70 mV in sodium solution (\bullet) and after 30 min in lithium solution (Δ). $S(f)$ was calculated at 4-Hz intervals from the ensemble average of spectra from 16 segments of noise containing 512 points sampled at 2 kHz (cut-off filter 800 Hz, 3dB down). The downward and upward error bars show 1 s.e.m. at 4 Hz for the Na and Li curves respectively. Points at frequencies above 8 Hz are a segment average of adjacent values of $S(f)$. The solid lines were obtained by least squares fits of the points to a single Lorentzian and the arrows denote the half-power frequencies (63 Hz in Li solution, 107 Hz in Na solution). Spectral densities were normalised for comparison. Asymptotic spectral densities were 0.18×10^{-21} A²s (lithium) and 0.32×10^{-21} A²s (sodium).

Table 1 The average lifetime (τ_n) and conductance (γ_g) of endplate channels in muscle fibres voltage-clamped at -50 mV in solutions containing 115 mM LiCl, NaCl or CsCl

Solution	Li	Na	Cs
n	7	7	4
τ_n (ms)	1.91 ± 0.07	1.38 ± 0.11	0.98 ± 0.05
γ_g (pS)	17.7 ± 1.8 (16.7 ± 1.5)	26.7 ± 0.80 (26.2 ± 1.1)	34.7 ± 2.1 (33.3 ± 2.6)

Results were obtained from power density spectra except for the values of γ_g in brackets which were calculated from noise variance. An average ± 1 s.e.m. was obtained from n experiments. (Temperature 20°C .)

sites of action of these permeant cations, it is clear that they have significant effects on endplate channels. An understanding of the cause of these effects should provide further information about processes that determine endplate channel lifetime and conductance.

This work was supported by a grant from the National Health and MRC of Australia. D.V.H. was supported by a grant from the Clive and Vera Ramaciotti Foundation. We thank Dr P. H. Barry, K. Takeda and D. J. Adams for useful discussions.

D. VAN HELDEN
O. P. HAMILL
P. W. GAGE

School of Physiology and Pharmacology,
University of New South Wales,
Kensington, New South Wales 2033,
Australia

Received 10 June; accepted 12 August 1977.

1. Takeuchi, A. & Takeuchi, N. *J. Physiol., Lond.* **154**, 52–67 (1960).
2. Katz, B. & Miledi, R. *J. Physiol., Lond.* **244**, 665–699 (1972).
3. Anderson, C. R. & Stevens, C. F. *J. Physiol., Lond.* **235**, 655–691 (1973).
4. Katz, B. & Miledi, R. *J. Physiol., Lond.* **230**, 707–717 (1973).
5. Colquhoun, D., Dionne, V. E., Steinbach, J. H. & Stevens, C. F. *Nature* **253**, 204–206 (1975).
6. Colquhoun, D. *A. Rev. Pharmac.* **15**, 307–325 (1975).
7. Gage, P. W. *Physiol. Rev.* **56**, 177–247 (1976).
8. Gage, P. W. & McBurney, R. N. *J. Physiol., Lond.* **244**, 385–407 (1975).
9. Gage, P. W., McBurney, R. N. & Van Helden D. J. *J. Physiol., Lond.* (in the press).
10. Robinson, R. A. & Stokes, R. H. *Electrolyte Solutions* (Butterworths, London 1959).

Evidence for the Clara cell as a site of cytochrome P450-dependent mixed-function oxidase activity in lung

THE cellular localisation of pulmonary mixed-function oxidase (MFO) activity has aroused considerable interest, especially because of the increasing incidence of lung disease of environmental origin—including cancers—and the realisation that many chemical toxins and carcinogens require MFO-catalysed activation. The precise identification of pulmonary cellular populations possessing MFO activity has previously met with little success, however, probably because of the complexity and heterogeneity of the lung, which has been estimated to contain over 40 different specific cell types¹. In this communication I describe studies with the pulmonary toxin, 4-ipomeanol (1-[3-furyl]-4-hydroxypentanone)², which indicate that a highly reactive metabolite of this naturally occurring furan derivative is preferentially formed and covalently bound in pulmonary non-ciliated bronchiolar (Clara)³ cells, leading to their necrosis. Since previous studies⁴ have established that the toxic metabolite formed *in vivo* during cytochrome P450-dependent oxidative metabolism of 4-ipomeanol is formed *in situ* in the lung, and does not reach the lung by way of the circulation, the present results indicate that the Clara cell is a primary locus of P450-dependent MFO enzymes in lung.

Covalently bound 4-ipomeanol metabolite was visualised in the lung using an autoradiographic technique and Fig. 1 shows examples of the striking appearance of the autoradiograms. As indicated by the accumulations of black photographic granules,

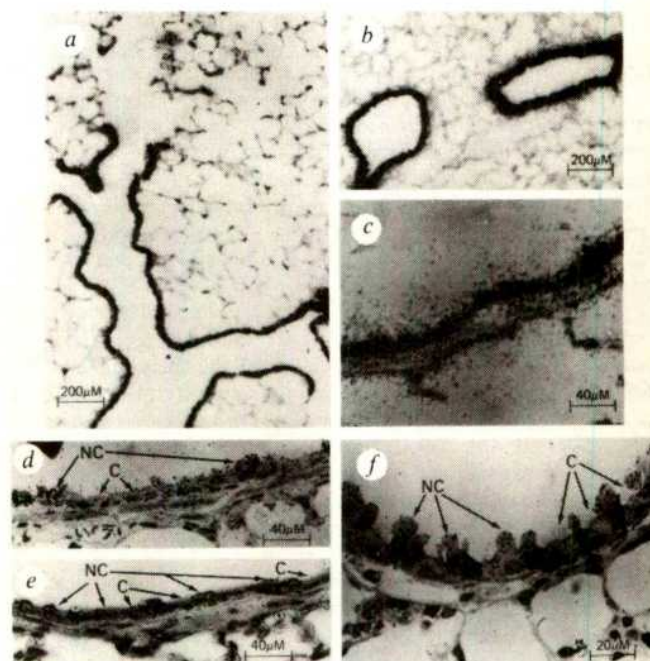


Fig. 1 Representative autoradiograms from lungs of rats (Sprague-Dawley, male, 100–120 g), mice (NIH-GP, male, 20–25 g), and hamsters (Golden Syrian, male, 60–70 g) given toxic doses of radiolabelled 4-ipomeanol (20 mg kg^{-1} rat or 35 mg kg^{-1} mouse and hamster) intraperitoneally. Generally-labelled ^3H -4-ipomeanol (60 mCi mmol^{-1}) was prepared by catalytic ($\text{Rh}/\text{Al}_2\text{O}_3$) tritiation with tritiated water. $^5\text{-}^{14}\text{C}$ -ipomeanol (2 mCi mmol^{-1}) was prepared using $1\text{-}^{14}\text{C}$ -propylene oxide². The animals were killed using pentobarbital 2 or 16 h after receiving the toxin and the lungs fixed *in situ* by intratracheal perfusion with 3% glutaraldehyde buffered with 0.1 M sodium cacodylate. Lungs were removed, cut, washed and dehydrated using a standard ethanol gradient, followed by a final wash with propylene oxide. Radioactivity remaining in the tissue after this procedure represented only the covalently bound 4-ipomeanol metabolite and could not be removed by further washing with aqueous or organic solvents or by organic solvent extraction of samples solubilised in 1 N sodium hydroxide. Samples of the specimens were embedded in paraffin or Epon 812 resin. Sections of 4–5- μm thickness were cut from the paraffin blocks and mounted on glass slides, and 2- μm thick sections were cut from the Epon blocks and mounted similarly. The slides were dipped in fresh Kodak NTB-2 photographic emulsion and placed in the dark for 2 or 4 weeks for tritium or ^{14}C -labelled samples, respectively. The exposed slides were developed and stained with toluidine blue. Frames a, b, c and d are rat lungs, frame e is hamster lung, and frame f is mouse lung. NC (arrows) identify non-ciliated bronchiolar (Clara) cells, and C (arrows) identify ciliated bronchiolar cells. The lung specimens were taken from animals 2 h after dosing with the toxin. Specimens shown in a, b, c and e were embedded in paraffin, the others were embedded in Epon resin. Scale bars: a, b, 200 μm ; c, d, e, 40 μm ; f, 20 μm .

the covalently-bound 4-ipomeanol metabolite was heavily concentrated in the lining cells of the airways. Similar results were obtained with both tritium and ^{14}C -labelled 4-ipomeanol, indicating that the binding did indeed represent covalently-bound metabolite, as previous biochemical studies have indicated, and was not due to nonspecific tritium exchange or carbon incorporation. Comparison of frames a, b and c from animals receiving the ^{14}C -labelled toxin, with d, e, and f from animals receiving the tritiated toxin, however, illustrates that greater resolution can be obtained using the tritium derivative, especially combined with Epon embedding and thinner sectioning. For example, in c, where the ^{14}C derivative was used, there seems to be some specific, but not precisely definable, cellular labelling; in d, e and f, however, it is possible to distinguish between the several types of bronchiolar lining cells³ and to appreciate the cellular specificity of the labelling.

In all three animal species tested, the Clara cells³ of the small airways were specifically alkylated by 4-ipomeanol (Fig. 1 d, e, f). In addition, in other specimens taken from animals 16 h after dosing with the toxin, a striking necrosis of the Clara cells was seen, whereas the adjacent ciliated bronchiolar cells, and the other major

pulmonary parenchymal cells were not necrotic (Fig. 2). Electron microscopic studies have confirmed these findings. It thus seems that the Clara cell is a specific cellular target of 4-ipomeanol. It is likely that pathological features of 4-ipomeanol poisoning which have been described previously in cattle⁵ and in experimental animals⁴, including pulmonary oedema, congestion and haemorrhage, are not primary events, but instead represent secondary or tertiary pathological changes. 4-Ipomeanol may serve as a useful tool for the elucidation of pathophysiological events related to Clara cell damage as well as the normal physiological role of the Clara cell.

To determine whether metabolism of 4-ipomeanol was required to produce the tissue-bound radioactivity, animals were pretreated with piperonyl butoxide, an inhibitor of the metabolism of 4-ipomeanol by both hepatic and pulmonary mixed-function oxidases⁴, before administration of the radiolabelled toxin. Histological examination of the lungs from these animals revealed a striking decrease in covalently-bound radioactivity in the bronchiolar cells (Fig. 3a, b) and the total absence of bronchiolar necrosis (not shown), even though both the blood and the pulmonary concentrations of the unmetabolised toxin were markedly elevated by the pretreatment (Fig. 3c). This experiment further illustrates that a highly reactive metabolite of 4-ipomeanol, which binds covalently with the target cells and which can be localised by the technique described here, is responsible for the Clara cell necrosis.

In studies of the dose dependency of bronchiolar binding and necrosis by 4-ipomeanol, it was shown that the sensitivity to the toxin decreases as airway size increases. With minimally toxic doses, covalently-bound radioactivity was maximally concentrated in the smallest airways and the subsequent development of bronchiolar necrosis was restricted to those areas. As doses were increased, proportionately larger airways were also labelled and eventually showed bronchiolar cell necrosis. It is possible that the seemingly greater sensitivity of the smaller airways is related to the fact that the relative proportion of Clara cells to the less sensitive ciliated bronchiolar cells increases as airway size decreases³.

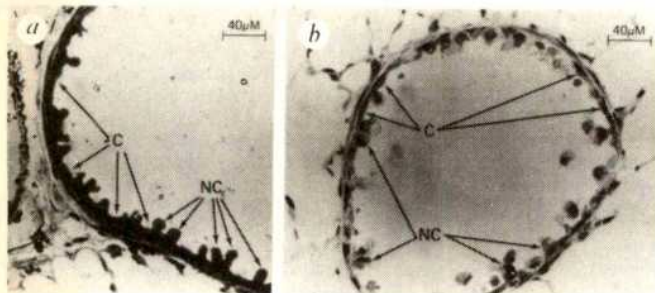


Fig. 2 a, Normal mouse bronchiole with well defined ciliated (C) and non-ciliated, or Clara (NC) cells. b, A typical pulmonary bronchiole from a mouse 16 h after i.p. administration of non-radiolabelled 4-ipomeanol (35 mg kg^{-1}). In b, the necrosis of Clara cells is apparent, and, although abnormal, ciliated cells still seem to be viable. Scale bars represent $40 \mu\text{m}$.

Previous attempts to localise pulmonary MFO *in vivo* have been inconclusive. Some investigators have suggested an alveolar cell locus⁶, and others have indicated that a bronchial cell localisation was likely⁷. One report indicated that pulmonary macrophages have little MFO activity⁸. Reid *et al.*⁹ found that bromobenzene, in addition to its toxic effects on the liver, also produces pulmonary bronchiolar necrosis that seems to be related to covalent binding to bronchioles of a reactive metabolite of the compound⁹. The reactive metabolite of bromobenzene, however, seems to be sufficiently stable to escape from its site of formation⁹. Thus, with this compound it was not possible to determine if the toxic metabolite binding to lung was formed in the liver or in the lung, or in both, and neither was it possible to determine the specific type of

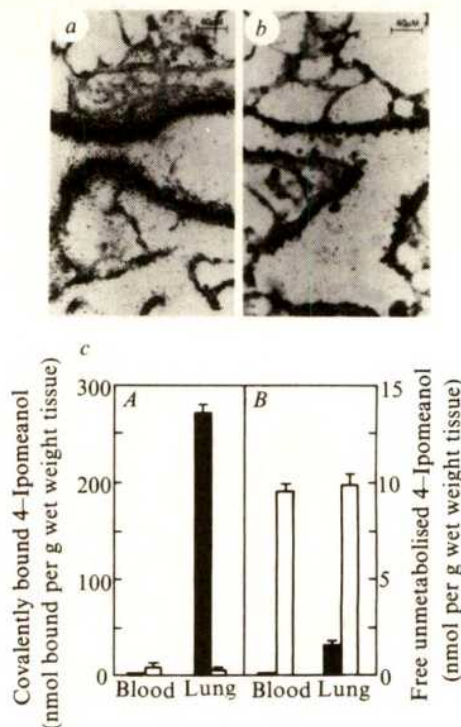


Fig. 3 Effect of piperonyl butoxide pretreatment ($1,600 \text{ mg kg}^{-1}$, i.p. 1 h previously) on bronchiolar alkylation (a, and b) and on blood and pulmonary levels of covalently-bound (solid columns) and free 4-ipomeanol (open columns) (c) in the rat. All tissues were removed and processed 2 h after administration of the ^{14}C -labelled toxin (20 mg kg^{-1} , i.p.). Covalently-bound toxin was assayed as described in ref. 4; free 4-ipomeanol was isolated using procedures described in ref. 2 and quantitated by liquid scintillation counting. Data in frame c are means ($\pm \text{S.E.M.}$) from groups of seven animals each. A, Control rats; B, treated rats. Scale bars represent $40 \mu\text{m}$.

bronchiolar cell to which the metabolite was bound, and which type of cell eventually became necrotic.

The physiological role of the Clara cell is unknown. Some argue that it is a source of pulmonary surfactant, whereas others feel its primary role is as a participant in the 'mucociliary elevator'. Pulmonary anatomists, however, have speculated that based on morphological features the Clara cell is metabolically a very active cell, with secretory functions. It is known to be richly endowed with smooth endoplasmic reticulum (SER)³ and this may be significant in the light of the present investigations, as SER is associated with MFO-activity in other cells, such as hepatocytes¹⁰. It is also interesting that the covalently bound 4-ipomeanol observed in the present experiments is most heavily concentrated in the cytoplasmic cap of the Clara cells, which is also the principal location of the SER³; nuclear labelling is minimal (Fig. 1f).

In summary, these studies indicate the Clara cell is an important potential site of oxidative metabolism of xenobiotics in the lung. Pathological changes of Clara cells, such as necrosis, or neoplastic transformation, which are produced by MFO-activated chemicals may therefore be explainable on that basis. Moreover, since the Clara cell possesses enzymes required for metabolic activation of certain chemical carcinogens, it must be considered a prime candidate as a cell of origin for at least some bronchogenic cancers. In continuing studies we hope further to define the biochemical characteristics of normal Clara cells, and to compare these properties with those of pulmonary tumours. The identification of biochemical similarities within specific histological types of human bronchogenic carcinomas, may implicate specific cell(s) of origin, and could possibly have important therapeutic use. In a tissue of relatively heterogeneous cellular composition such as the lung, cell types with enzymes capable of activating specific carcinogens may give rise to tumour cell populations uniquely susceptible to cytotoxins activated by the same enzymes. The possible use of specific bronchiolar alkylating agents, with 4-ipomeanol as the

prototype, should be explored as a new approach to chemotherapy of bronchogenic cancer of the lung.

MICHAEL R. BOYD

Clinical Pharmacology Branch,
National Cancer Institute, and
Section on Clinical Pharmacology,
Laboratory of Chemical Pharmacology,
National Heart, Lung and Blood Institute,
Bethesda, Maryland 20014

Received 14 June; accepted 9 August 1977.

1. Sorokin, S. P. in *Morphology of Experimental Respiratory Carcinogenesis* (eds Nittesheim, P., Hanna, M. G., Jr & Deatherage, J. W., Jr) 3-41 (US Atomic Energy Commission, Washington, DC, 1970).
2. Boyd, M. R., Burka, L. T., Harris, T. M. & Wilson, B. J. *Biochim. biophys. Acta* **337**, 184-195 (1974).
3. Jeffery, P. K. & Reid, L. J. *Ann.* **120**, 295-320 (1975).
4. Boyd, M. R. *Env. Hlth Persp.* **16**, 127-138 (1976).
5. Peckham, J. C., Mitchell, F. E., Jones, O. H. & Douppnik, B. J. *Am. J. Med. Assoc.* **160**, 169-172 (1972).
6. Wattenberg, L. W. & Leong, J. L. *J. Histochem. Cytochem.* **10**, 412-420 (1962).
7. Grasso, P., Williams, M., Hodgson, R., Wright, M. G. & Gangoli, S. D. *Histochem. J.* **3**, 117-126 (1971).
8. Hsok, G. E. R., Bend, J. R. & Fouts, J. R. *Biochem. Pharmacol.* **21**, 3267-3277 (1977).
9. Reid, W. D., Ilett, K. F., Glick, J. M. & Krishna, G. *Am. Rev. resp. Dis.* **107**, 539-551 (1973).
10. Fouts, J. R. *Biochim. biophys. Res. Commun.* **6**, 373-378 (1961).

Identification of a membrane-embedded segment of the large polypeptide chain of (Na⁺, K⁺)ATPase

THE sodium pump or (Na⁺, K⁺)ATPase couples hydrolysis of ATP to the active transport of Na⁺ and K⁺ across the cell membrane. The firm association of the enzyme with the membrane allows its purification from the outer renal medulla by selective extraction of other membrane proteins using sodium dodecyl sulphate (SDS)¹. Two polypeptide components remain in the purified preparation of (Na⁺, K⁺)ATPase. A catalytic protein with molecular weight (MW) about 100,000 (100 K) which constitutes 60-70% of the total protein and a smaller sialoglycoprotein of unknown function^{2,3}. The arrangement of these proteins within the membrane is an important but poorly understood aspect of the Na pump structure. Presumably the catalytic regions of the enzyme are in contact with the aqueous phase at the membrane surface, while other portions of the protein are in intimate contact with the lipid and account for its firm anchorage. The lipid associated domains of the pump are of special interest because in addition to a purely structural role, they may also form parts of the ion conducting pathways. We have been able to identify presumptive intramembranous portions of the Na pump protein by labelling them covalently from within the membrane with 5-[¹²⁵I]-iodonaphthyl-1-azide (INA). This label is a very hydrophobic light-sensitive compound developed previously for this purpose and tested with other systems⁴⁻⁷. A segment(s) of approximate MW 12 K derived from the large polypeptide chain has been identified by combining the INA-labelling technique with extensive proteolysis of the membrane bound enzyme. Graded proteolysis of the labelled (Na⁺, K⁺)ATPase has also permitted an estimate of the position of this segment within the large chain.

In the dark INA is unreactive and partitions (> 98%) into membranes^{8,9}. Subsequent irradiation at 310 nm (ϵ 21,400) converts INA into a reactive nitrene which inserts covalently into the protein and lipid components of the (Na⁺, K⁺)ATPase preparation (Fig. 1a). The distribution of radioactivity between protein and lipid could be controlled by varying the concentration ratio of label to enzyme. At low INA-to-protein ratios (roughly 0.5 mol INA per mol of enzyme) the label was attached almost exclusively to the large chain (Fig. 1a). In this experiment inhibition of the (Na⁺, K⁺)ATPase activity was less than 10%. The sialoglycoprotein was barely labelled and less than 20% of the radioactivity was associated with the lipid. Evidence for a membrane-bound segment of the large

chain was obtained by extensive digestion of labelled (Na⁺, K⁺)ATPase with trypsin (Fig. 1b) or thermolysin (not shown). In either case radioactivity was transferred essentially quantitatively from the large chain to a fragment(s) of MW about 12 K. After the extensive proteolysis only 45-50% of the original protein remains in the membrane whereas less than 10% of the radioactivity could be released from the membrane by

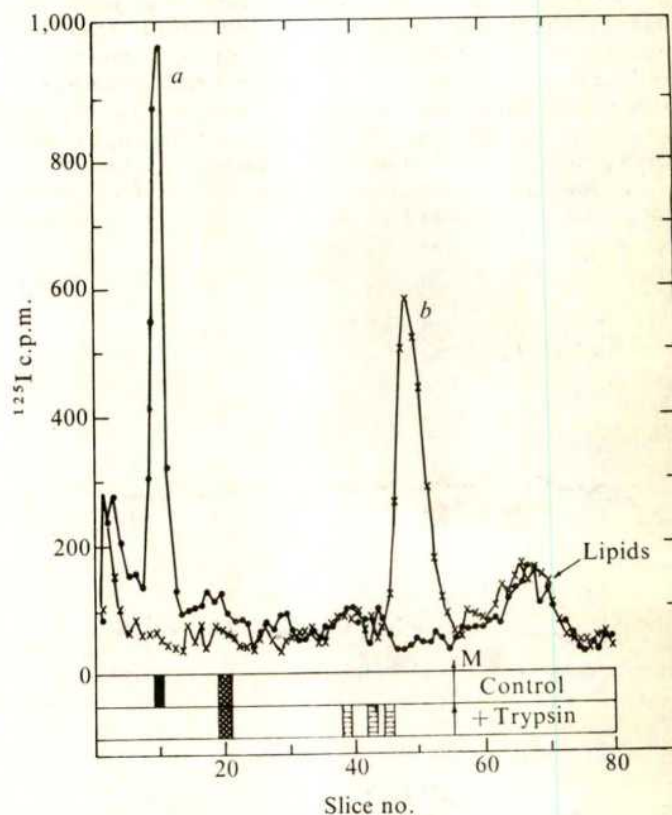


Fig. 1 Labelling of (Na⁺, K⁺) dependent ATPase with 5-[¹²⁵I]-iodonaphthyl-1-azide and effect of extensive trypsinolysis of labelled membranes. 0.6 mg of (Na⁺, K⁺)ATPase prepared from pig kidney¹ (specific activity 32.2 IU per mg protein) was suspended in 1 ml of a medium containing histidine, 25 mM, pH 7.5; EDTA, 1 mM and NaCl 100 mM. 5-[¹²⁵I]-iodonaphthyl-1-azide (0.2-0.3 µg of material with SA 1-3 × 10⁸ c.p.m. per µmol INA) prepared by methods described in ref. 7, was added in the dark in 10 µl of ethanolic solution. The membranes plus label were incubated at 37 °C for 5 min in the dark to allow full partitioning of the INA into the membranes. The suspension was then irradiated for 2 min with light mainly of wavelengths 313 and 365 nm produced by a mercury lamp HB200. The mercury lines below 300 and above 400 nm were excluded by passing the light through a Corning 7-60 band pass filter and Schott WG 305 cut off filter. After irradiation a small sample of the membranes was removed for measurement of (Na⁺, K⁺)ATPase activity¹⁰ and the rest was dialysed overnight at 0 °C against 1 l of solution containing histidine 25 mM, pH 7.5; EDTA 1 mM; bovine serum albumin 1 mg ml⁻¹. Radioactive diffusible byproducts of INA removed by this procedure amounted to about half of the total radioactivity added initially. For extensive proteolysis, portions of dialysed labelled enzyme containing 100 µg of protein were incubated for 1 h at 37 °C in 120 µl of medium containing imidazole 25 mM, pH 7.5, KCl 150 mM, CaCl₂, 1 mM, and trypsin 10 µg Sigma Type III. Then 1 ml of the medium (minus trypsin) was added to trypsinised and control membranes and these were centrifuged at 40,000 r.p.m. for 1 h in a Beckman type 65 rotor. About 10% of the total radioactivity was found in the supernatant, but there was no significant difference between the control and trypsinised enzyme. The pellets were dissolved in 100 µl SDS 2%, mercaptoethanol 1% and the dissolved material electrophoresed on polyacrylamide gels in SDS essentially as described in ref. 10. After staining with Coomassie blue and destaining in methanol acetic acid (see ref. 10) which also removed excess radioactive byproducts, the gels were cut into slices 1 mm thick, and the ¹²⁵I counted. The figure shows the distribution of radioactivity and position of Coomassie blue staining bands in duplicate gels. The arrows and letter M mark the tracking dye front. a, Control; b, following extensive trypsin digestion.

centrifugation. The large polypeptide chain disappeared and peptides with MW 58 K, 23 K, 20 K and 17 K staining with Coomassie blue were observed; staining in the region containing the radioactivity was weak or absent (see Fig. 1). The specificities of trypsin and thermolysin are very different and so it is quite likely that undigested peptides are protected from complete proteolysis by their association with the lipids.

After exposure of the enzyme at a much higher concentration of INA (namely 25–50 mol INA per mol of enzyme) the lipids became labelled to a level 6–8-fold greater than the protein and incorporation was also seen in the glycoprotein. In these conditions the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ activity was almost completely inhibited and radioactivity equivalent to roughly one molecule of INA was incorporated into the large chain per mol of enzyme. Exhaustive trypsinisation of these membranes resulted in essentially the same pattern of the label distribution as shown in Fig. 1b.

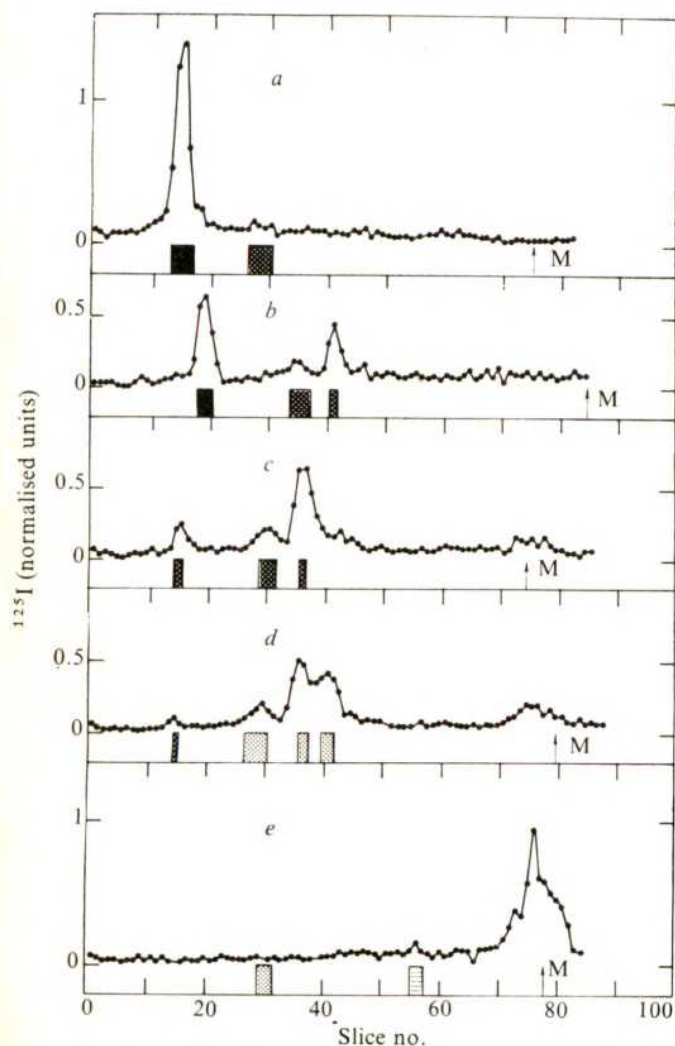


Fig. 2 Distribution of radioactivity in fragments produced by controlled trypsinolysis in K^+ medium of INA labelled $(\text{Na}^+, \text{K}^+)\text{ATPase}$. $(\text{Na}^+, \text{K}^+)\text{ATPase}$ was labelled and dialysed as in Fig. 1. Aliquots containing 50–75 μg were incubated with trypsin at 37°C in a total volume of 0.5 ml in the following conditions: trypsin $\sim 3 \mu\text{g}$; KCl , 150 mM; imidazole 17 mM, pH 7.5; CaCl_2 , 1 mM. In b, CaCl_2 was absent and sample e contained 10 μg of trypsin. Trypsin inhibitor (0.5 ml of $20 \mu\text{g ml}^{-1}$) was added to the control sample (a) before addition of trypsin, and to the other samples after incubation with trypsin for the following times: b, 10 min; c, 15 min; d, 40 min and e, 60 min. The samples were then taken through the same steps as in Fig. 1. In these experiments the gels were run for 8–9 cm to facilitate observation of intermediate radioactive peaks. The lipids have migrated out of the gel into the lower bathing solution. Units of radioactivity have been normalised to account for variations in the total amount of protein applied to the gels in the different samples.

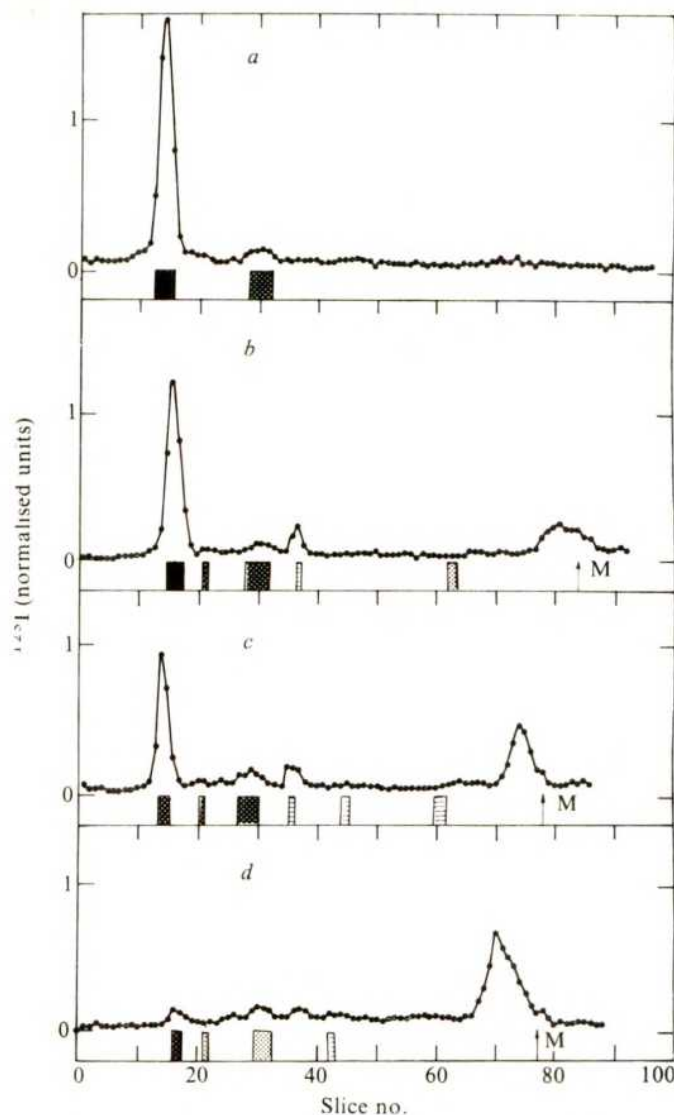


Fig. 3 Distribution of radioactivity in fragments produced by controlled trypsinolysis in a Na^+ medium of INA labelled $(\text{Na}^+, \text{K}^+)\text{ATPase}$. $(\text{Na}^+, \text{K}^+)\text{ATPase}$ was labelled and dialysed as in Fig. 1. Aliquots containing 50–75 μg were incubated with trypsin at 37°C in a total volume of 0.5 ml in the following conditions: trypsin, 3 μg ; NaCl , 150 mM and imidazole 17 mM, pH 7.5. Sample e also contained CaCl_2 1 mM. 0.5 ml of trypsin inhibitor $20 \mu\text{g ml}^{-1}$ was added to the control a before addition of trypsin and to the other samples after the following incubation times; b, 20 min; c, 40 min and d, 60 min. All other procedures were as in Figs 1 and 2.

The preferential insertion into the large chain at low concentrations of INA and inhibition of the $(\text{Na}^+, \text{K}^+)\text{ATPase}$ could imply contrary to expectation, that the label binds specifically to catalytic areas of the protein. Such areas are often hydrophobic clefts in enzyme surfaces. This possibility is unlikely for the following reasons. All catalytic functions are destroyed by relatively limited proteolysis^{8,9} but, as seen above, exhaustive proteolysis did not release the label. Furthermore, binding to the ATP binding site of a fluorescent ATP analogue, formycin triphosphate¹¹ was unaffected by incorporation of sufficient idonaphthyl groups to inhibit activity by 70%, and ATP did not alter incorporation of label into the protein when it was present during irradiation (not shown). We did not study the mechanism of inhibition any further. But, the observation that INA does not interfere with ATP binding is compatible with the notion that it may inhibit catalysis by perturbing protein–lipid interactions, since it has been shown that ATP binding is unaffected after inactivation of $(\text{Na}^+, \text{K}^+)\text{ATPase}$ by complete delipidation¹².

We have examined labelling of the large protein in Na^+ - or K^+ -containing media. The enzyme adopts specific Na^+ - or K^+ -dependent conformations^{8,12-14} and so it was interesting to ask whether these different states are accompanied by a change in accessibility of the protein to INA in the bilayer. In six separate experiments with different concentration ratios of label to protein we observed 10–25% greater incorporation into the large chain in the presence of KCl than with NaCl. This may mean that the conformational change following binding of K^+ leads to an altered relationship of protein to lipid.

In attempts to localise the 12 K fragment we exploited the fact that major fragments of the large chain can be produced by controlled tryptic cleavage in the presence of NaCl or KCl because the conformational response of the protein to cation binding is reflected in exposure of different bonds to tryptic attack^{8,9}. Thus, fragments with MW 58 K and 46 K were formed by primary cleavage of the large chain in presence of KCl. A fragment of 78 K was seen after digestion in the presence of NaCl. The distribution of INA in protein fragments generated by controlled trypsinolysis of enzyme labelled at low INA concentrations is shown in Figs 2 and 3. These results show clearly that the binding of INA does not prevent the conformational response of the large chain to Na^+ and K^+ . In the presence of KCl (Fig. 2) most of the radioactivity of the large chain was transferred to the 46 K fragment, but some labelling of the 58 K fragment was also seen. Comparison of radioactivity in the peaks with the content of protein obtained from absorbance scans of the gels showed that about 80% of the label was transferred to the 46 K fragment and only 20% to the 58 K fragment. Subsequently a 36 K fragment and the terminal 12 K peptide appear. The 46 K and the 36 K fragments therefore include segments which are embedded in the bilayer. The low concentration of label in the 58 K fragment may be due either to labelling of different parts of the large chain or to variation of the position of the tryptic split within the large chains in the preparation.

In the presence of NaCl (Fig. 3), the distribution of label was much more distinct. The 78 K fragment was completely devoid of radioactivity and the terminal 12 K fragment seems to arise almost by primary cleavage of the large chain. A minor fraction of the label, at most 2%, was found in a 37 K fragment. This implies that the radioactive 12 K segment or segments are localised near one end of the large chains which give rise to the 78 K fragment in the presence of NaCl.

Our results support previous evidence that iodonaphthylazide acts as a hydrophobic probe which labels membrane proteins from within the bilayer. The selectivity of the label for a small segment of the large peptide does not imply that other regions of the protein are not embedded in the membrane, for the labelled segment may hinder access of the label to other areas of the large peptide or indeed to the glycoprotein component. Preferential binding to this segment, or kinetic factors could explain the discrimination against unlabelled regions of protein and lipid side chains. Our work illustrates that combining specific labelling from within the lipid core with proteolytic digestion at the membrane surface may be an important tool for identifying segments of membrane proteins in intimate contact with the lipids.

We thank Dr T. Bercovici for ¹²⁵I-INA and Mrs Rivka Goldshlegger and Annie Sloth for excellent technical assistance. P.L.J. was a recipient of a short-term fellowship from EMBO.

STEVEN J. D. KARLISH

Biochemistry Department, Weizmann Institute of Science,
Rehovot, Israel

PETER LETH JORGENSEN

Institute of Physiology,
University of Aarhus, Aarhus, Denmark

CARLOS GITLER

Membrane Department, Weizmann Institute of Science,
Rehovot, Israel

Received 7 July; accepted 12 August 1977.

1. Jorgensen, P. L. *Biochim. biophys. Acta* **356**, 36–52 (1974).
2. Kyte, J. J. *Biol. Chem.* **247**, 7642 (1972).
3. Jorgensen, P. L. *Biochim. biophys. Acta* **356**, 53–67 (1974).
4. Klip, A. & Gitler, C. *Biochem. biophys. Res. Commun.* **60**, 1155–1162 (1974).
5. Gitler, C. and Klip, A. in *Perspectives in Membrane Biology* (eds Estrado, O. S. & Gitler, C.) 149 (Academic, New York, 1974).
6. Sigrist-Nelson, K., Sigrist, H., Bercovici, T. & Gitler, C. *Biochim. biophys. Acta* **468**, 163–176 (1977).
7. Bercovici, T. & Gitler, C. *Biochemistry* (submitted for publication).
8. Jorgensen, P. L. *Biochim. biophys. Acta* **401**, 399–415 (1975).
9. Jorgensen, P. L. *Biochim. biophys. Acta* **446**, 97–108 (1977).
10. Jorgensen, P. L. *Meth. Enzym.* **32B**, 277–290 (1974).
11. Karlsh, S. J. D., Yates, D. W. & Glynn, I. M. *Nature* **263**, 251–253 (1976).
12. Jensen, J. & Ottolenghi, P. *Biochem. J.* **159**, 815–817 (1976).
13. Hegevary, C. & Post, R. L. *J. biol. Chem.* **246**, 5234–5240 (1971).
14. Norby, J. G. & Jensen, J. *Biochim. biophys. Acta* **233**, 104–116 (1971).

Synthesis of haemoglobin Wayne in erythroid cells

HETEROZYGOTES for haemoglobin (Hb) Wayne possess two minor haemoglobin components that migrate more rapidly than HbA on electrophoresis at pH 8.6. Each of the minor haemoglobin components contains an abnormal α chain in which the carboxyl-terminal tripeptide sequence, Lys-Tyr-Arg, has been replaced by an octapeptide¹. The slower of the two components, henceforth designated Wayne-Asn, has the following octapeptide sequence: Asn-Thr-Val-Lys-Leu-Glu-Pro-Arg, whereas the faster one (Wayne-Asp) has exactly the same sequence except that asparagine at position 139 is replaced by aspartic acid. (These components, formerly designated¹ Wayne-1 and Wayne-2, respectively, have been renamed for clarity.) This is the first variant described in which deamidation of the gene product is believed to occur. The purpose of this study was to demonstrate the proposed deamidation and to explain another interesting feature of the Hb Wayne phenotype in heterozygotes: namely, the presence of the variant haemoglobins in markedly reduced quantities relative to HbA (3% and 4%, respectively, for Hb Wayne-Asn and Hb Wayne-Asp).

Peripheral blood red cells from two heterozygotes with Hb Wayne and bone marrow cells from one of them were incubated for 60 min in the presence of ³H-labelled leucine. A fraction of the labelled haemolysates was converted into globin immediately by precipitation in acid-acetone. The α/β ratios obtained, based on total counts and the specific activities, indicate that the synthesis of α chains is equal to that of the β chains in the peripheral blood and bone marrow (balanced synthesis). Moreover, a free α chain peak could not be demonstrated after chromatography of the haemolysates on Sephadex G-100 gel filtration columns.

In order to compare more precisely the specific activities of the various α chains (Table 1), it was necessary to first purify the haemoglobins in the labelled haemolysates by column chromatography of the haemolysates on carboxymethyl cellulose². This was followed by preparative isoelectric focusing of the peaks containing the Hb Wayne fractions using LKB columns and finally globin chain separation³. The Wayne chain fractions obtained were shown to be free of contamination by peptide chromatography of tryptic digests.

In the peripheral blood incubations the amount of radioactivity corresponding to the α^{Wayne} chains was less than 11% of the total α chain radioactivity (Table 1). The corresponding value in the bone marrow was 11.4%. These figures were considerably lower than the 25% expected on the basis of the two loci believed to be present for α chains. The $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{A}}$ specific activity ratio was 2.40 to 2.46 in the peripheral blood and 3.1 in the bone marrow, suggesting instability of the $\alpha^{\text{Wayne-Asn}}$ chains. In both peripheral blood and bone marrow, the specific activity of the $\alpha^{\text{Wayne-Asp}}$ chains was lower than that of the $\alpha^{\text{Wayne-Asn}}$ chains (Table 1). Time course experiments using peripheral blood showed no change in the $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{Wayne-Asp}}$ ratio from 4 min to 3.5 h (not shown).

In order to evaluate the difference in labelling of the two types of variant α chains a pulse-chase experiment was performed. Peripheral blood red cells from a heterozygote were incubated in the presence of ³H-leucine for 15 min (pulse) after which they were washed and a portion was immediately frozen. The remaining cells

Table 1 Incorporation of ^3H -leucine into α and β chains following incubation of red cells for 60 min

	α/β ratios of globin from crude haemolysates	Specific activity	Radioactivity of purified α chains					
			Specific activity (c.p.m. per mg)		% Of total α chain radioactivity			
	Total counts		$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$	α^{A}	$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$	α^{A}
Peripheral blood (WS)	0.97	0.94	644	115	262	8.36	1.80	89.84
Peripheral blood (GS)	1.04	0.96	968	202	403	6.96	1.95	91.09
Bone marrow	1.12	0.91	40,039	837	12,880	11.16	0.27	88.57

A fraction of the crude haemolysates was converted to globin immediately by precipitation in acid acetone. The globin obtained was chromatographed on columns of Sephadex G-100 in 20% HCOOH^{15} . After chromatography globin fractions were recovered by freeze drying. Another fraction of the haemolysate was used for purification of the various haemoglobin components for accurate determination of radioactivity in the α^{Wayne} chains. Globin chain separation was carried out according to Clegg *et al.*³ Aliquots of 1 ml of each fraction were added to 10 ml Instagel (Packard) and counted by scintillation spectroscopy. The total radioactivity under each peak was summated. The various chain components were then pooled, desalted on Biogel P2 columns equilibrated with 0.5% formic acid, and lyophilised. The chains were re-dissolved in distilled water and the protein concentration determined by the Lowry method²⁹.

were reincubated in a medium containing unlabelled leucine (chase) for 1, 4 and 6 h. The specific activities of the α^{A} and β chains remained unchanged during the chase period as did that of the $\alpha^{\text{Wayne-Asn}}$ and $\alpha^{\text{Wayne-Asp}}$ chains (Table 2), suggesting that during the short period of the chase no detectable conversion or degradation of the abnormal chains relative to each other or to α^{A} occurs. If deamidation is a slow process, then a greater proportion of Hb Wayne-Asn relative to Hb Wayne-Asp should be present in reticulocytes when compared with the more mature red cells. A reticulocyte fraction was prepared from the peripheral blood of a heterozygote by means of a discontinuous Ficoll density gradient⁴. Isoelectric focusing in polyacrylamide gels of the reticulocyte haemolysate revealed a markedly decreased proportion of Hb Wayne-Asp when compared to the haemolysate prepared from unfractionated peripheral blood. Moreover, the proportion of Hb Wayne-Asn in reticulocytes was approximately twice as much as in peripheral blood (Fig. 1).

The above data support the hypothesis that $\alpha^{\text{Wayne-Asn}}$ chains are synthesised initially and subsequently converted to $\alpha^{\text{Wayne-Asp}}$ chains. Robinson and others⁵⁻⁸ have shown that specific glutamyl and asparaginyl residues have characteristic rates of deamidation that are determined by the nature of neighbouring amino acid residues in both the primary and tertiary structures. The discovery of Hb Providence, a β chain variant present as two components, one having asparagine instead of lysine at position 82 and the other having aspartic acid at the same position, indicates that deamidation is not unique to Hb Wayne⁹⁻¹⁰. It is also possible that Hb J-Singapore¹¹ is not a double substitution variant but a neutral substitution at $\alpha^{79} \text{Ala} \rightarrow \text{Gly}$ followed by deamidation at α^{78} to yield aspartic acid¹².

It is interesting to note that $\alpha^{\text{Wayne-Asp}}$ chains account for a higher proportion of the total α -chain radioactivity in peripheral blood than in bone marrow (Table 1); the $\alpha^{\text{Wayne-Asn}}/\alpha^{\text{Wayne-Asp}}$ ratio was 0.5 in the former and 0.06 in the latter. A possible explanation for all of these findings is that the asparagine at residue 139 of $\alpha^{\text{Wayne-Asn}}$ forms a cyclic imide, as proposed by Bornstein¹³, while the chains are in the nascent chain or monomer conformation. While in this conformation the cyclic imide is rapidly hydrolysed to the aspartyl form. Once the $\alpha^{\text{Wayne-Asn}}$ chain is incorporated into the Hb Wayne tetramer further cyclisation is blocked by steric

hindrance but those cyclised chains that had formed are hydrolysed very slowly. In the bone marrow any $\alpha^{\text{Wayne-Asp}}$ monomer chains produced by deamidation of $\alpha^{\text{Wayne-Asn}}$ monomers might be rapidly degraded by proteolytic enzymes that are not active in mature erythrocytes^{14,15}.

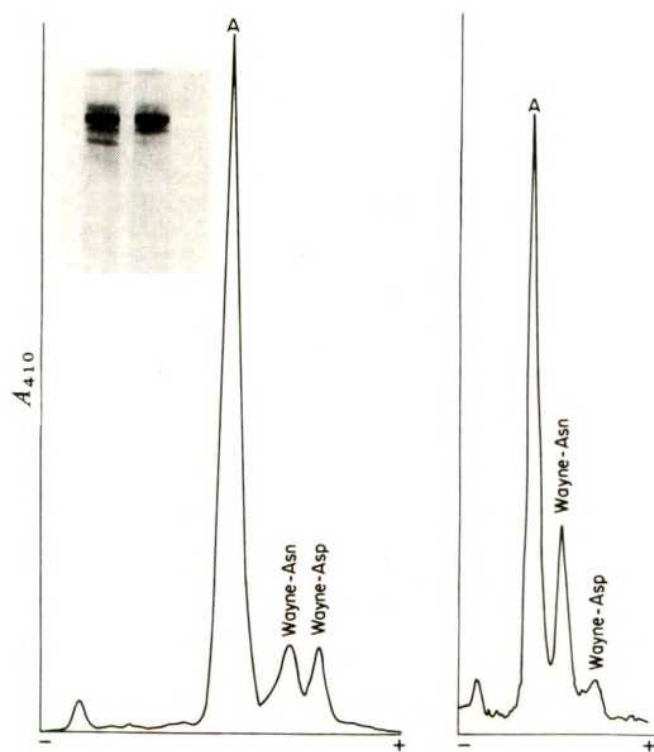


Fig. 1 Isoelectric focusing in polyacrylamide gels of peripheral blood haemolysate (left) and reticulocyte haemolysate (right). The densitometric tracings were made on unstained gels at 410 nm using a Gilford recording spectrophotometer.

Table 2 α Chain specific activities during a pulse chase experiment

Incubation time	Specific activity (c.p.m. per mg)		$\alpha^{\text{Wayne-Asp}}/\alpha^{\text{A}}$	$\alpha^{\text{Wayne-Asp}}/\alpha^{\text{A}}$	$\alpha^{\text{Wayne-Asn}}/\alpha^{\text{Wayne-Asp}}$
	$\alpha^{\text{Wayne-Asn}}$	$\alpha^{\text{Wayne-Asp}}$			
15 min pulse	1,510	275	1.80	0.33	5.49
1 h chase	1,631	286	1.84	0.32	5.70
4 h chase	1,572	283	1.73	0.31	5.55
6 h chase	1,590	269	1.79	0.30	5.91

The presence of Hbs Wayne as minor components is shared by Hbs Constant Spring¹⁶⁻¹⁸, Koya Dora¹⁹, Icaria²⁰ and Seal Rock²¹, other variants characterised by elongated chains. Because the α_{Wayne} chains comprise only 11% of the total α chains synthesised during the period of incubation, instability of the chains does not fully account for the low proportion of Hb Wayne. A delay in translation of the α_{Wayne} chains as a result of a deficiency of tRNA for one or more of the amino acids corresponding to the abnormal sequence cannot be excluded, although such a delay has not been found in any of the abnormal haemoglobins that are present in reduced amount and that have been studied²²⁻²⁵, including Hb Constant Spring¹⁶. Perturbation of termination of translation *per se* seems also an unlikely explanation since the synthesis of Hb Cranston, a frameshift mutant of the β chain, is not so severely impaired²⁶. Yet another possible explanation, that the mRNA has been rendered unstable by the deletion responsible for the frame shift, has been excluded by the demonstration that no more Hb Wayne components are synthesised in bone marrow than in peripheral reticulocytes.

The expectation that an α -chain mutant would direct the synthesis of 25% of the α -chain mRNA is based on the unproven assumption that there are two loci that are equally active in directing α -chain synthesis. This assumption is partly based on the fact that individuals heterozygous for Hbs J-Buda and G-Pest²⁷ possess close to 25% of these variants, along with approximately 50% of Hb A, as well as the fact that a large number of other α -chain structural mutants comprise 20-25% of the haemoglobin of heterozygotes. Haemoglobin synthesis studies have not been performed on most of these variants. Consequently their true rate of synthesis is not known. Therefore, the reduced production of the Constant Spring and α_{Wayne} chains could also be explained on the basis of multiple α -chain loci that are not equally active in α -chain synthesis. Such a hypothesis has been invoked to explain a greater than expected amount of Hb J-Mexico in heterozygotes²⁸. It is possible to envision one major locus, another of intermediate activity, and a third locus accounting for only a minor proportion of alpha chains. The mutation in α_{Wayne} would correspond to the intermediate locus and that of $\alpha_{\text{Constant Spring}}$ would correspond to the minor locus. A complete understanding of these complex abnormalities requires study of additional informative mutants.

This work was supported in part by USPHS grants GM15419 and NIH 5-T32-01723.

S. M. HANASH
W. P. WINTER
D. L. RUCKNAGEL

University of Michigan Medical School,
Department of Human Genetics,
Ann Arbor, Michigan 48109

Received 18 July; accepted 10 August 1977.

- Seid-Akhavan, M., Winter, W. P., Abramson, R. K. & Rucknagel, D. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 882-886 (1976).
- Huisman, T. H., Martis, E. A. & Dozy, A. J. *Lab. clin. Med.* **52**, 312-327 (1958).
- Clegg, J. B., Naughton, M. A. & Weatherall, D. J. *J. molec. Biol.* **19**, 91-108 (1966).
- DeSimone, J., Kieve, L. & Shaeffer, J. J. *Lab. clin. Med.* **84**, 517-524 (1974).
- Robinson, A. B. *Proc. natn. Acad. Sci. U.S.A.* **71**, 885-888 (1974).
- McKerrow, J. H. & Robinson, A. B. *Science* **183**, 85 (1974).
- Lai, C. Y., Chen, C. & Horecker, B. L. *Biochem. biophys. Res. Commun.* **40**, 461-468 (1970).
- Midelfort, C. F. & Mehler, A. H. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1816-1819 (1972).
- Moo-Penn, W. J. *et al. J. biol. Chem.* **251**, 7557-7562 (1976).
- Charache, S., Fox, J., McCurdy, P., Kazanian, H. & Winslow, R. J. *clin. Invest.* **59**, 652-658 (1977).
- Blackwell, R. O., Wong Hock Boon, C. S. & Weng, M. I. *Biochim. biophys. Acta* **278**, 482-490 (1972).
- Lehmann, H. & Huntsman, R. G. *Man's Haemoglobins* 2nd edn. 478 (Lippincott, Philadelphia, 1974).
- Bornstein, P. *Biochemistry* **9**, 2408-2420 (1970).
- Chalevelakis, G., Clegg, J. B. & Weatherall, D. J. *Proc. natn. Acad. Sci., U.S.A.* **72**, 3853-3857 (1975).
- Wood, W. G. & Stamatoyannopoulos, G. *J. clin. Invest.* **55**, 567-578 (1975).
- Clegg, J. B., Weatherall, D. J. & Milner, P. F. *Nature* **234**, 337-340 (1971).
- Clegg, J. B. & Weatherall, D. J. *Ann. N. Y. Acad. Sci.* **232**, 168-178 (1974).
- Kan, Y. W., Todd, D. & Dozy, A. M. *Br. J. Haematol.* **28**, 103-107 (1974).
- DeJong, W. W., Meera Khan, P. & Bernini, L. F. *Am. J. hum. Genet.* **27**, 81-90 (1975).
- Clegg, J. B. *et al.* **251**, 245-247 (1974).
- Bradley, T. B., Wohl, R. C. & Smith, G. J. *Clin. Res.* **23**, 131a (1975).
- Rieder, R. F. *J. clin. Invest.* **51**, 364-372 (1972).
- Rieder, R. F. *J. clin. Invest.* **50**, 388-395 (1971).
- Farace, M. G. & Bank, A. *Biochim. biophys. Acta* **312**, 591-597 (1973).
- Rieder, R. F. & James, G. W. *Blood* **47**, 489-494 (1976).
- Franklin Bunn, H., Schmidt, G. F., Haney, D. N. & Dluhy, R. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3609-3613 (1975).
- Hollan, S. R. *et al. Nature* **235**, 47-50 (1972).
- Trabuchet, G., Pagnier, J., Benabadi, M. & Labie, D. *Hemoglobin* **1**, 13-25 (1976/77).
- Lowry, O. H., Rosebrough, N. J., Farr, A. & Randall, R. S. *J. biol. Chem.* **193**, 265-275 (1951).

Red cell charge is not a function of cell age

REPORTS^{1,2} that the oldest circulating human erythrocytes have an electrophoretic mobility up to 30% lower than the youngest cells have been widely accepted, particularly since such findings suggest a plausible mechanism for the removal of the oldest cells from circulation. For those studies^{1,2} the red cells were separated on the basis of age by using the accepted relationship that older cells are denser on the average than younger cells. We report here that electrophoretic mobility studies conducted independently by conventional electrophoresis, streak width measurements and electrophoretic light scattering have shown the mobilities to be the same for red cell fractions of differing density and hence age *in vivo*. These data suggest that hypotheses which invoke a role for decreasing surface charge density in the mechanism of senescent red cell recognition *in vivo* are unsound.

The non-random nature of the red cell elimination process has led to suggestions that membrane determinants such as red cell surface charge³ and/or antibody binding to the cell surface⁴ may provide specificity as a result of age-dependent alterations which enhance cell adhesion to phagocytic cells.

The observation that various sialoglycoproteins are rapidly eliminated from the blood following removal of their sialic acid⁵ has prompted considerable speculation on the role of sialic acid loss in the ageing and elimination of red cells^{6,7}. In some instances, normal lifespans have been observed for sialic acid deficient red cells⁸⁻¹⁰. But, the sialic acid content of old (dense) red cells is lower than young (less dense) cells¹¹⁻¹³. As the major source of negative surface charge on human red cells is the carboxyl group of membrane-bound *N*-acetylneuraminic acid¹⁴, an age-related decrease in electrophoretic mobility^{1,2} is consistent with a net loss of sialic acid per unit of membrane surface area.

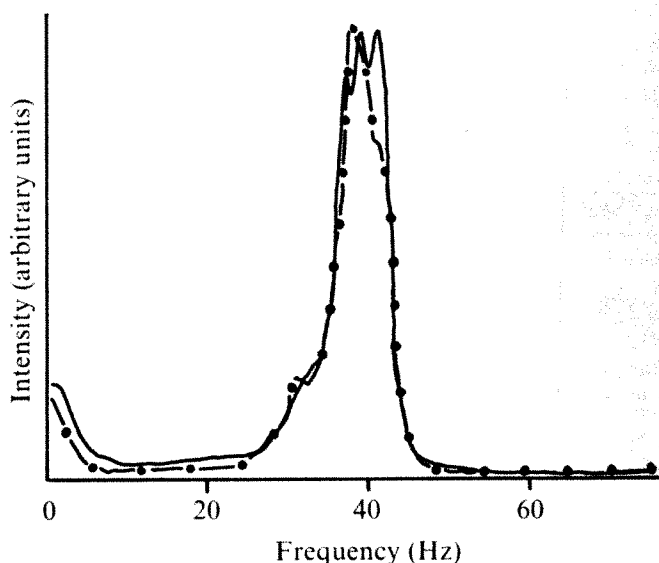


Fig. 1 Electrophoretic light scattering spectra¹⁹ for least dense (top) and most dense (bottom) human red cell subpopulations at 25 °C in 0.0145 M NaCl-4.5% w/v sorbitol-0.007 M bis-Tris buffer at pH 7.3. The top and bottom fractions each representing ~2% of the whole population were obtained by centrifugation of the cells in their own plasma for 30 min, 140,000g at 4 °C followed by three washes in the electrophoresis buffer. The mean Doppler shift for both spectra is 39.5 Hz, which corresponds to an electrophoretic mobility of $2.75 \pm 0.05 \mu\text{m s}^{-1} \text{ V}^{-1} \text{ cm}$ after a correction for electro-osmosis (9.8 Hz). Experimental parameters were: scattering angle, 14.9 °; laser wavelength, 514.5 nm; electric field, 16.2 V cm⁻¹ and frequency resolution of ~0.03 Hz. In these conditions a 20% difference in mobility would produce a difference in shift frequency of ~6 Hz. The spectra shown for the subpopulations were indistinguishable from that of the whole population (not shown).

Table 1 Streak width and aspartate aminotransferase activities¹⁷ for whole red cell populations and extreme density fractions representing 2–7% of the whole populations

Sample no.	Aspartate aminotransferase (IU per 10 ¹⁰ RBC)			Unfractionated	Electrophoretic streak width (mm)		
	Unfractionated	Top	Bottom		Top	Bottom	Top and bottom
1	0.95	1.66	0.40	0.79	1.27	1.01	0.88
2	1.88	7.15	0.87	0.61	0.56	0.64	0.66
3	2.96	5.22	0.95	0.84	1.00	0.97	1.16
4	0.87	2.12	0.58	0.57	0.68	0.62	0.74
5	1.53	1.26	0.49	0.62	0.64	0.67	0.60
5a	1.53	1.13	0.58	0.62	0.62	0.64	0.62
6	1.20	1.79	0.89	0.79	0.98	0.81	1.07
6a	1.20	1.60	1.02	0.79	0.78	0.79	0.80

Samples 1, 4, 5 and 6 were obtained from adults, samples 2 and 3 from the umbilical cord of infants at birth. All samples were fractionated by repeated centrifugation for 30 min at 2000g on phthalate ester mixtures¹⁸ except for samples 5a and 6a which were obtained by ultracentrifugation at 50,000g, 20 °C for 2 h. Cell suspensions (2% v/v) in pH 7.4, 16.5 mM Tris-acetate buffer containing 5% sucrose were electrophoresed at 21 °C, 80 V cm⁻¹. Streak widths are to be compared with the 13.8 mm total distance of migration for a mobility of 2.3 $\mu\text{m s}^{-1} \text{V}^{-1} \text{cm}$ RBC, red blood cell.

To detect any significant dispersity in electrophoretic mobility which accompanies changes in density and *in vivo* ageing of red cells, the extreme ends of the red cell density distribution were examined for evidence of differences in electrophoretic mobility. Using various red cell density fractionation procedures, studies conducted independently in our three laboratories with different instrumentation and conditions do not show any experimentally significant electrophoretic mobility differences for the extreme red cell subpopulations.

Electrophoresis of erythrocyte fractions by the continuous-flow streak deflection method¹⁵ was carried out in the conditions previously reported¹⁶ using the Kolin endless belt electrophoresis apparatus. If present, heterogeneity in electrophoretic mobility of cell populations is evidenced by progressive sample band broadening on passage through the electrical field of the instrument and by actual band splitting for samples with multimodal mobility distributions. Examination of the banding behaviour for whole and density-fractionated human red cell populations (Table 1) gave no indication of electrophoretic inhomogeneity. The maximum observed bandwidths were consistent with about a 5% range in electrophoretic mobility for mixtures of cells made from the density extremes. The band or streak width expected on the basis of Yaari's data² for 'top' and 'bottom' cell mixtures is about five times the observed value. Furthermore the band should have shown signs of observable splitting as the run progressed. Marked

differences in red cell aspartate aminotransferase activity between the fractions indicated that significant cell separation according to cell age had been achieved¹⁷.

Electrophoretic light scattering spectra (Fig. 1) for 'top' and 'bottom' fractions of red cells obtained by ultracentrifugation also failed to show significant differences between the mean mobilities or the mobility distributions.

Analytical electrophoretic mobility data for samples of top and bottom cell populations obtained by two different density fractionation techniques also showed no differences either at high or low ionic strength (Table 2). Extreme subpopulations representing as little as 1% (not shown) were not found to differ.

In each of the described electrophoresis systems the expected mobility differences² for red cells from the extremes of the density distribution should have been detected with ease. We conclude from the lack of any clear indications of mobility heterogeneity in these studies which involved different density separation and electrophoretic monitoring techniques that no measurable differences in mobility are correlated with red cell density through the central ~95% of the density distribution. This is in contrast to the observations of Yaari² which showed electrophoretic mobility to be a monotonic function of cell density through this range.

Electrophoretic mobility is recognised as a measure of net surface charge density²³. A constant electrophoretic mobility for red cells of different densities implies that the effective net number

Table 2 Mobility data and descriptive indices for human red cells fractionated with phthalate esters¹⁸ and by high-speed centrifugation²⁰

Subpopulation	MCHC (g per 100 ml)	NANA (fg per cell)	GOT (IU per cell $\times 10^{11}$)	Electrophoretic mobility ($\mu\text{m s}^{-1} \text{V}^{-1} \text{cm}$)	
				0.15 M NaCl	0.03 M NaCl
Phthalate ester					
<i>a</i> Top 6%	32.8	20.1	—	$-1.10 \pm 0.06(30)$	$-1.72 \pm 0.11(56)$
Bottom 10%	40.6	18.3	—	$-1.10 \pm 0.06(30)$	$-1.72 \pm 0.09(46)$
<i>b</i> Top 5%	35.8	20.8	16.9	$-1.09 \pm 0.07(60)$	$-1.71 \pm 0.09(50)$
Bottom 6%	41.4	19.6	6.6	$-1.11 \pm 0.05(32)$	$-1.72 \pm 0.09(28)$
<i>c</i> Top 7%	34.0	21.2	13.5	$-1.09 \pm 0.07(20)$	$-1.74 \pm 0.06(20)$
Bottom 2%	39.9	16.8	4.9	$-1.12 \pm 0.08(20)$	$-1.73 \pm 0.12(20)$
Murphy fractionation					
<i>a</i> Top 5%	33.4	18.4	9.4	$-1.10 \pm 0.07(20)$	$-1.82 \pm 0.07(20)$
Bottom 5%	40.1	16.6	4.3	$-1.11 \pm 0.07(20)$	$-1.84 \pm 0.09(20)$
<i>b</i> Top 5%	31.4	17.8	10.7	$-1.08 \pm 0.04(20)$	$-1.77 \pm 0.10(20)$
Bottom 5%	35.8	15.9	2.0	$-1.10 \pm 0.06(28)$	$-1.78 \pm 0.07(20)$
<i>c</i> Top 5%	34.3	17.8	9.7	$-1.05 \pm 0.08(20)$	$-1.72 \pm 0.09(20)$
Bottom 5%	41.7	16.2	4.8	$-1.07 \pm 0.08(20)$	$-1.70 \pm 0.09(20)$
Pooled ratios	0.843	1.13	2.98	0.985	0.999
Top/bottom	± 0.026	± 0.07	± 1.36	± 0.009	± 0.008

Whole blood samples were collected by venipuncture from four adult subjects. Fractions were washed once in 2–4 volumes of autologous plasma, three times in > 20 volumes of pH 7.4, 0.144 M NaCl–0.01 M potassium phosphate (PBS). Mean cell haemoglobin concentration (MCHC) was calculated from haemoglobin assayed as cyanmethaemoglobin and microhaematocrit values after centrifugation for 5 min at 15,000g. *N*-acetylneuraminic acid (NANA) was assayed in supernatants²¹ after incubation of $\sim 2 \times 10^9$ RBC per ml in PBS plus ~ 100 Behringwerke units of *Vibrio cholera* neuraminidase for 1 h at 37 °C. Aspartate aminotransferase (glutamic oxaloacetic transaminase, GOT) was assayed spectrophotometrically²² and activity is expressed in international units (IU) where one unit represents the conversion of 1 μmol of NADH to NAD⁺ in 1 min at 25 °C. Mobilities were measured at 25 °C in cylindrical chambers equipped with Ag/AgCl electrodes²³ or Pt electrodes²⁴, and operated at voltage gradients < 5 V cm⁻¹ and currents < 3 mA to minimise joule heating. Mean mobilities and standard deviations are given for the number of cells in parentheses. The two ionic strength media listed were buffered to pH 7.2 \pm 0.2 with NaHCO₃ and 0.03 M NaCl contained 4.0% (w/v) sorbitol to maintain medium tonicity.

of negative charges per unit area of membrane surface as observed by electrophoresis remains constant even though the total number of charge groups, such as sialic acid, on the cell surface may be decreased as, for example, by membrane loss.

This work was supported by USPHS grants CA 13955 and HL 18284 and NSF grant PCM 72-05133. J.Y.J. was a Killam Postdoctoral Research Fellow. B.R.W. is an Alfred P. Sloan Research Foundation Fellow. We thank Frank Nordt and Bonnie Voyda for technical assistance.

S. J. LUNER
DOROTHY SZKLAREK

Department of Pediatrics,
University of California at Los Angeles,
Center for the Health Sciences,
Los Angeles, California 90024

R. J. KNOX
G. V. F. SEAMAN

Department of Neurology,
University of Oregon Health Sciences Center,
Portland, Oregon 97201

J. Y. JOSEFOWICZ
B. R. WARE

Department of Chemistry,
Harvard University,
Cambridge, Massachusetts 02138

Received 17 June; accepted 8 August 1977.

1. Danon, D. & Marikovsky, Y. *C. r. hebdo. Acad. Sci., Paris* **253**, 1271-1272 (1961).
2. Yaari, A. *Blood* **33**, 159-163 (1969).
3. Danon, D., Marikovsky, Y. & Skutelsky, E. *Red Cell Structure and Metabolism* (ed. Ramot, B.) 23-28 (Academic, New York, 1971).
4. Kay, M. M. B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3521-3525 (1975).
5. Ashwell, G. & Morell, A. G. *Adv. Enzym.* **41**, 99-128 (1974).
6. Lloyd, C. W. *Biol. Rev.* **50**, 325-350 (1975).
7. Bocci, V. *Experientia* **32**, 135-140 (1976).
8. Chien, S. *et al. Blood* **43**, 445-460 (1974).
9. Luner, S. J., Sturgeon, P., Szklarek, D. & McQuiston, D. T. *Vox Sang.* **29**, 440-449 (1975).
10. Myllylä, G. *et al. Vox Sang.* **20**, 7-23 (1971).
11. Greenwalt, T. J. & Steane, E. A. *Br. J. Haematol.* **25**, 207-215 (1973).
12. Baxter, A. & Beeley, J. G. *Biochem. J.* **3**, 134-136 (1975).
13. Cohen, N. S., Ekholm, J. E., Luthra, M. G. & Hanahan, D. J. *Biochim. biophys. Acta* **419**, 229-242 (1976).
14. Cook, G. M. W., Heard, D. H. & Seaman, G. V. F. *Nature* **191**, 44-47 (1961).
15. Luner, S. J. & Szklarek, D. *Biophys. J.* **16**, 168a (1976).
16. Sturgeon, P., Kolin, A., Kwak, K. S. & Luner, S. J. *Hematologia* **6**, 93-108 (1972).
17. Sass, M. D., Vorsanger, E. & Spear, P. W. *Chin. chim. Acta* **10**, 21-26 (1964).
18. Danon, D. & Marikovsky, Y. *J. Lab. clin. Med.* **64**, 668-674 (1964).
19. Ware, B. R. *Adv. Colloid Interface Sci.* **4**, 1-44 (1974).
20. Murphy, J. R. *J. Lab. clin. Med.* **82**, 334-341 (1973).
21. Aminoff, D. *Biochem. J.* **81**, 384-392 (1961).
22. Henry, R. J., Chiamori, N., Golub, O. J. & Berkman, S. *Am. J. clin. Path.* **34**, 381-398 (1960).
23. Seaman, G. V. F. *The Red Blood Cell* (ed. Surgenor, D. M.), 1135-1229 (Academic, New York, 1975).
24. Bingham, A. D., Flemans, R., Heard, D. H. & Seaman, G. V. F. *Nature* **182**, 642-644 (1958).

Ion-induced changes in head group conformation of lecithin bilayers

THE structure and orientation of the phosphocholine polar group in lecithin-containing membranes has been discussed extensively. Studies of egg-yolk lecithin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), using neutron diffraction and ^1H and ^{31}P nuclear magnetic resonance (NMR), have led to the conclusion that the phosphocholine dipole is orientated parallel to the membrane surface¹⁻⁴. On the other hand, ^1H and ^{31}P NMR spectra of sonicated DPPC vesicles in the presence of trivalent ions suggested that the choline group is extended approximately perpendicular⁵ or folded parallel⁶ to the bilayer surface. We have, therefore, repeated our previous ^1H and ^{31}P NMR measurements in the presence of trivalent ions and report here that the addition of these shift reagents induces large changes in the spectral parameters, that is, the ^1H quadrupole splittings and the ^{31}P chemical shift anisotropy. These observations can only be interpreted in terms of specific ion-induced changes in the choline head group conformation of DPPC.

Typical spectral changes observed on addition of EuCl_3 , LaCl_3 and $\text{K}_3\text{Fe}(\text{CN})_6$ to unsonicated dispersions of DPPC are shown in Figs 1-3. The observed changes cannot

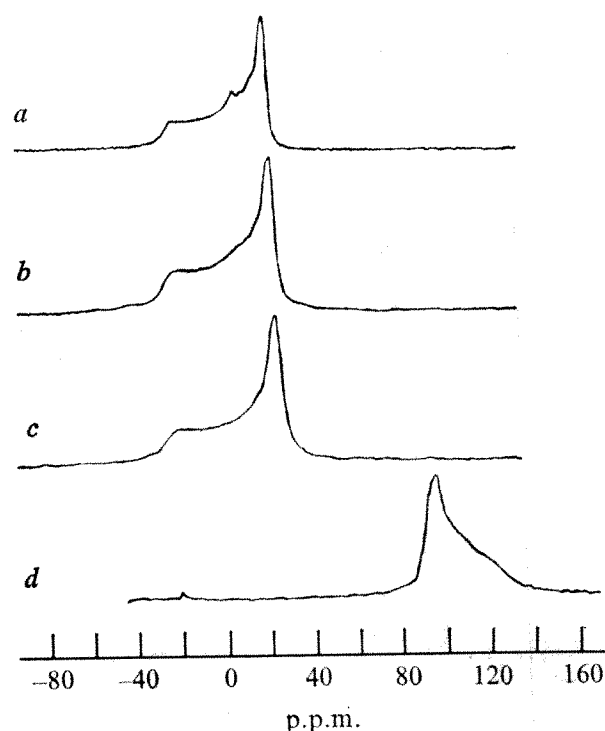


Fig. 1 Phosphorus-31 NMR spectra of unsonicated multibilayers of DPPC complexed with trivalent ions at 48 °C. *a*, No ions; *b*, $\text{Fe}(\text{CN})_6^{3-}$; *c*, $\text{La}(\text{III})$; *d*, $\text{Eu}(\text{III})$. Solutions of $\text{K}_3\text{Fe}(\text{CN})_6$, LaCl_3 , and EuCl_3 were prepared as described in ref. 5. Approximately 200 mg of DPPC were mixed with 0.5 M ion solutions to yield a final concentration of 50 wt % H_2O and a DPPC to ion mole ratio of 2.7. The samples were then sealed in glass ampoules and placed in 10-mm NMR tubes. The NMR spectra were obtained in the pulsed Fourier transform mode at 36.4 MHz using a Bruker HX-90 spectrometer/B-NC-12 data system equipped with a deuterium lock and home-built quadrature phase detection. Approximately 5,000 free induction decays were averaged to obtain these spectra. A proton-decoupling field of 10 W tuned to the CH_2 resonance of the choline head group moiety was applied. The chemical shifts are relative to 85% orthophosphoric acid. The residual chemical shift anisotropy ($\Delta\sigma$) is given approximately by the separation between the edges of the powder spectra.

be ascribed to general ionic strength effects, since no spectral changes are apparent in control studies using NaCl. Different patterns of spectral behaviour are observed at all of the head group segments studied. Binding of $\text{La}(\text{III})$ or $\text{Fe}(\text{CN})_6^{3-}$ has little effect on the shape of the ^{31}P NMR spectra. But on addition of $\text{Eu}(\text{III})$ a large chemical shift is observed which is accompanied by an apparent reversal in the sign of the chemical shift anisotropy. The effects of ion binding on the quadrupole splittings of the $^1\text{NCH}_2\text{CD}_2$ and $^1\text{NCD}_2\text{CH}_2$ segments (the α and β positions, respectively) tend to be generally in the same direction, but the magnitude of the observed changes is ion-specific. The observed quadrupole splitting at the α position is decreased, whereas that at the β position is increased on ion binding. Little change is apparent at the $(\text{CD}_3)_3\text{N}^+$ segment (data not shown). The data are summarised in Table 1.

The mole ratios of ion to phospholipid used in this work are higher than those used by Hauser *et al.*⁵, however, concentration dependence studies reveal significant effects at ion : phospholipid mole ratios as low as 1 : 10. In the case of $\text{Eu}(\text{III})$ binding, two components are seen in the ^{31}P NMR spectra in certain conditions—the predominant spectral component is shown in Fig. 1. It is also possible that there is more than one component on binding of $\text{La}(\text{III})$ or $\text{Fe}(\text{CN})_6^{3-}$, as the lack of a chemical shift in the ^{31}P NMR spectra upon binding of these ions would make it difficult to resolve components with similar chemical shift anisotropies.

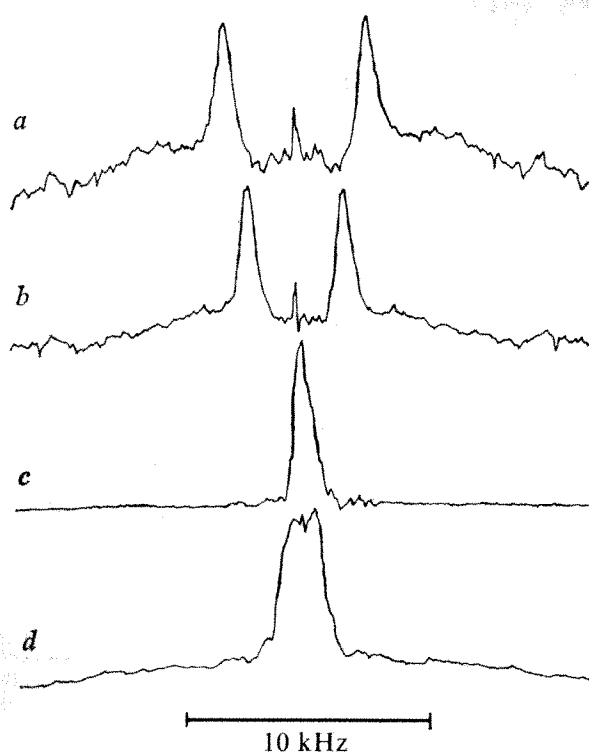


Fig. 2 Deuterium NMR spectra of unsonicated multibilayers of $^+NCH_2CD_2$ -DPPC complexed with trivalent ions at 59 °C. *a*, No ions; *b*, $Fe(CN)_6^{3-}$; *c*, $La(III)$; *d*, $Eu(III)$. Selectively deuterated DPPC was prepared as described in ref. 3. The spectra were recorded at 13.8 MHz with a fluorine lock; otherwise the experimental conditions are identical to those described in the legend to Fig. 1. Approximately 6,000–10,000 free induction decays were averaged to obtain the spectra. The absolute value of the residual quadrupole splitting ($\Delta\nu$) is given by the separation between the two largest peaks in the powder-type spectra. Note that the spectra are recorded at different gains.

Since DPPC dispersions complexed with trivalent ions undergo phase transitions at temperatures near the gel to liquid crystalline phase transition of pure DPPC (ref. 7 and H. U. Gally, personal communication), it is unlikely that the bilayer structure is disrupted on binding of any of the ions used here. The static electric field gradient tensor of the 2H labels primarily reflects the electronic structure of the bonds containing these atoms and is not affected by ion binding to the phosphocholine groups. The observed changes in the residual quadrupole splitting must, therefore, be due to changes in the head group motion and conformation. The exact nature and extent of these conformational changes can only be determined from a quantitative analysis of the 2H and ^{31}P NMR data, which will not be pursued here. The interpretation of the ^{31}P NMR data is not unequivocal, as it is possible that the contact shift due to

$Eu(III)$ binding⁵ may be different for the different components of the chemical shielding tensor. In principle, this could account for the different ^{31}P NMR spectral changes associated with $Eu(III)$ and $La(III)$ binding and would be consistent with the similar 2H NMR spectral changes observed on binding of these ions. The binding of $Eu(III)$ and $La(III)$ to phospholipids is believed to be very similar³. An alternative explanation, however, is that the contact shift is similar or identical for all the principal components of the chemical shielding tensor, in which case the ^{31}P NMR spectral changes upon $Eu(III)$ binding would reflect changes in molecular motion at the phosphate segment. Further work, for example, determination of the ^{31}P static chemical shift tensor of DPPC complexed with shift reagents, is needed before a firm conclusion can be reached.

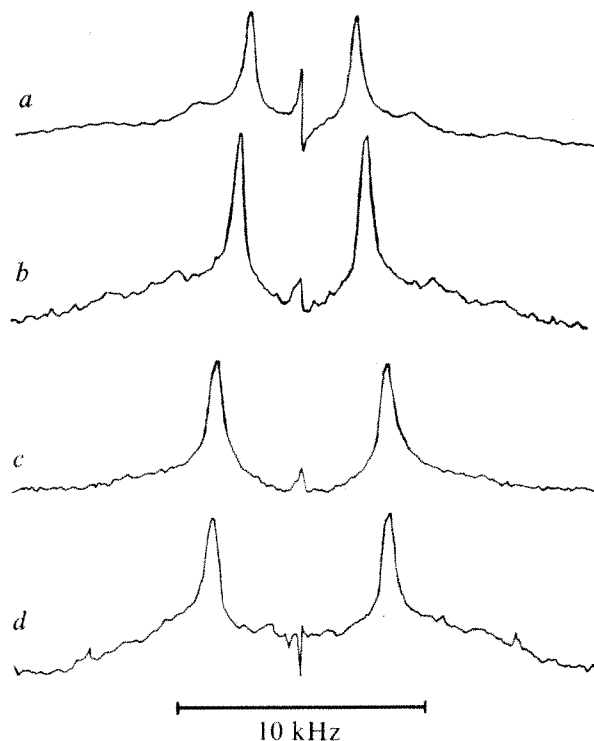


Fig. 3 Deuterium NMR spectra of unsonicated multibilayers of $^+NCD_2CH_2$ -DPPC complexed with trivalent ions at 59 °C. *a*, No ions; *b*, $Fe(CN)_6^{3-}$; *c*, $La(III)$; *d*, $Eu(III)$. Experimental conditions as in Figs 1 and 2.

The data presented here suggest that caution should be exercised in applying the results of NMR structural analysis using paramagnetic ions to phospholipid bilayers free of complexed ions. The observation of specific ion-induced changes is consistent with the idea that the phosphocholine group of DPPC is not in a fixed, rigid conformation^{3,4,8} and further suggests that structures deduced from data for binding of several ions^{5,6} may represent averages over several different conformations. These results are a direct demonstration of ion-induced changes in the head group conformation of phospholipids. Similar conclusions regarding lanthanide binding have been reached by Hauser *et al.* (manuscript in preparation). From a biological point of view, it will be interesting to see if divalent cations such as Ca^{2+} induce similar spectral changes and whether these changes can be quantitatively interpreted in terms of changes in head group conformation.

We thank Dr R. J. P. Williams for helpful comments. This work was supported by grant 3.008.76 from the Swiss National Science Foundation (J.S.) and a postdoctoral fellowship from the U.S. National Institute of General Medical Sciences (M.F.B.).

Table 1 Effect of trivalent ions on 2H quadrupole splittings ($\Delta\nu$) and ^{31}P chemical shift anisotropy ($\Delta\sigma$) of the phosphocholine group of DPPC

Segment	No. of ions added	$Eu(III)$ $\Delta\sigma$ (p.p.m.)	$La(III)$	$Fe(CN)_6^{3-}$
PO_4^-	-44	+44	-54	-51
		$ \Delta\nu $ (kHz)		
$^+NCH_2CD_2OP$	5.9	~ 1	~ 0	3.9
$^+NCD_2CH_2OP$	4.4	7.2	6.9	5.1

Ion solutions were prepared as described in ref. 5. $[DPPC]/[ion] = 2.7-3.0$. $[H_2O] = 50 \pm 0.3\%$ by weight. Temperature, 59 °C.

MICHAEL F. BROWN
JOACHIM SEELIG

Abt. Biophysikalische Chemie,
Biozentrum der Universität Basel,
Klingelbergstrasse 70,
CH-4056 Basel, Switzerland

Received 4 July; accepted 4 August 1977.

1. Worcester, D. L. & Franks, N. P. *J. molec. Biol.* **100**, 359–378 (1976).
2. Yeagle, P. L., Hutton, W. C., Huang, C. & Martin, R. B. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3477–3481 (1975).
3. Gally, H.-U., Niederberger, W. & Seelig, J. *Biochemistry* **14**, 3647–3652 (1975).
4. Seelig, J., Gally, H.-U. & Wohlgenuth, R. *Biochim. biophys. Acta* **467**, 109–119 (1977).
5. Hauser, H., Phillips, M. C., Levine, B. A. & Williams, R. J. P. *Nature* **261**, 390–394 (1976).
6. Barsukov, L. I., Shapiro, Y. E., Viktorov, A. V., Volkova, V. I., Bystrov, V. F. & Bergelson, L. D. *Biorganich. Khim.* **2**, 1404–1416 (1976).
7. Simon, S. A., Lis, L. J., Kaufman, J. W. & MacDonald, R. C. *Biochim. biophys. Acta* **375**, 317–326 (1975).
8. Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F. & Smith, I. C. P. *Biochemistry* **15**, 954–966 (1976).

Replication of linear adenovirus DNA is not hairpin-primed

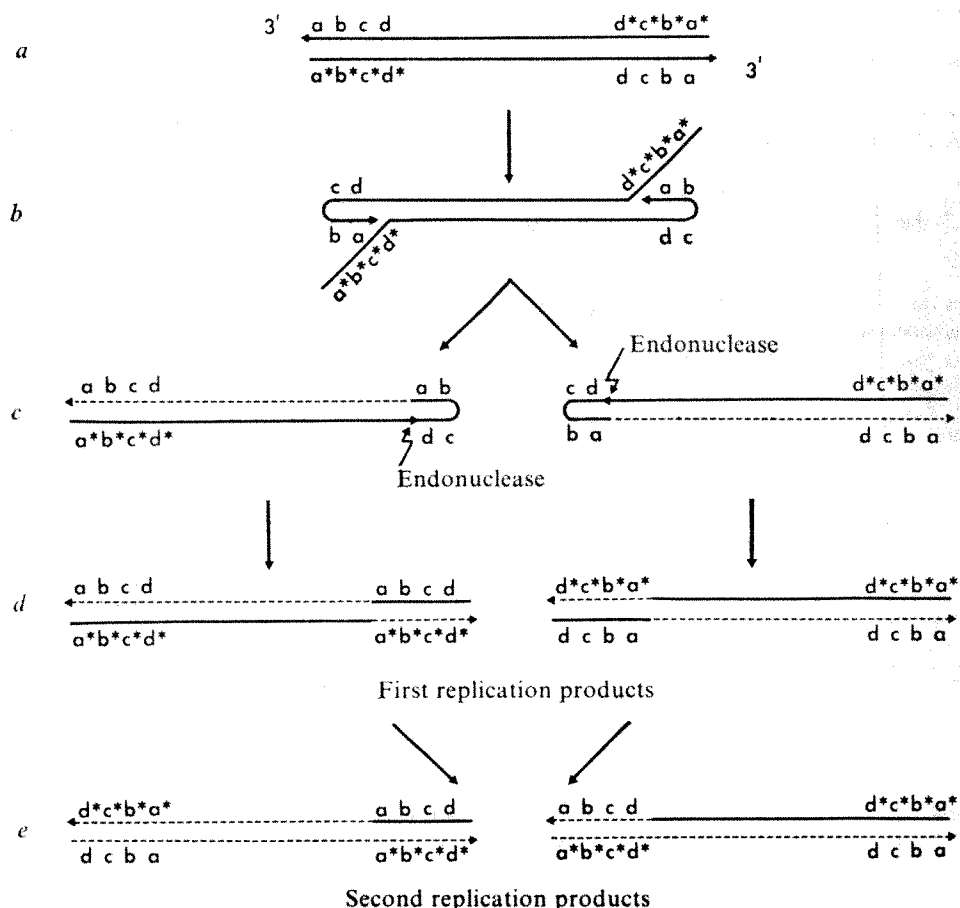
THE mechanism of replication of the 5' ends of linear DNA chromosomes remains unclear^{1,2}, as the known DNA polymerases, which act in the 5' to 3' direction, require a free 3'-OH as a primer for chain elongation. If a short ribonucleotide sequence primed DNA synthesis, its subsequent removal would leave no primer for gap fill synthesis at the 5' end. Most linear bacteriophage DNA molecules therefore have terminal repeats or single stranded complementary ends, which allow formation of circular or concatemeric replication intermediates¹. Several interesting models have been proposed to explain replication of the 5' ends of eukaryote chromosomes and linear DNA molecules which cannot circularise. Cavalier-Smith³ proposed that chromosome ends can form self-complementary hairpin loops,

allowing the parental 3' end to act as a primer for DNA polymerase, followed by endonuclease action and repair synthesis. This model has been modified by Bateman⁴ and Tattersall and Ward⁵ have extended it to propose a rolling hairpin model for the replication of parvovirus and linear chromosomal DNA. There is now considerable evidence for hairpin priming of replication of the DNA of minute virus of mice (MVM) and adeno-associated virus (AAV)^{6–7}, and hairpin priming may well be a general mechanism for replication of linear DNA molecules that are unable to circularise or form concatemers^{3,5,7}. Roberts⁸ and Wu *et al.*⁹ have proposed a hairpin-primed model for replication of adenovirus DNA, which is summarised in Fig. 1. We present evidence here which is not consistent with a hairpin-primed model for replication of adenovirus DNA.

Human adenovirus DNA is a linear duplex and has ends with properties consistent with an inverted terminal repetition that is not palindromic¹⁰. J. R. Arrand and R. J. Roberts (manuscript in preparation) have sequenced the inverted terminal repetition in adenovirus type 2 and have shown it to be a unique, non-palindromic sequence of 102 base pairs (sequence a b c in Fig. 1) with an unpaired modified 5' terminal deoxycytidine. Covalently attached to each 5' end of the DNA is a 55,000 molecular weight (MW) protein¹¹, possibly linked to the DNA by the unpaired deoxycytidine residue. The DNA replicates by a strand displacement mechanism and the origins and termini of DNA replication are at the molecular ends of the chromosome^{12–14}.

One consequence of the hairpin-primed model (Fig. 1) is that with each round of replication, the terminal sequence must be inverted, resulting in four types of molecules differing in the order of the terminal sequences. As the adenovirus termini are not palindromic, the first round of replication products would have terminal repeats of two types and 50% of intracellular viral DNA should therefore anneal

Fig. 1 Model for self-primed initiation of adenovirus DNA replication by a hairpin mechanism. *a*, Virion parental DNA (solid lines) showing inverted terminal repetition (sequence a b c); *b*, hairpin formation at the 3' end of either strand (sequence a complementary to sequence d); *c*, daughter strand synthesis in the 5' to 3' direction using the 3'-OH from the parental strand as primer, and site-specific endonuclease introducing a ss nick near sequence d; *d*, strand displacement within hairpin and completion of terminal 3' ends; *e*, products of a second round of replication by the same mechanism. Model proposed by Roberts⁸ and Wu *et al.*⁹. The events shown could occur at the end of a round of replication (to complete the 5' ends of progeny strands) rather than at initiation as shown.



to form circles after digestion of the 3' ends with exonuclease III. We find this is not the case. Viral DNA was extracted from HEK cells infected with human adenovirus type 5 (Ad5). After digestion of 0.1%, 0.75%, 1.3% and 1.6% of the double-stranded molecules in one experiment and 0.1%, 0.7%, 1.1% and 1.5% in a second experiment, the intracellular viral DNA was annealed. Circular molecules that sedimented faster than linear Ad5 DNA were not observed (Fig. 2). Samples from one experiment were also examined in the electron microscope and no circular molecules were observed. In a control experiment with T7 DNA¹⁵, using similar digestion and annealing conditions, circle formation was observed in DNA digested to 0.9% of the molecule (Fig. 2) and 89% of molecules were circular by electron microscopy, whereas T7 DNA failed to circularise when digested to only 0.15%. These results indicate that non-inverted terminal repetitions of the type shown in Fig. 1d do not occur within the terminal 260 bases of replicating Ad5 DNA, as has been reported previously for mature DNA isolated from virions^{15,16}. As the terminal sequence is not palindromic (J. R. Arrand and R. J. Roberts, in preparation), we conclude that inversion of the termini during replication, which is a logical consequence of the hairpin-primed model for non-palindromic termini (Fig. 1), does not occur during adenovirus DNA replication. It is interesting to note that there is evidence for a terminal palindromic base sequence in AAV DNA⁶, supporting other evidence for a hairpin-primed mechanism for AAV DNA replication.

The hairpin-priming model also requires covalent addition of progeny DNA to a hairpin on the parental strand (Fig. 1). Some or all of the pulse-labelled viral DNA should therefore sediment faster than virion DNA in alkali and should contain sequences that spontaneously renature on rapid transfer to conditions in which duplex DNA is stable. But, fast sedimenting pulse-labelled adenovirus DNA has not been observed in alkaline gradients^{2,10,17}, and we have been unable to detect spontaneously renaturing sequences in replicating adenovirus DNA by chromatography on hydroxylapatite (data not shown). This may be because the replication intermediates predicted by the model are highly transient, but such intermediates were observed during AAV DNA replication^{8,18}.

We tested a further prediction of the hairpin-primed model for adenovirus DNA replication which is independent of the exact nature of the terminal sequences and whether self-priming occurs at initiation or termination of synthesis. It can be seen from Fig. 1 that the model predicts a strand switch in which the original 3' terminus becomes the 5' end of progeny strand, to be replaced by newly replicated DNA at the 3' end of the parental strand. Thus all newly replicated molecules should have newly replicated DNA at both 3' ends of the double-stranded DNA. This was tested experimentally by pre-labelling Ad5 DNA with ³H-thymidine and then transferring to medium containing ³²P and 5-bromodeoxyuridine (BUdR) (Fig. 1). If the model is correct, molecules that had undergone one round of replication in BUdR, isolated as a peak of hybrid (LH) density in CsCl, should have all their 3' ends labelled with ³²P. But, 50% of the 3' ends of LH DNA should be labelled with ³H rather than ³²P if there is no terminal strand switch during replication. The nature of the label at the 3' ends of LH DNA isolated from infected cells by CsCl equilibrium density centrifugation and hydroxylapatite chromatography was examined by exonuclease III digestion. Figure 3 shows the release of ³H-thymidine acid-soluble counts plotted as a function of the release of ³²P acid-soluble counts by exonuclease III digestion. The points form a straight line through zero, without the delay in release of ³H predicted by the model. The 95% confidence limits for the intercept of the curve indicate that a strand switch of less than 16

bases cannot be excluded, but such a short switch is not consistent with terminal sequence data (J. R. Arrand and R. J. Roberts, in preparation). Figure 3 also shows the expected curve if a strand switch of 120 base pairs occurred (that is, the smallest possible hairpin from sequence data). In this case, the intercept would be 0.68% ³²P counts released and zero ³H counts released, but this is clearly not the observed result. The discrepancy between our result and that predicted by the model is unlikely to be an experimental artefact. Contamination of LH DNA with ³H-LL DNA was not detectable by recentrifugation. Recombination between replicating molecules was not detectable as ³²P radioactivity entering the LL (³H) peak. Continued incorporation of ³H after the shift to ³²P, BUdR medium was prevented by adding a 33-fold excess of BUdR over the original ³H-thymidine concentration and addition of 5-fluorodeoxyuridine. Nearly all molecules labelled in a 3-h period would be mature, without gaps at which exonuclease could act, and completed molecules were further selected by using only the centre of the peak of LH molecules, and centrifuging the sample a second time in CsCl.

We have presented evidence that hairpin priming does not

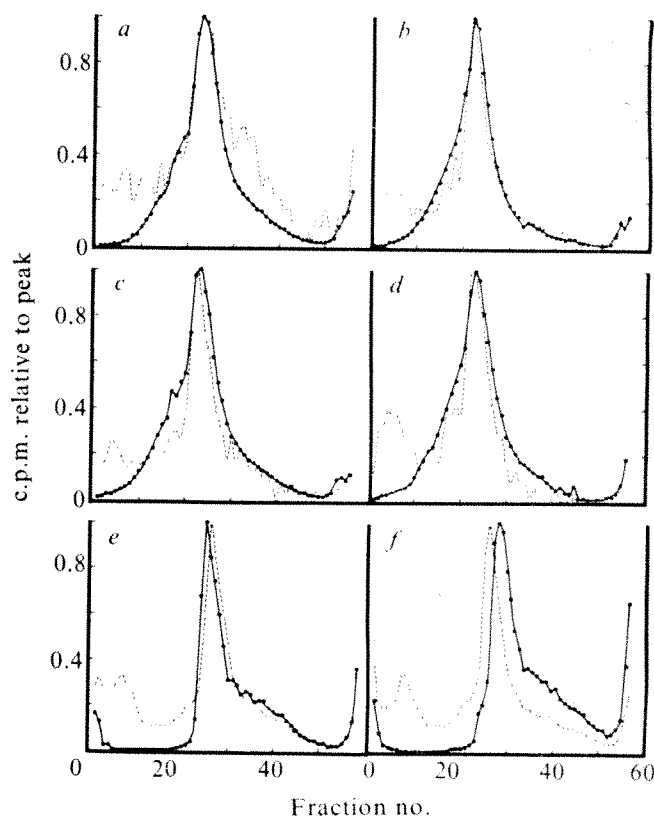


Fig. 2 Neutral sucrose gradient sedimentation of pulse-labelled Ad5 DNA and T7 DNA after exonuclease III digestion and annealing. Human (HEK) cells were infected with 5 plaque-forming units per cell Ad5 and labelled with ³H-thymidine from 16 to 17 h after infection. Viral DNA, isolated by a modification of the Hirt procedure²², neutral sucrose gradient centrifugation and hydroxylapatite chromatography, was digested for various times with *E. coli* exonuclease III, annealed for 30 min at 65 °C and slowly cooled to room temperature and centrifuged through a 5–20% neutral sucrose gradient (SW27, 18 h, 16,000 r.p.m. at 4 °C). ³²P labelled Ad5 DNA was added as marker (○); ³H-thymidine (●). Assuming Ad5 DNA contains approximately 35,000 base pairs, the extent of digestion from each 3' end would be 0.1% = 15 bases (a); 0.7% = 120 bases (b); 1.1% = 190 bases (c) and 1.5% = 260 bases (d). Lower panels: T7 DNA control digested to 0.15% (e) and 0.9% (f), annealed and centrifuged in similar conditions (●), with ³²P CELO virus DNA as marker (MW 29 × 10⁶)¹⁵. Sedimentation was left to right.

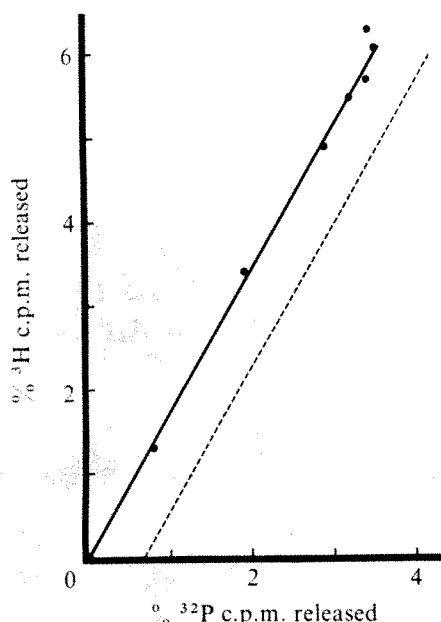


Fig. 3 Release of ^3H and ^{32}P acid soluble counts from LH DNA by exonuclease III digestion. Ad5 infected HEK cells were pre-labelled with ^3H -thymidine ($20 \mu\text{Ci ml}^{-1}$, $5 \times 10^{-7} \text{ M}$) from 6 to 16 h after infection and then washed and labelled with ^{32}P and $5 \mu\text{g ml}^{-1}$ BUdR, ($1.6 \times 10^{-5} \text{ M}$) in the presence of $0.5 \mu\text{g ml}^{-1}$ 5-fluorodeoxyuridine from 16 to 19 h after infection. Viral DNA was isolated by a modification of the Hirt procedure²², ribonuclease treated and centrifuged to equilibrium in a CsCl gradient (initial density 1.730 g ml^{-1}) in a Ti50 rotor at 33,000 r.p.m. for 64 h at 20°C . The centre of the LH DNA peak was collected and recentrifuged in a similar equilibrium gradient, purified by hydroxylapatite chromatography and digested with *E. coli* exonuclease III. ●, % Release of ^3H counts plotted against percentage release of ^{32}P counts; dashed line, expected curve if the strand switch in Fig. 1 involves 120 base pairs (see text).

occur in adenovirus DNA replication. Wu *et al.*⁹ observed some type of secondary structure at the ends of adenovirus type 2 DNA in the electron microscope, but they could not conclude whether their data supported the hairpin structure shown in Fig. 1. There is no other published evidence for hairpin priming in adenovirus DNA replication, although there is considerable evidence for such a mechanism of replication at the 5' ends of other linear viral DNA chromosomes^{5-7, 18-21}. Although the absence of hairpin-priming in adenovirus DNA replication does not indicate the mechanism by which the 5' ends of progeny DNA are replicated, we have proposed an alternative model for adenovirus DNA replication in which the terminal 55,000 MW protein binds deoxycytidine and recognises the inverted terminal repetition, providing the free 3'-OH to prime DNA synthesis¹¹. This model is consistent with data presented above and all known data concerning adenovirus DNA replication, and suggests a function for both the 55,000 MW protein and terminal repetition. It will be interesting to determine whether replication of the ends of eukaryote chromosomes is primed by hairpin loops as originally proposed by Cavalier-Smith³, by a protein-bound nucleotide as we have proposed for adenovirus¹¹, or by some other mechanism as yet unknown.

We thank Dr H. B. Younghusband for helpful discussions and electron microscopy, Dr I. R. Lehman for *E. coli* exonuclease III and Miss A. Tandy for technical assistance.

B. W. STILLMAN
A. J. D. BELLETT

Department of Microbiology,
John Curtin School of Medical Research,
Australian National University,
Canberra, ACT 2601 Australia

A. J. ROBINSON

Department of Molecular Virology,
Imperial Cancer Research Fund Laboratories,
Lincoln's Inn Fields,
London WC2, UK

Received 13 July; accepted 12 August 1977.

1. Watson, J. D. *Nature new Biol.* 239, 197-201 (1972).
2. Bellett, A. J. D. & Younghusband, H. B. *J. molec. Biol.* 72, 691-709 (1972).
3. Cavalier-Smith, T. *Nature* 250, 467-470 (1974).
4. Bateman, A. J. *Nature*, 253, 379-380 (1975).
5. Tattersall, P. & Ward, D. C. *Nature* 263, 106-109 (1976).
6. Straus, S. E., Sebring, E. D. & Rose, J. A. *Proc. natn. Acad. Sci. U.S.A.* 73, 742-746 (1976).
7. Hauswirth, W. W. & Berns, K. I. *Virology* 78, 488-499 (1977).
8. Roberts, R. J. (in the press).
9. Wu, M., Roberts, R. J. & Davidson, N. *J. Virol.* 21, 766-777 (1977).
10. Philipson, L., Pettersson, U. & Lindberg, U. *Virology Monographs* no. 14 (Springer, Vienna and New York, 1975).
11. Rekosh, D. M. K., Russell, W. C., Bellett, A. J. D. & Robinson, A. J. *Cell* 11, 283-295 (1977).
12. Horwitz, M. S. *J. Virol.* 18, 307-315 (1976).
13. Weingartner, B., Winnaker, E. L., Tolun, A. & Pettersson, U. *Cell* 9, 259-268 (1976).
14. Sussenbach, J. S. & Kuijk, M. G. *Virology* 77, 149-157 (1977).
15. Younghusband, H. B. & Bellett, A. J. D. *J. Virol.* 8, 265-274 (1971).
16. Green, M. *et al. Proc. natn. Acad. Sci. U.S.A.* 57, 1302-1309 (1967).
17. Horwitz, M. S. *J. Virol.* 8, 675-683 (1971).
18. Fife, K. H., Berns, K. I. & Murray, K. *Virology* 78, 475-487 (1977).
19. Bourguignon, G. J., Tattersall, P. J. & Ward, D. C. *J. Virol.* 20, 290-306 (1976).
20. Kocot, F. J., Carter, B. J., Garon, C. F. & Rose, J. A. *Proc. natn. Acad. Sci. U.S.A.* 70, 215-219 (1973).
21. Denhardt, D. T., Eisenberg, S., Bartok, K. & Carter, B. J. *J. Virol.* 18, 672-684 (1976).
22. Hirt, B. *J. molec. Biol.* 26, 365-369 (1967).

Benzo[a]pyrene diol epoxide covalently binds to deoxyguanosine and deoxyadenosine in DNA

CHEMICAL carcinogens, for example, benzo[a]pyrene (BaP), are metabolised to reactive intermediates which bind covalently to cellular macromolecules¹⁻³ and the extent of binding seems to correlate with the carcinogenic potency of the hydrocarbons¹. Through various mutagenicity, metabolism and binding studies, the intermediate which undergoes formation of a stable covalent complex with DNA has been identified as 7 β ,8 α -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP diol epoxide)⁴⁻⁹. The structure of a covalent adduct formed between this hydrocarbon and poly(G) has also been established⁸⁻⁹. We report here the identity of several adducts obtained by reacting (\pm)BaP diol epoxide with tritium-labelled DNA. Four differently labelled lots of DNA were synthesised *in vitro* with DNA polymerase I by incorporating in each case three unlabelled and one tritium labelled nucleoside triphosphate. Through the use of this unambiguous labelling technique we have demonstrated that activated BaP forms two adducts with deoxyguanosine, two with deoxyadenosine and possibly one with deoxycytidine, while reaction with deoxythymidine was not detected. This approach also allowed the relative percentage of each adduct to be calculated. The deoxyguanosine adducts predominated and constituted 92% of the total stable covalent adducts formed.

(\pm)BaP diol epoxide was synthesised as previously described^{10,11}. Covalent binding of adducts to calf thymus DNA was carried out at neutral pH by enzymatic activation of ^3H -BaP or reaction of unlabelled or ^{14}C -(\pm)BaP diol epoxide directly with the nucleic acid. The phosphodiester backbone of the DNA was enzymatically hydrolysed and the products were chromatographed on Sephadex LH-20. Hydrophobic material eluting from the column was concentrated and further analysed by high pressure liquid chromatography (HPLC).

Figure 1a shows a HPLC profile of hydrolysis products resulting from a co-injection of unlabelled DNA reacted with (1) ^3H -BaP plus microsomes and (2) unlabelled (\pm)BaP diol epoxide. Chromatography of DNA-BaP diol epoxide adducts alone results in a fluorescence trace identical with that of Fig. 1a. But, chromatography of the microsome catalysed adducts alone results in detection of

peaks 1, 4, 5 and 7 only. Therefore, the reaction between (\pm)BaP diol epoxide and DNA yields three more adducts (peaks 2, 3 and 6) than BaP plus microsomes and DNA.

The bases to which (\pm)BaP diol epoxide binds were identified by reacting ^{14}C -(\pm)BaP diol epoxide with each of four samples of ^3H -labelled DNA. The DNA samples were labelled unambiguously with ^3H in either deoxyguanosine,

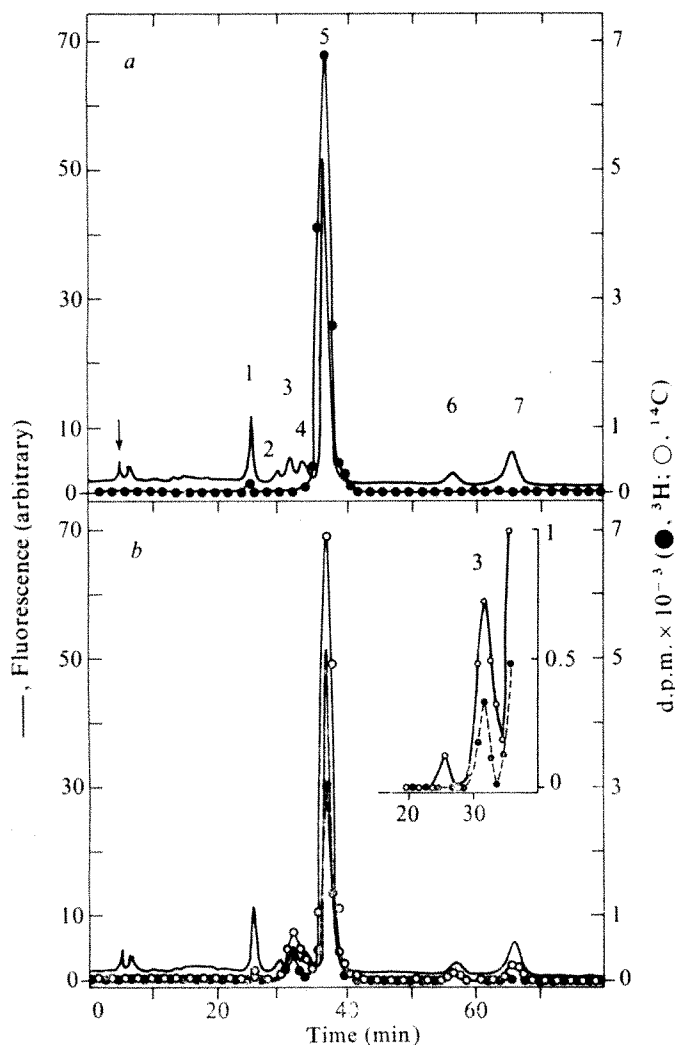


Fig. 1 HPLC profiles of BaP and (\pm)BaP diol epoxide-DNA adducts were carried out on a Varian model 8500 fitted with two columns (Waters μ Bondapak C_{18} , 4 mm \times 30 cm) in series and eluted with 50% methanol water. BaP covalent adducts were isolated after reaction of [^3H]BaP with DNA in the presence of NADPH and rat liver microsomes from animals pre-treated with 3-methylcholanthrene. Alternatively, unlabelled or ^{14}C -(\pm)BaP diol epoxide was reacted directly with unlabelled or ^3H -labelled DNA in the same conditions as the enzyme activation of the hydrocarbon. The unlabelled or ^{14}C -(\pm)BaP diol epoxide was used at the same concentration as the ^3H -BaP. The ^3H -labelled DNA's were enzymatically made by incorporation of three unlabelled and one ^3H -labelled nucleoside triphosphate with DNA polymerase from *Escherichia coli*. Calf thymus DNA, untreated, was used as template. The labelled nucleoside triphosphate was used undiluted and this procedure resulted in DNA with a specific activity of $14 \mu\text{Ci mg}^{-1}$. Four DNA samples were synthesised with ^3H located in either (1) deoxyguanosine, (2) deoxyadenosine, (3) deoxycytidine or (4) deoxythymidine. After reaction of the hydrocarbon with DNA any protein was removed by phenol extraction. The DNA was precipitated with ethanol and the precipitate heated to remove intercalated hydrocarbon. The DNA was enzymatically hydrolysed and hydrophobic material isolated by Sephadex LH-20 chromatography. The HPLC profiles represent adducts obtained from LH-20 columns. *a*, Co-injections of ^3H -BaP plus microsomes reacted with unlabelled DNA and unlabelled (\pm)BaP diol epoxide reacted with unlabelled DNA and *b*, ^{14}C -(\pm)BaP diol epoxide reacted with ^3H -deoxyguanosine-labelled DNA.

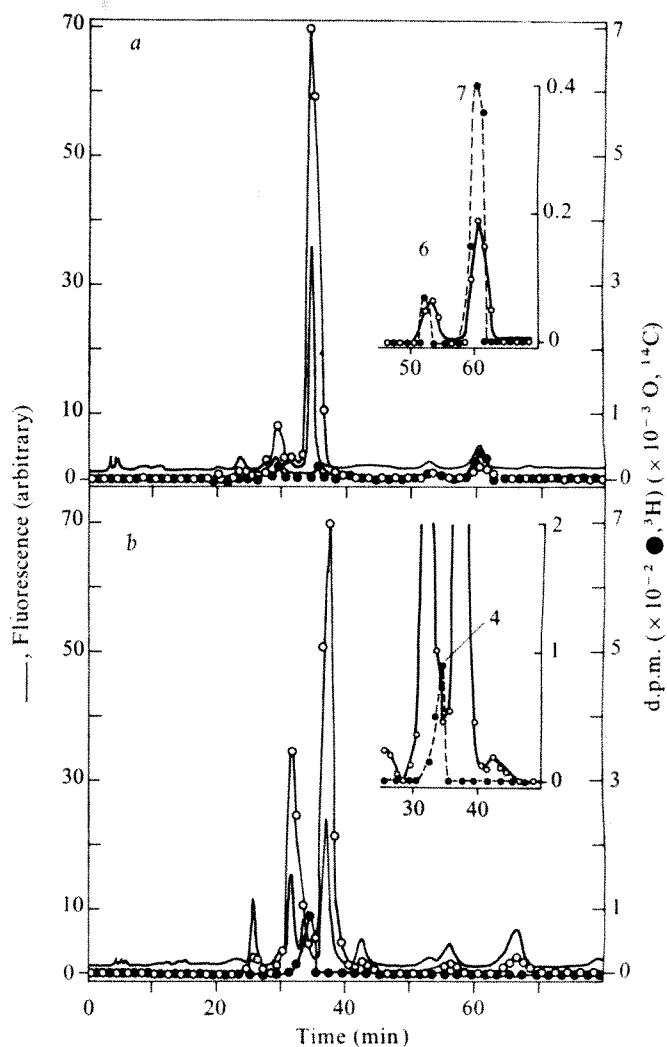


Fig. 2 HPLC profile of LH-20 samples isolated as described in Fig. 1 after reaction of ^{14}C -(\pm)BaP diol epoxide with *a*, ^3H -deoxyadenosine labelled DNA and *b*, ^3H -deoxycytidine labelled DNA.

deoxyadenosine, deoxycytidine or deoxythymidine. The four samples of labelled DNA were prepared by incorporating three unlabelled triphosphates and one ^3H -labelled triphosphate into calf thymus DNA by *Escherichia coli* DNA polymerase I. The reaction conditions were essentially as previously described¹², except that the DNA template was untreated and the tritiated nucleoside triphosphate was not diluted with cold material. The labelled DNA was ethanol-precipitated, dialysed, reacted with ^{14}C -(\pm)BaP diol epoxide and the covalent adducts were isolated after hydrolysis and Sephadex LH-20 chromatography. The results of three of the four double label experiments are presented in Figs 1*b*, 2*a* and 2*b*. HPLC of these samples resolved five ^{14}C -labelled adducts. Figure 1*b* represents reaction of ^{14}C -(\pm)BaP diol epoxide with ^3H -deoxyguanosine labelled DNA. Peaks 3 and 5 contain both ^3H and ^{14}C and therefore represent hydrocarbon-deoxyguanosine adducts. From the specific activities of both the hydrocarbon and base a 1:1 ratio of the two components was calculated. Similar inspection of Fig. 2*a* and *b* identifies peaks 6 and 7 as deoxyadenosine adducts while peak 4 may be a deoxycytidine complex. Assignment of this adduct must remain tentative because of the lack of complete symmetry and coincidence of the ^3H and ^{14}C . Reaction of deoxythymidine was not observed despite our ability to detect approximately 1 adduct per 2.5×10^7 base pairs. The relative percentage of each adduct formed is shown in Table 1. Binding occurred to the extent of 92% deoxyguanosine, 5% deoxyadenosine, 3% deoxycytidine

Table 1 Relative distribution of (\pm) BaP diol epoxide adducts with the bases of DNA

Peak no.*	1†	2	3	4	5	6	7
Base	—	?	dG ₁	dC ¹ ‡	dG ₂	dA ₁	dA ₂
Per cent							
total based on§							
³ H	—	—	10	2.4	86	0.1	1.4
¹⁴ C	—	—	10	3	82	1	4

*Peak nos refer to Fig. 1a.

†Peak 1 is probably a hydrolysis product of (\pm) BaP diol epoxide.

‡Assignment of this adduct is tentative.

§Computed from Fig. 1b.

and 0% deoxythymidine. These results are not in accord with those obtained by reaction of (\pm)BaP diol epoxide with model nucleic acids¹³, where the hydrocarbon reacted with poly(G), poly(A) and poly(C) to a similar extent. The reactions with model systems were carried out in 50% acetone which would have denatured the nucleic acids¹³, while the conditions used here were more favourable to maintaining helical conformation. The differences in reaction conditions may explain the relative lack of binding asymmetry found with the model systems.

Two adducts were obtained for both deoxyguanosine and deoxyadenosine and this could also be the case for deoxycytidine since peak 2 was not identified and could represent a second deoxycytidine adduct. The formation of two adducts with each base could have resulted either from the reaction with both stereoisomers of (\pm)BaP diol epoxide or alternatively a single isomer forming *cis* and *trans* addition products. Reaction of (\pm)BaP diol epoxide with poly(G) results in predominately *trans* addition of equal amounts of both stereoisomeric hydrocarbons⁶. We also analysed the adducts formed between (\pm)BaP diol epoxide and poly(G) by HPLC and circular dichroism spectroscopy. In confirmation of the results of Weinstein *et al.*⁶, we found that two major adducts were formed in about equal amounts and correspond to reaction of (+) and (−)BaP diol epoxide. In addition, two minor adducts were found which could represent (+) and (−)BaP diol epoxide reacting by *cis* addition of the guanine base.

Only one deoxyguanosine adduct was formed with the microsome system. This probably represents *trans* addition of only one enantiomer since enzymatic formation of BaP diol epoxide is stereoselective¹⁴. Two adducts were formed by reaction of (\pm)BaP diol epoxide with DNA and by analogy with the poly(G) system correspond to adduct formation with the (+) and (−) enantiomers of the hydrocarbon. In contrast to the poly(G) system where diastereomers are formed in approximately equal amounts, the DNA adducts occur at 90% and 10% for deoxyguanosine and 80% and 20% for deoxyadenosine. The amounts of the two adducts with deoxyguanosine and deoxyadenosine tend to equalise on binding of the hydrocarbon to heat denatured DNA. Also, there is an increase in the overall proportion of deoxyadenosine adducts. These results suggest that the asymmetric binding of the two stereoisomers of (\pm)BaP diol epoxide is less with single-stranded DNA than with double-stranded DNA. The preferential reaction of one enantiomer with DNA may be related to the dissymmetry of the right-handed helix, while the increase in deoxyadenosine adducts may result from topological changes in DNA conformation. Thus, the rates, sites and amounts of products obtained by reacting activated BaP with nucleic acids are probably controlled to a large degree by the amount of helical form and the conformation of the polymer. Of the adducts which have not been characterised the identity of peak 2 remains unknown while peak 1 co-chromatographs by HPLC with a hydrolysis product of (\pm)BaP diol epoxide. The high-resolution mass spectrum of the main deoxyguanosine adduct (peak 5) is

consistent with a structure in which the 10-position of the hydrocarbon is attached to the *N*²-exocyclic amine of the base. This structure is analogous to that obtained by reacting (\pm)BaP diol epoxide with poly(G)⁶. Quantitatively the most important adduct is binding to the exocyclic amine of guanine. Since (\pm)BaP diol epoxide formed adducts only with those bases containing a free amino group it is possible that this is the preferred binding site of the hydrocarbon.

In addition to the results reported here two other studies have indicated that BaP activation occurs by formation of the diol epoxide^{4,6}. But, evidence for other, as yet uncharacterised, intermediates has been presented^{15,16}. The difference in these results and ours may lie in the reaction conditions used. We find that magnesium chloride at 1 and 5 mM inhibits microsomal mediated binding of BaP to DNA by 60% and 75%, respectively. Since magnesium chloride was used in these studies^{15,16} it might account for the sixfold lower binding which was obtained compared with our results. If magnesium chloride specifically affected BaP diol epoxide binding another type of adduct could accumulate which would otherwise be a minor component. Even if other binding adducts do occur the importance of studying BaP diol epoxide rests on the observation that it is the most biologically active metabolite of BaP yet described^{8,21,22}.

In addition to the base adducts reported here, (\pm)BaP diol epoxide catalyses DNA and RNA strand scission¹⁷. Despite the fact that 250–300 base adducts are formed for each strand scission¹⁷ the relative importance, and indeed physiological significance, of each of these processes are unknown. Ethyl nitrosourea forms alkyl phosphotriesters and strand scissions which are lethal¹⁸. Since (\pm)BaP diol epoxide undergoes similar reactions this could account for some of the cytotoxic properties of BaP¹⁹. Furthermore, the excision repair of adenine carcinogen adducts in a number of cases is known to occur at a greater rate than those of guanine²⁰. The *N*²-exocyclic amine adduct of deoxyguanosine-BaP diol epoxide may therefore have a relatively long biological half life. As cancer induction occurs over a long period of time this may be an important adduct in the biological transformation caused by BaP.

We thank A. L. Burlingame for the high resolution mass spectrum and NCI for the ¹⁴C-(\pm)BaP diol epoxide. This work was supported in part by the US ERDA Division of Biological and Environmental Research and NCI, contract Y01-CP50203.

T. MEEHAN
K. STRAUB
M. CALVIN

Laboratory of Chemical Biodynamics,
University of California,
Berkeley, California 94720

Received 3 June; accepted 15 August 1977.

- Brookes, P. & Lawley, P. D. *Nature* **202**, 781–784 (1964).
- Gelboin, H. V. *Cancer Res.* **29**, 1272–1276 (1969).
- Heidelberger, C. *A. Rev. Biochem.* **44**, 79–121 (1975).
- Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. *Nature* **252**, 326–328 (1974).
- Meehan, T., Straub, K. & Calvin, M. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1437–1441 (1976).
- Weinstein, I. B. *et al. Science* **193**, 592–595 (1976).
- Koreeda, M., Moore, P. D., Yagi, H., Yeh, H. J. & Jerina, D. M. *J. Am. chem. Soc.* **98**, 6720–6722 (1976).
- Huberman, E., Sachs, L., Yang, S. K. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 607–611 (1976).
- Nakanishi, K. *et al. J. Am. chem. Soc.* **99**, 258–260 (1977).
- McCaustland, D. J. & Engel, J. F. *Tetrahedron Lett.* **30**, 2549–2552 (1975).
- Yagi, H., Hernandez, O. & Jerina, D. M. *J. Am. chem. Soc.* **97**, 6881–6883 (1975).
- Richardson, C. C., Inman, R. B. & Kornberg, A. *J. molec. Biol.* **9**, 46–69 (1964).
- Jennette, K. W. *et al. Biochemistry* **16**, 932–938 (1977).
- Yang, S. K., McCourt, D. W., Roller, P. P. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2594–2598 (1976).
- King, H., Thompson, M. & Brookes, P. *Cancer Res.* **34**, 1263–1269 (1975).
- Thompson, M., King, H., Osborne, M. & Brookes, P. *Int. J. Cancer* **17**, 270–274 (1976).
- Gamper, H. B., Tung, A., Straub, K., Bartholomew, J. C. & Calvin, M. *Science* **197**, 671–674 (1977).
- Singer, B. *Nature* **264**, 333–339 (1976).
- Landolph, J. R., Bartholomew, J. C. & Calvin, M. *Cancer Res.* **36**, 4143–4151 (1976).
- Dipple, A. & Roberts, J. J. *Biochemistry* **16**, 1499–1503 (1977).
- Wislocki, P. G. *et al. Biochem. biophys. Res. Commun.* **68**, 1006–1012 (1976).
- Newbold, R. F. & Brookes, P. *Nature* **261**, 52–54 (1976).

matters arising

General relativistic incompressibility

COOPERSTOCK and Sarracino¹ have attempted to redefine the concept of incompressibility in general relativity. Their result, if correct, would lead to a higher allowable maximum redshift from the surface of a bound object than is usually said to be permitted². We point out here a serious physical difficulty associated with their work. In calculating the mass of an equilibrium configuration in general relativity, the local mass density (a scalar quantity) can always be related to the pressure through an equation of state established in an inertial reference frame, without regard to the local gravitational potential. Nonetheless, Cooperstock and Sarracino choose to define a constant proper mass density $\rho_{\text{proper}} \equiv \rho(r)/g_{rr}^{1/2} = \text{constant} = a$ (say). They then compute a stellar mass from the relation

$$M = \int_0^r \rho_{\text{proper}} 4\pi r^2 g_{rr}^{1/2} dr \quad (1)$$

where g_{rr} is the radial component of the metric tensor. This definition of the mass is correct, being simply the general relativistic expression with $\rho_{\text{proper}} g_{rr}^{1/2}$ in place of $\rho(r)$.

As $\rho(r)$ is already a scalar, however, ρ_{proper} has no well defined physical significance. The division of the integral for mass into a product of a 'proper' volume and 'proper' density is arbitrary and misleading. Cooperstock and Sarracino, in asserting that the global contribution of gravitation to the overall mass-energy of a star will affect the local stress tensor, have missed the point of the equivalence principle, the very foundation of general relativity. The validity of this proposition guarantees that ρ itself (not $\rho/g_{rr}^{1/2}$) is what one would measure when applying an 'ergometer' to a small piece of matter, whether it was inside a neutron star or in empty space. In principle, the source stress tensor $T_{\mu\nu}$ appearing on the right hand side of the field equations $G_{\mu\nu} = kT_{\mu\nu}$ should contain all sources of mass-energy except gravitation. In practice, the gravitational binding energy of two neutrons, whether 10^{28} or 10^{-13} cm apart is negligible. Therefore ρ is the physical quantity relevant for local dynamical effects in neutron stars.

Nonetheless, following the procedure of Tolman³, one can treat the relation $\rho(r) = ag_{rr}^{1/2}$ as a defining equation for the radial variation of $\rho(r)$ and then deduce the equa-

tion of state $p = p(\rho)$. In that case, the equation of hydrostatic equilibrium is still given by⁴

$$\frac{dp}{dr} = -\frac{GM(r)\rho(r)}{r^2} \left[1 + \frac{p(r)}{\rho(r)c^2} \right] \times \left[1 + \frac{4\pi r^3 p(r)}{M(r)c^2} \right] \left[1 - 2GM(r)/rc^2 \right]^{-1} \quad (2)$$

Substituting $ag_{rr}^{1/2}$ for $\rho(r)$ and noting that in a physically allowable object p must be positive, all the terms on the right hand side of equation (2) must be positive, so that the pressure decreases with radius, that is $dp/dr < 0$. Now the numerical results of Cooperstock and Sarracino¹ show that $p(r)$ decreases with increasing $g_{rr}^{1/2}$, that is

$$dp/d(g_{rr}^{1/2}) < 0$$

But since $\rho(r) = ag_{rr}^{1/2}$ must be a positive quantity, $dp/d\rho < 0$. This is an unstable and unphysical situation. In order to have microscopic stability⁴, one requires $dp/d\rho > 0$. Furthermore, dividing dp/dr by $dp/d\rho$, one finds $dp/dr > 0$. It is hard to imagine that a physically realistic star with ρ increasing outward can be made. Though the resulting configuration is in equilibrium since it is a solution to the general relativistic hydrostatic equilibrium equation, it is unstable. Therefore, the resulting star constructed from Cooperstock and Sarracino's definition of incompressibility in general relativity is physically unrealisable. It seems that the redshift limit $z = 2$ set by Bondi² remains the largest allowable surface redshift consistent with both general relativity and microscopic stability.

K. BRECHER

Department of Physics,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

I. WASSERMAN

Department of Physics,
Harvard University,
Cambridge, Massachusetts 02138

¹ Cooperstock, F. I. & Sarracino, R. S. *Nature* **264**, 529 (1976).

² Bondi, H. *Proc. R. Soc. A* **282**, 303 (1964); *Lectures on General Relativity*, Brandeis Summer Institute in Theoretical Physics, 1964 (eds Deser, S. & Ford, K. W.) (Prentice-Hall, Englewood, 1965).

³ Tolman, R. C. *Phys. Rev.* **55**, 364 (1939).

⁴ Weinberg, S. *Gravitation and Cosmology* (Wiley, New York, 1972).

COOPERSTOCK AND SARRACINO REPLY—For Brecher and Wasserman, ρ_{proper} would have "well defined physical significance" only if it were completely invariant. In earlier correspondence, we had directed them to the equivalence principle, noting that this

principle renders such invariance *a priori* unattainable. The equivalence principle implies that the gravitational field can be locally transformed away by free-fall. That is not the point. The point is that with respect to a frame at rest relative to a spherically-symmetric body, ρ_{proper} certainly is well defined. It assumes the form $\rho g_{rr}^{-1/2}$ in Schwarzschild coordinates. This form has been justified by Misner and Sharp^{1,2} from dynamical considerations. We have justified the form from static considerations, and the extension has been made to charged fluid spheres (F.I.C. and R.S.S., in preparation, also, F.I.C. and V. de la Cruz, in preparation). In another coordinate system, say isotropic coordinates, it would not assume this form, but rather be determined by the integrand of the energy integral of the body over proper volume

$$M(r) = \int_0^r \rho_{\text{proper}} dV_{\text{proper}}$$

All energy, including gravitational energy, contributes to the total mass of the body and hence ρ_{proper} , which includes all energy, is the physically relevant quantity in general relativity. The failure to recognise its central role has been perpetuated by Newtonian conditioning.

On the one hand, Brecher and Wasserman assert that the validity of the equivalence principle guarantees that ρ and not ρ_{proper} is what their "ergometer" will measure. On the other hand, they then say that the gravitational binding energy of two neutrons, even 10^{-13} cm apart, is very small at any rate. If the energy is not there in principle, why worry about it in practice?

We feel that Brecher and Wasserman miss the point again. Certainly the gravitational binding for two neutrons is negligible. But, we are not concerned here about two neutrons nor indeed, necessarily about neutrons. We are concerned about conditions where large amounts of matter are being compressed towards their limit and gravitational energy is very significant indeed. This significance is made quite evident in the distinction between the bodies which satisfy the $\rho = \text{constant}$ and $\rho_{\text{proper}} = \text{constant}$ equations of state. We are not "asserting that the global contribution of gravitation to the overall mass-energy of a star will affect the local stress tensor ($T_{\mu\nu}$) . . .". We are asserting that the local contribution of gravitational energy, in addition to $T_{\mu\nu}$, determines the physically relevant proper total energy density.

Without entering the controversy regard-

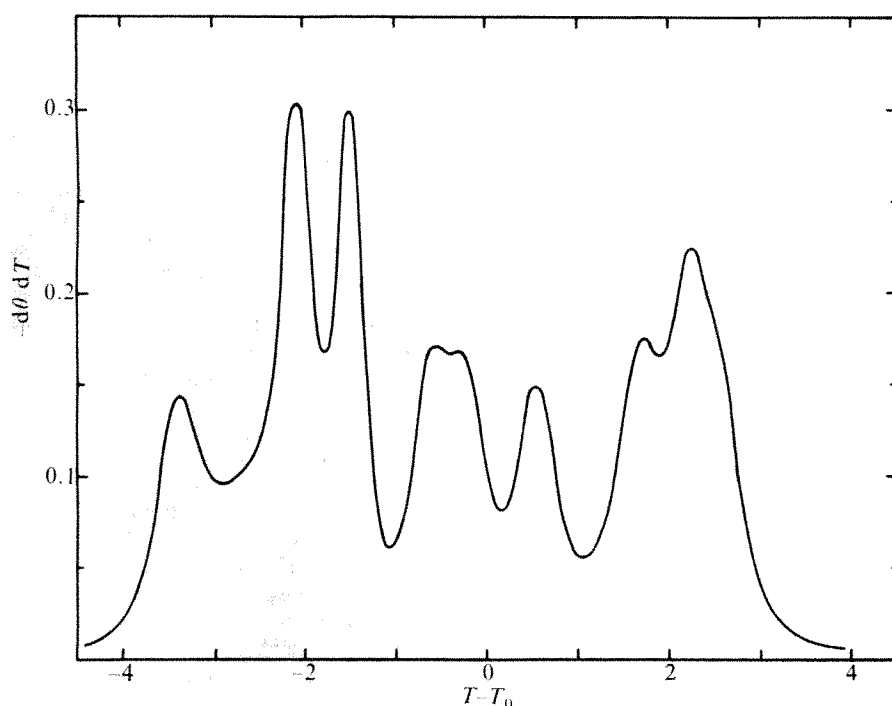


Fig. 1 Calculated differential melting curve for the block-random DNA sequence containing 4,002 base pairs. It consists of three blocks 1,334 base pairs long with GC contents of 0.43, 0.50 and 0.57. The sequences within each of these blocks were obtained using a random digits generator. The calculations were performed by the method of Poland⁵ for a standard set of the DNA thermodynamic parameters (see for example ref. 7). $\sigma = 5 \times 10^{-5}$; $T_{AT} = 340$ K; $T_{GT} = 380$ K; $U_{AT} = 8$ kcal mol⁻¹; loop-weighting exponent $\alpha = 1.5$. The negative value of the derivative of the degree of helicity θ with respect to temperature is plotted as ordinate and the difference between the temperature and the value $T_0 = 360$ K being an abscissa.

ing superluminal sound, we can certainly state that $d\rho/d\rho_{\text{proper}}$ is non-negative. One does not have to pursue the analysis of Brecher and Wasserman to determine that $d\rho/dr > 0$ for the $\rho_{\text{proper}} = \text{constant}$ equation of state. This is obvious from Buchdahl's proof. The point is that ρ_{proper} does not increase with r . To paraphrase Brecher and Wasserman, we would find it hard to imagine that a physically realistic star with ρ_{proper} increasing outward can be made. Clearly our equation of state was chosen as the limiting case which fulfills this requirement.

F. I. COOPERSTOCK
R. S. SARRACINO

Department of Physics,
University of Victoria,
Victoria, British Columbia,
Canada V8W 2Y2

⁵ Misner, C. W. & Sharp, D. H. *Phys. Rev.* **136B**, 571 (1964).

⁶ Misner, C. W. *Phys. Rev.* **137B**, 1360 (1965).

⁷ Cooperstock, F. I. & de la Cruz, V. (to be published).

The nature of the fine structure of DNA melting curves

SEVERAL reports¹⁻⁶ have suggested that the differential melting curves of DNA from various bacteriophages (including λ , T7, S_D, PM2 and fd) have a clear-cut fine structure. This fine structure is seen as a series of sharp peaks

with half-width of about 0.3° . Experimental results obtained in the various laboratories correlate well and thus one can suggest that fine structure of the differential melting curves is a general feature of all DNA's containing several tens of thousands or less of base pairs. It is clear also that a particular profile of the differential melting curve reflects a particular sequence of base pairs. Wada *et al.*⁶ have gone further and concluded that the fine structure is evidence of the existence in DNA of homostability regions characterised by highly homogeneous sequences largely different from random ones. They came to this conclusion because of their failure to obtain theoretically differential melting curves resembling experimental curves for the case of a random sequence of base pairs (see refs 2 and 4).

In contrast to the findings of Wada *et al.* we have shown⁷ that the existing helix-coil transition theory predicts the effect of fine structure in the differential melting curves for purely random sequences. The theory explains all main features of the observed effect including the width of the individual peaks, the dependence of the effect on number of base-pairs in the genome, on the state (closed or open) of circular DNA and so on (for details see ref. 5).

It has long been known that in many instances DNA consists of blocks

which differ from one another in their GC content, but inside such blocks the distribution of base pairs has been thought to be quasirandom (see for example ref. 7). λ DNA presents a classic example of such block sequences. It is clear that DNA with a block sequence will melt within broader temperature interval and simply because of this it will exhibit much greater fine structure than DNA of the same length but of random sequence (that is, consisting of a single block). In fact, in random DNA the different peaks overlap each other and smooth off the fine structure. Experimentally this tendency is shown dramatically when quasirandom T7 DNA is compared with the block λ DNA which is virtually as long but has a differential melting curve with very much more distinct fine structure.

An even more impressive example of a differential melting curve with very distinct peaks was presented by Wada *et al.*⁶. They measured the differential melting curve for the replicative form of fd DNA. This DNA consists of only about 6,000 base pairs but its differential melting curve contains as many as seven well resolved peaks.

To simulate something similar to this we have generated a sequence containing 4,002 base pairs divided in three blocks each consisting of 1,334 base pairs with the GC contents of 0.57; 0.50 and 0.43. These values were chosen so that the resultant melting curve for our artificial sequence should be as wide as the experimental curve for fd DNA. Within each block a sequence was obtained with the aid of a generator of random digits. The theoretical differential melting profile for such sequence is shown in Fig. 1. This profile consists of at least six well resolved peaks and bears a strikingly close resemblance to the experimental profile presented by Wada *et al.*⁶.

Thus we conclude that the fine structure of differential melting curves can be explained quite plausibly by the theory of helix-coil transition under the assumption that only quasirandom or block-quasirandom sequences exist. So the observed sharp peaks on the differential melting curves can not be considered as evidence for existing homostability regions, that is, sufficiently long sequences consisting of repeating short segments which contain a fixed number of AT and GC pairs as Wada *et al.*⁶ have claimed.

M. D. FRANK-KAMENETSKII
A. V. VOLOGODSKII

Department of Biology,
I. V. Kurshatov Institute
of Atomic Energy,
Moscow, 123182, USSR

- ¹ Yabuki, S., Gotoh, O. & Wada, A. *Biochim. biophys. Acta* 395, 258–273 (1975).
- ² Gotoh, O., Husimi, Y., Yabuki, S. & Wada, A. *Biopolymers* 15, 655–670 (1976).
- ³ Ansevin, A. T., Vizard, D. L., Brown, B. W. & McConathy, J. *Biopolymers* 15, 153–174 (1976).
- ⁴ Vizard, D. L. & Ansevin, A. T. *Biochemistry* 15, 741–750 (1976).
- ⁵ Lyubchenko, Yu. L., Frank-Kamenetskii, M. D., Vologodskii, A. V., Lazurkin, Yu. S. & Gauze, G. G., Jr *Biopolymers* 15, 1019–1036 (1976).
- ⁶ Wada, A., Tachibana, H., Gotoh, D. & Takanami, M. *Nature* 263, 439–440 (1976).
- ⁷ Lazurkin, Yu. S., Frank-Kamenetskii, M. D., & Trifonov, E. N. *Biopolymers* 9, 1253–1306 (1970).
- ⁸ Poland, D. *Biopolymers* 13, 1859–1871 (1974).

Crustal and *in situ* origin of Norwegian eclogites

KROGH¹ in a study of Norwegian eclogites concludes that all these eclogites can represent crustal rocks metamorphosed *in situ* during subduction to depths of 60–85 km. To show that eclogites are metamorphosed *in situ* demands that their conditions of formation are replicated in the adjacent amphibolite, or occasionally granulite facies, gneisses¹. In the western parts of Krogh's area eclogite equilibration conditions are $T \sim 800^\circ\text{C}$, $P \sim 23$ kbar¹. There are, however, no gneiss parageneses compatible with such conditions in which plagioclase is unstable². Thus applying geobarometric techniques^{3,4} to a pegmatite, said to be genetically related to adjacent eclogite⁵, maximum pressures are 12 kbar (ref. 3) or 13.2 kbar (ref. 4) for an assessed $T = 700^\circ\text{C}$. The occurrence of margarite in meta-anorthosites associated with eclogite⁶ gives maximum pressures of about 9 kbar⁷.

For Norwegian eclogites to be crustal demands that either their bulk chemical variations are controlled only by low- P fractionation, or that transitions from parent basalts and so on can be seen. I know of no unequivocal examples of the latter.

Krogh¹ does not seem to distinguish between the country-rock eclogites⁸ he describes and eclogites within ultramafic masses^{9,10}. He ignores critical evidence from the latter association that clinopyroxene, orthopyroxene and garnet show exsolution relationships^{9,10} which suggest high T - P conditions (1,100–1,600 $^\circ\text{C}$, 20–40 kbar) not compatible with crustal metamorphism or a subduction regime. He also ignores the description of orthopyroxene exsolving garnet from a country-rock eclogite¹¹ (recent work¹² suggests $T = 1,200$ – $1,370^\circ\text{C}$, $P = 30$ – 33 kbar).

Krogh demonstrates that there are regional variations in conditions of eclogite equilibration and that these conditions vary with time. He does not show how the eclogites reached this equilibrium. Exsolution data suggest that some eclogites at least,

formed from high- P liquids^{10,11} and are neither crustal nor *in situ*.

Paradoxically continent–continent collision processes¹ also seem a likely scenario for such rocks though dominated by deep level obduction¹² rather than subduction processes¹.

M. A. LAPPIN

Department of Geology
& Mineralogy,
Marischal College,
Aberdeen, UK

- ¹ Krogh, E. J. *Nature* 267, 17–19 (1977).
- ² Kushiro, I. *Miner. Soc. Am. Spec. Paper* 2 179–192 (1969).
- ³ Ghent, E. D. *Am. Miner.* 69, 710–714 (1976).
- ⁴ Schmid, R. & Wood, B. J. *Contr. Miner. Petrol.* 54, 255–280 (1976).
- ⁵ Green, D. H. & Mysen, B. O. *Lithos* 5, 147–161 (1972).
- ⁶ Lappin, M. A. *Norsk geol. Tidsskr.* 46, 439–495 (1966).
- ⁷ Chatterjee, N. J. *Am. Miner.* 69, 669–709 (1976).
- ⁸ Carswell, D. A. *Norsk geol. Tidsskr.* 53, 65–78 (1973).
- ⁹ Lappin, M. A. *J. Petrol.* 15, 567–601 (1974).
- ¹⁰ Carswell, D. A. *Earth planet. Sci. Lett.* 20, 347–352 (1973).
- ¹¹ Lappin, M. A. *Miner. Mag.* 39, 313–330 (1973).
- ¹² Lappin, M. A. & Smith, D. C. *J. Petrol.* (in the press).

KROGH REPLIES—From the first sentences and the conclusion of my article¹, it should be quite clear that I am dealing with eclogites within gneisses. Low- P (<10 kbar) fractionation may, in fact, be responsible for chemical variations within eclogites². Several lines of evidence also suggest that some of the eclogites may represent meta-sediments³. Transitions from parental low- P igneous to eclogitic parageneses are in fact, described from several localities (refs 4–7 and Bryhni & Wickström personal communication).

Gneisses from Hareidland contain plagioclase–clinopyroxene symplectites along with garnet + Ksp ± bto + rutile^{2,8}, thus indicating an originally eclogitic paragenesis in which plagioclase was unstable.

Analyses of co-existing garnet + clinopyroxene pairs from granulites at Måløy show that the $K_D^{\text{garnet-clinopyroxene}}$ values here are equal to those in adjacent eclogites (L. Malinconico, personal communication). This indicates similar metamorphic conditions in these two rock types.

Margarite in meta-anorthosite associated with eclogites is suggested by Lappin⁹ to be related to local amphibolitisation of the eclogites.

The pegmatite referred to¹¹ may well be from the field and petrographic descriptions⁸ post-eclogitic. Lappin's¹¹ limitations on the maximum pressure attained by the gneisses are thus related to later, post-eclogite events.

I have demonstrated the variation in metamorphic conditions with time by studying the chemical zoning of the minerals of the eclogites.

The inferred increase in P and T from, for example, 9.6 kbar, 505 $^\circ\text{C}$ to 17.6 kbar, 720 $^\circ\text{C}$ (ref. 1, Table 1) must be regarded as a rather marked variation. I further reported relict blueschist facies mineralogy in eclogites from Sunnfjord. In these samples, the cores of both garnet and amphibole have compositions typical for blueschist facies¹⁰, while the rim compositions are more typical for eclogites within gneisses^{10,12}. Furthermore, inclusions of low- P metamorphic mineral assemblages are fairly common in the garnets of eclogites from Nordfjord^{1,5,13} and Sunnfjord¹, indicating that prograde metamorphism has produced the eclogite mineralogy.

These variations all indicate that a large increase in P and T occurred with time at individual localities, as well as from east to west across the Gneiss Region.

With regard to the exsolution phenomena in pyroxenes^{14–16}, the exsolution history is still ambiguous. The existing chemical data are not satisfactory since the chemical zoning in the exsolved phases is not investigated. These data^{14–16}, however, still tend to give equilibrium conditions that fall within the regional P - T pattern defined for the whole area¹. A more extensive discussion of the crustal and mantle derivation of Norwegian eclogites will soon be available⁵.

The evidence of mineral zoning in individual samples, and the regional variation in equilibrium conditions strongly argues that the eclogites I have studied were formed by *in situ* prograde metamorphism of crustal rocks. This does not exclude the possibility that some of the components of this terrane, such as the ultrabasic masses, may have been introduced from the mantle^{5,11,16}.

ERLING J. KROGH

Mineralogisk-Geologisk Museum,
Sargst. 1,
Oslo 5,
Norway

- ¹ Krogh, E. J. *Nature* 267, 17–19 (1977).
- ² Mysen, B. O. & Heier, K. S. *Contr. Miner. Petrol.* 36, 73–94 (1972).
- ³ Bryhni, I. *Norges geol. Undersøkelse*, 264, 67 (1966).
- ⁴ Griffin, W. L. & Raheim, A. *Lithos* 6, 21–40 (1973).
- ⁵ Bryhni, I., Krogh, E. J. & Griffin, W. L. *N. Jb. Miner. Abh.* 130 (in the press).
- ⁶ Gjelsvik, T. *Norsk geol. Tidsskr.* 30, 33–134 (1952).
- ⁷ Griffin, W. L. *Mem. geol. Soc. Am.* 135, 37–63 (1972).
- ⁸ Green, D. H. & Mysen, B. O. *Lithos* 5, 147–161 (1972).
- ⁹ Lappin, M. A. *Norsk geol. Tidsskr.* 46, 439–495 (1966).
- ¹⁰ Coleman, R. G., Lee, D. E., Beatty, L. B. & Brannock, W. W. *Geol. Soc. Am. Bull.* 76, 483–508 (1965).
- ¹¹ Lappin, M. A. *Nature* 269, 730 (1977).
- ¹² Mottana, A. & Edgar, A. D. *Lithos* 3, 37–49 (1969).
- ¹³ Bryhni, I. & Griffin, W. L. *Contr. Miner. Petrol.* 32, 112–125 (1971).
- ¹⁴ Carswell, D. A. *Earth planet. Sci. Lett.* 20, 347–352 (1973).
- ¹⁵ Lappin, M. A. *Miner. Mag.* 39, 313–330 (1973).
- ¹⁶ Lappin, M. A. *J. Petrol.* 15, 567–601 (1974).

reviews

Wonderful contrivances

N. Feather

Perpetual Motion: The History of an Obsession. By Arthur W. J. G. Ord-Hume. Pp. 235. (Allen and Unwin: London, 1977.) £50.

THE subtitle of this book *The History of an Obsession* is significant in its place: the study is one of unrelieved failure (and a modicum of fraud); of inevitable failure when the products of obsessive fantasy are examined for sober fact. It is also apposite in another connection. In his Acknowledgements and Preface, and casually in asides throughout the text, the author gives a brief history of his own thralldom to his subject.

Mr Ord-Hume is a qualified mechanical engineer, with a successful career as a small-aircraft designer behind him, whose first encounter with the perpetual-motion saga was at his father's knee: "[My Father] used to tell me about it all when I was very small." Then, at university, his persistent importuning of one professor in particular at last brought the injunction never "to mention the subject again". In later life Mr Ord-Hume began a file of literature references to perpetual motion, as a by-product of research for other writings. The file grew and grew. Finally, a few years ago, the BBC's producer of *Horizon* planned a programme on perpetual motion, and he was asked to help. As he reports: "For several weeks I became thoroughly steeped in this subject, exploring in depth the references which I had filed away over the years . . . [the] material ended up in the form which you now have in your hands".

The form, alas, is not entirely prepossessing, there are footnotes with references to cited literature, there are four pages of bibliography and an index of more than six pages, indeed the trappings of a scholarly work, but the writing is far from scholarly. (On the author's behalf it should, however, be said that it was not his intention to write academically—rather to provide "a subjective history of the quest for the impossible".) Mr Ord-Hume should at least, however, have supervised his indexer. A single person referred to in different places in the text under slightly different guises (as Edward J.

Wood, rather than as Wood *simplificiter*, or as Edward Somerset, rather than as the Marquis of Worcester) is apt to be indexed under each guise, without cross-reference—and the author himself might well have considered the item "Ord-Hume, author, quoted" as supererogatory!

More serious, for whatever readership he was consciously writing, Mr Ord-Hume should have made sure that he got his elementary physics right. His version of Newton's first and third laws will pass muster, but his second law will not—"When a force acts upon a body in motion, the effect of this action is the same, in magnitude and direction, as if it acted on the body at rest." His version of the second law of thermodynamics reads: "heat cannot be increased without the expenditure of more work: it cannot run uphill".

There is much, then, to irritate the knowledgeable, or confuse the tyro, in this book, but there is also a wealth of solid information, and a gallery of illustrations (some ninety of them)

of the strange and wonderful contrivances from none of which, in the upshot useful work was obtained without drawing on an external source of energy (some, in fact, were never intended to do external work—the self-winding clocks, for example). The illustrations alone provide interest and instruction; it is convenient to have them in a slender (and not immoderately priced) volume. Apart from this aspect, however, the serious engineer will probably in the end find it necessary to seek out the two-volume work of Dircks (vol. 1, 1861; vol. 2, 1870) in the library, if he wishes to pursue the subject in depth.

Mr Ord-Hume may claim to have brought the story up-to-date by the inclusion of Strutt's radium clock, the Wilberforce pendulum and the breeder reactor as objects of discussion—but again, I fear, he has garbled the physics in the process. □

N. Feather is Emeritus Professor of Natural Philosophy at the University of Edinburgh, UK.

Plant cell and tissue culture

Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Edited by J. Reinhart and Y. P. S. Bajaj. Pp. 803. (Springer: Berlin, Heidelberg and New York, 1977.) DM190; \$83.60.

PLANT CELL and tissue culture is developing into an active and exciting area of research and is permitting novel lines of investigation in the various plant sciences. *In vitro* techniques are being widely used: on the one hand they enable manipulations of microbial genetics, physiology and biochemistry to be accomplished with higher plant materials; whereas on the other hand, they prove a direct tool for the propagation of useful agricultural varieties. A major volume attempting a contemporary review of the field should reflect a fair measure of this excitement and potential. Indeed, there are some excellent and readable reviews in *Plant Cell, Tissue and Organ Culture* which capture interest and stimulate thinking. Several reviews of plant cell culture techniques

are particularly notable. These excellent gems are, however, set with uninspired efforts to make up this collection of thirty-five articles. Several of the reviews are either very poorly written or ramble from the declared topics.

The whole volume can be compared to that paragon of biological reviews, *The Bacteriophage Lambda*, published by Cold Spring Harbor Laboratories. One profound difference is immediately evident. Although the *Lambda* book seems to be a product of firm editorial control, this present book does not seem to have had such direction and, consequently, reads as if it were written by committee. Further, the *Lambda* book focuses on an organism, and thus, has a unifying theme, whereas this volume attempts to cover not only many organisms but also the entire complex phenomenology of plant cell, tissue and organ culture. Perhaps this is too much to ask of one book. We are left with the impression that the immediate details of the technology of *in vitro* plant culture overshadowed a coherent presentation of the underlying

biological principles. In a sense, the volume doesn't constitute a book (although bound as one) but a collection of reviews on *in vitro* plant culture, which could be the tissue culture chapters from books on specific crops, cytogenetics, development or other aspects of plant biology.

A closer look at a few of the chapters is instructive. Chapter 1 is called "Regeneration of Plants, Vegetative Propagation and Cloning". The authors of ten individual articles which comprise this chapter discuss many other aspects of plant cell and tissue culture besides these topics. A good number of them cover in elementary detail the very basics of plant cell culture, such as media composition and preparation of the explant; others attempt to cover all aspects of plant cell culture, including such items as haploids, mutagenesis, disease resistance and economics. The present chapter title is perhaps inappropriate and misleading, and could better be "Current Status of Cell and Tissue Culture with Regard to Selected Crops". This first chapter might have been organised around fundamental aspects of regeneration. What induces organogenesis and embryogenesis? What do we know and what do we need to know? What approaches are currently being taken to get answers to these questions? Then there could have been a final section on current applications of the *in vitro* techniques and further prospects. In organising the chapters by crops, the editors have permitted redundancy and inconsistency in the degree of detail.

Chapter 3, entitled "Cytology, Cytogenetics and Plant Breeding", doesn't

deal with plant breeding at all; that is, it doesn't give examples of breeding schemes or new varieties or individual plant breeders who utilise tissue culture techniques. There is also an inappropriate mixing of whole plant and tissue culture experiments. The reviews are typically a cataloguing of experiments, with little or no attempt to pull these findings together into a hypothesis (let alone, a theory!).

Chapter 4, "Protoplasts, Somatic Hybridisation and Genetic Engineering", contains very detailed reviews of the subjects it considers. In particular, each article tends to have full discussions of the experimental technique involved, although occasionally, at the expense of a critical evaluation of the implications of the data obtained. There is no mention of either recombinant DNA or crown gall—disappointing, since these are two very promising approaches to genetic engineering. Each article unfortunately contains small sections which repeat the material discussed as the main topic of another section.

Chapter 6, entitled "Cell Culture and Secondary Products", is very straightforward with three subsections. These include culture techniques, secondary products, and tissue culture and pharmacy. A nice addition to the extensive secondary products list in section two would have been the area of importance of these products. The author of section three was the only one to make a real effort to discuss the exciting range and importance of plant secondary products. This chapter contains a great deal of information which should be of value to

those contemplating, or presently involved in secondary product research, but may be a confusing compendium of chemicals to others. The excitement of the area—where it's been, where it is and where it's going—was not obvious.

Some positive suggestions may be in order. The editors might have written a reasonably in-depth introduction to each major section. This could have served the function of bringing loose ends together and pointing out unifying concepts. If the book is ever revised, as such a work might be, it could be offered in several smaller volumes, even in paperback. This would allow investigators to buy just those sections which are of most interest to them. In fact, each chapter could have been one paperback. The redundant sections would then have become useful, the price could have been cut considerably, and the material might have reached a wider market.

In spite of its deficits, *Plant Cell, Tissue and Organ Culture* will find its way into a great number of laboratories working with plant cell culture. It does contain a copious quantity of factual information and thus fills a need. It is a shame that the publishers have decided to fix the price so high, and that copyright laws restrict photocopying. A photocopy at one quarter the expense is attractive—but we don't want to introduce scientists into a life of crime!

Peter S. Carlson

Peter S. Carlson is John Hannah Professor of Agriculture at Michigan State University.

Atomic collision theory

Potential Scattering in Atomic Physics. by P. G. Burke. Pp. viii + 138. (Plenum: New York and London, 1977). \$27.

ALTHOUGH many of the theoretical ideas underlying the scattering of electrons by atoms were developed in the 1930s, particularly by Sir Harrie Massey and his collaborators, it was not until the development of electronic computation in the early 1950s that really extensive calculations could be carried out to test these ideas. It was a happy chance that the start of Professor Burke's career (under Sir Harrie Massey at University College, London) coincided with this development, of which he took the greatest advantage. Now he is not only one of the leading authorities on the theory

of atomic collisions, but also on the complex techniques involved in the computation of cross-sections which are both of intrinsic interest and of practical importance in the theory of plasmas, of astrophysics and in parts of theoretical chemistry. With the author's reputation in mind, one turns to this little book with high hopes, which are not disappointed. Here is a marvellously lucid and concise account of the elementary parts of atomic collision theory admirably serving as an introduction to the subject.

The book starts with an account of scattering by short range spherically symmetrical potentials, which is then generalised to include long range Coulomb potentials and spin-dependent potentials. Following this basic material, elastic scattering of electrons by atoms or ions is discussed and it is shown how, at low energies, the effective range and quantum defect theories can be developed. This leads naturally into a discussion of resonance scattering

with a brief account of some of the analytical properties of the scattering matrix. Some of the most fruitful techniques for the many electron problem have been based on variational methods and these are discussed in some detail, together with the associated problem of finding bounds to the phase shifts. The book concludes with chapters on the Born series and its convergence, and on those approximations that have been developed for use at high energies, including the well known Glauber approximation.

Although much of this book could be read with profit by final year undergraduates, it will be of most use to those students who are starting research in atomic physics or quantum chemistry, for whom it will serve as an elegant introduction to the original literature and to the advanced treatises.

B. H. Bransden

B. H. Bransden is Professor of Theoretical Physics at the University of Durham, UK.

Crab biology

The Biology of Crabs. By G. F. Warner. Pp. xii+202. (Paul Elek: London, 1977.) £6.95.

It is no easy task to write a book about crabs which will both appeal to and be bought by a wide audience. One approach is to produce a short cheap book which will enter the undergraduate market as a supplement to more general texts, a second is to aim for a more definitive work which will become a necessary standby for every research worker involved with the Brachyura. Dr Warner has made a brave attempt to bridge this gap.

First, I must indulge in that measure of nit-picking required of a reviewer to prove that the book has been read. On p81, *Inarchus* should read *Inachus*, and on p139 *Metopaulus* should be *Metopaulias*. More seriously, on p121, $a > 1$ for positive allometry and $a < 1$ for negative allometry, and not the converse as is stated. Generally, however, the book is accurate, very well written, and complemented by a set of extremely clear diagrams which are either original or redrawn.

My main criticisms deal with sins of omission rather than of commission—with the balance of the material presented in relation to the book as a whole. Naturally the criticisms will reflect my own interests and prejudices, just as the content of the book reflects those of the author.

In the first chapter on anatomy and basic function, the stomach is discussed and illustrated at length, whereas the entire reproductive system is dismissed in a half-page of text. In the third chapter compound eye theory is considered in some detail without being related specifically to the Brachyura. In the chapter on rhythms, tidal and diurnal cycles are covered fully, but annual cycles of reproduction and moulting are glossed over—by no means all temperate crabs have synchronised annual breeding periods.

The chapters on life styles and food and feeding are fascinating but all too short, whereas that on social behaviour is particularly detailed in comparison. In chapter 8 on life histories some mention of how growth can be determined by Hiatt's method or by suture tags would be worth including, and a simple diagram would profitably replace most of the long verbal description of the mechanism of autotomy. The penulti-

mate chapter attempts to bring some order to the confused state of brachyuran phylogeny. I must personally disagree strongly with Glaessner's viewpoint, and emphasise that the affinity of the Raninidae and Tymolinae are with the primitive Brachyura, and that of the Oxystomata with the other advanced Brachyura.

There is sufficient background information in this book to make it intelligible to the non-specialist, and because of this it is certainly a useful source of facts and ideas for the undergraduate who wishes to seek examples of biological theories in action. The ecological diversity, yet basically conservative morphology, of the Brachyura makes them an excellent medium for this purpose. The book is of more limited use to the research worker because of the background content and the restricted length. Few aspects are dealt with in real depth, and the number of references is small in this context. But it is the only book on crab biology available in English. It is also a readable book, and will certainly provide a good introduction to the group.

Richard G. Hartnoll

Richard G. Hartnoll is Senior Lecturer in Marine Biology at the University of Liverpool, UK.

Defining the virus

The Virus: A History of the Concept. By Sally Smith Hughes. Pp. xix+140. (Heinemann Educational: London; Science History Publications: New York, 1977.) £3.90; \$6.95.

FIFTEEN years ago, it was relatively easy to define a virus. Since then, it has become increasingly difficult. The 1960s were the hey-day of the virion—that is, the definitive, observable infective particle of a virus. It was a circumscribed, compact, convenient entity and, for any one virus, once it had been described, the virion was essentially characterised.

Eighty years ago the subject was shrouded in confusion because of the lack of knowledge. Paradoxically, it has become more difficult again now, because of a plethora of knowledge. For example, the distinction between viral and cellular DNA in the DNA tumour viruses has become increasingly blurred, and, so far as the bacterial viruses are concerned, the plasmids have obstructed the once clear picture of phages as bacterial viruses.

The concept of the virion as the essential and definable stage in the

biology of any virus was not easily won, and the slow climb from confusion with the bacteria to the molecular biology of the 1960s makes an interesting story. Needless to say, it was not a smooth one, and it went by fits and starts rather than by steady progress. The 1890s were a watershed, when the agents of tobacco mosaic and foot-and-mouth disease were characterised as novel and even bizarre.

Dr Hughes spent three years as a historian in an academic department of virology, and her book records well the period leading up to the 1890s, and in particular the words and thoughts of Ivanovski and Beijerinck, their relative contributions to the subject, and something of how they lived, moved and had their being. (Who would have dreamed that Beijerinck's father was a tobacco-nist?) The story after the turn of the century is told somewhat more briefly, and supplemented by useful tables and appendices. The whole book is well produced and remarkably low-priced. One of its attractions is that most of the story can be read in the course of an evening. Strongly recommended.

A. P. Waterson

A. P. Waterson is Professor of Virology at the Royal Postgraduate Medical School, London, UK.

BOOKS

ON PURE
AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £7.00
Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H.K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Ancient supernovae

The Historical Supernovae. By D. H. Clark and F. R. Stephenson. Pp. x + 233. (Pergamon: Oxford and New York, 1977.) Hardback £8.50; paperback £3.95.

THE history of astronomy is a history of man struggling with new ideas and painstakingly trying to make accurate observations of celestial objects. Ideas were easier and their reporting is more widespread; flat earths, geocentric systems, perfectly smooth and spherical moons and planets, crop up in many of the contemporary books and manuscripts. Reports of observations are rarer and less easy to decipher. Some examples are Eratosthenes's estimation of the size of the Earth, the measurement of the Earth-Moon distance by Aristarchus of Samos, and the catalogue of 1,080 stars made by Hipparchus which not only gave the celestial latitude and longitude of each star but also divided them into six magnitudes according to their brightness.

Legend has it that one of the factors that motivated Hipparchus to perform this task was the appearance of a nova in Scorpio in 134 BC. Interestingly, two of the greatest astronomers of the past millennium, Tycho Brahe and Johannes Kepler, were also fascinated by the new stars which appeared in AD 1572 and 1604. Novae, supernovae and supernovae remnants also seem to have fascinated David Clark of the Mullard Space Science Laboratory, University College, London, and Richard Stephenson of the Institute of Lunar and Planetary Sciences, University of Newcastle upon Tyne, and motivated them to write this book; we should be thankful they did. Their task was one of the more difficult tasks in the study of the history of astronomy, the careful and painstaking perusal of historical records for references to new stars.

The book is divided into twelve chapters. The first sets the scene and convinces the reader as to the reasons why supernovae are so important. Not only are they one of the more spectacular of stellar events but also their remnants, the ejecta of the explosion, are amongst the most unusual and exciting astrophysical phenomena. Supernovae are thought to be responsible for pulsars, black holes, runaway stars, extended sources of radio emission, beautiful expanding nebulous objects, galactic X-ray sources and possibly gravitational radiation. About one supernova occurs in our galaxy every 120 years, the high degree of obscuration cutting down the number observed from Earth. Luckily, observa-

tions of supernovae and their remnants in other galaxies has increased our knowledge of these objects enormously.

The second chapter reviews the historical sources used—Far Eastern histories and diaries (remarkably detailed records from Chinese professional astronomers/astrologers going back to about 200 BC), Arabic astrological works, medieval European monastic chronicles and post-Renaissance European scientific writings. Chapter three considers three types of new stars recognised by Far Eastern observers—the quest stars, rayed stars and sweeping stars, the latter two being tail-less and tailed comets. It also contains a catalogue of pre-telescope novae and supernovae complete with durations and approximate celestial galactic coordinates. Chapter four discusses the characteristics of supernovae remnants and lists the rem-

nants within 10 kpc of the Sun. Chapters 5 to 11 present a detailed discussion of the new stars of AD 185, 386, 393, 1006, 1181, 1572 and 1604, the prime supernovae candidates. The book ends with the authors' thoughts on the evolution of supernova remnants and a review of the possible effects a nearby supernova would have on the Earth and its environment.

At times, this book tends to exude the musty, mothbally smell of a doctoral thesis, an impression that is not helped by the fact that the book is typed, all 232 camera-ready pages of it. It is still, however, a fascinating account of these enigmatic early observations and is well worth reading.

David W. Hughes

David W. Hughes is Lecturer in Astronomy and Physics at the University of Sheffield, UK.

X-ray structural analysis

Structure Determination by X-Ray Crystallography. By M. C. F. Ladd and R. A. Palmer. Pp. 393. (Plenum: New York and London, 1977.) \$35.40.

THE jacket description claims this book to be "an indispensable guide for advanced undergraduates and beginning postgraduates", as well as being of interest to the large number of workers using crystallographic structure determination as a research tool. It is the first reasonably comprehensive textbook on the subject to be published for several years, and its emphasis differs somewhat from its companions.

The introductory chapters on crystal geometry are extremely thorough; the detailed analysis of space groups and their symbols, in particular, should prevent much of the confusion which a newcomer to the field experiences on first meeting them. The following chapter describes preliminary examination of crystals, and is less satisfactory in that it gives considerable space to techniques not frequently used (optical properties, indexing of oscillation photographs) yet only three-and-half pages to Weissenberg and precession methods, one or both of which is used in virtually every structure determination. There is, furthermore, no mention of the determination of crystal system and unit cell dimensions from Weissenberg photographs.

Chapter 4 derives structure factor equations from first principles; as throughout the book, the derivations

are clearly expressed and attractively presented, and the worked examples (particular space groups, systematic absences) provide considerable illumination of the principles involved.

The detailed methods of X-ray structure analysis are introduced gently and relatively early via simple (but real) examples involving consideration only of special positions; it is doubtless encouraging to the beginner to see 'real' structure determinations before considering more complex topics such as Fourier series and Patterson functions. These are introduced in chapter 6, again clearly derived and with several detailed examples of the application of Patterson methods, leading naturally to a discussion of heavy atom methods and difference syntheses.

The major disappointment is the relegation of direct methods to a chapter headed "Some Further Topics". In recent years, many advances have been made in the theory and practice of direct methods, and yet no material is presented which is not in Stout and Jensen's *X-Ray Structure Determination* (Macmillan, 1968). Some mention should have been made of non-centrosymmetric direct methods and the MULTAN philosophy, at the very least.

One might disagree with the authors' choice of material and emphasis (which they defend in the Preface), but one cannot criticise the quality of presentation. It must be asked, however, whether this book represents any substantial advance on other (cheaper) books already available on the subject; regrettably, it does not.

P. G. Jones

P. G. Jones is Lecturer in the University Chemical Laboratories, Cambridge, UK.

obituary

W. W. Bishop

DR. WALTER WILLIAM BISHOP, Head of the Department of Geology at Queen Mary College, University of London, died suddenly at his home on 19 February 1977, at the age of 45.

A native son of Birmingham, he graduated from the University there in 1952 with an honours degree in geography followed by a Certificate in Education. However, his interests turned towards geology and it was in this field that he received his Ph.D. degree at Birmingham in 1956. He went immediately to Africa as geologist with the Uganda Geological Survey and, through contacts with E. J. Wayland and L. S. B. Leakey, became involved with studies of Pleistocene deposits and the geomorphological evolution of the region.

By 1959 he had demonstrated the natural origin of the supposed "Kafuan" culture and provided evidence for morphological and tectonic control of the events that had led to the erection of a rigid climatic framework that now seemed insupportable. The fossil-bearing Miocene sediments also attracted his attention and his first African paper was a brief account of Miocene mammals from Napak. Further field work in Uganda and Kenya later led him to realise the important genetic relationships that existed between the Miocene fossil deposits and the volcanic cones throughout the region.

Thus, within three years of his arrival, was established the pattern of his many important contributions on the stratigraphy, geomorphology and volcanics of the Tertiary and Quaternary in East Africa, as well as his fruitful association with archaeologists, anthropologists and palaeontologists concerned with mammalian and hominoid evolution.

In 1959 he went to the University of Glasgow as Assistant Lecturer in Geology and Assistant Curator at the Hunterian Museum, but after three years returned to East Africa as Director of the Uganda Museum in Kampala and Lecturer in Geology at Makerere College, University of East Africa. These years were very productive and Bill Bishop soon became the recognised authority on the Tertiary and Quaternary of the region. At the Museum he was responsible for several new developments, such as the establishment of a Museum Education

Service. He organised a number of expeditions, including the Baker Centenary Expedition (1963) to study the geomorphological evolution of the Lake Albert rift valley, which led to a new picture of the stratigraphy and to important fossil collections from the Pliocene-Pleistocene Kaiso Beds.

The prospect of a teaching position tempted him back to London in 1965 as Lecturer in Geology at Bedford College, where Professor Basil King was already interested in research on the volcanics of the rift valley and directed the East Africa Geological Research Unit. This unit undertook responsibility for systematic geological mapping in the Lake Baringo basin, where important sedimentary deposits were found sandwiched within a long volcanic sequence; these beds provided valuable fossil material bridging a former gap between the middle Miocene and the late Pliocene. Radiometric dating was applied to the entire Miocene succession, thus establishing for the first time a good chronology for the faunas.

After moving to Queen Mary College in 1974, Professor Bishop continued his East African field work, partly in association with Richard Leakey, but also began work in Pakistan where a group from Yale University was re-examining the Miocene-Pliocene Siwalik sediments and their critical hominoid fossils. He was invited to become Director of the Peabody Museum at Yale and his death occurred only a few months before he was due to take up this appointment. Not long before he had been honoured by the award of the Prestwich Medal of the Geological Society of London in recognition of the outstanding contributions that had made him "a world leader in the very active field of African geology."

Always a co-operative, enthusiastic and helpful person, Bill Bishop was deeply involved in local, national and international bodies ranging from student societies to the Presidency of the INQUA Sub-Commission for Quaternary Stratigraphy of Africa. He served the Geological Society of London as a Council member, as its Secretary, and as Scientific Editor of the *Journal*, doing a great deal to build up both the Society and its publication. He was joint organiser of two symposia sponsored by the Wenner-Gren Foundation for Anthropological Research and edited the two books that have

become standard references for the time scale of hominoid evolution. In 1975 he organised a highly successful three day symposium on a similar theme for the Geological Society of London, as yet unpublished.

Dr Bishop was a lively and attractive speaker and was guest lecturer at many institutions. At meetings, his tact and insight smoothed over potential conflicts and his humour was infectious. His performances in amateur dramatics and his admirable voice in light opera will long be remembered, as well as the witty ditties with which he sometimes enlivened scientific meetings. His death is not only a great loss to science but also a severe personal loss to his many friends and colleagues throughout the world. His happy family life with his wife and two sons did much to sustain him in his busy existence and his love of natural history remains imprinted in them as his science does in his students and his colleagues.

H. B. S Cooke

J. A. V. Butler

JOHN ALFRED VALENTINE BUTLER, F.R.S., Emeritus Professor of Physical Chemistry at the University of London, died on 16th July after a short illness.

'J.A.V.', as his friends knew him, was born on St. Valentine's day in 1899. His early education was at Cheltenham Grammar School, and he took his first degree in chemistry at Birmingham University. After a short stay at the University College of Swansea he spent 12 years as a lecturer at the University of Edinburgh. Although he was a physical chemist, we know that during this period he came into contact with Edgar Stedman who, in the late thirties, was beginning his work on the composition of the cell nucleus. From the few comments we heard J.A.V. make about his relationship with Edgar Stedman at that time, we gathered that it was not always harmonious. Nevertheless an obvious interest in the chemistry of the cell nucleus was planted at that time, and was to emerge later.

In 1939 a Rockefeller fellowship took him to the U.S.A. where he also served as an executive officer in the British Commonwealth Scientific Office in Washington.

In 1946 he returned to England to

work at the Courtauld Institute of Biochemistry in London for three years, and in 1949 came to the Chester Beatty Research Institute to apply his physical chemical knowledge and techniques to the study of DNA. This began an interesting and exciting 17 years, during which he studied many aspects of the chemistry of the components of the cell nucleus.

It was in 1953 that I first met J.A.V., and being a very junior member of the technical staff at that time I had little to do with him in an official capacity. However, within six months he was telling me in considerable detail why my geraniums were dying, and the need for repotting them at frequent intervals. Although I believe he was basically a shy man, he was friendly with all members of his staff, and never hesitated to allow the students a day off for the Varsity Rugby match at Twickenham.

He will be remembered also, I am sure, by his friends at the Institute, for his absent-mindedness. I have seen him return from an academic board meeting with two hats, one on his head and one in his hand, hotly pursued by the rightful owner of the second hat. On another occasion, deep in conversation with a colleague in a café, he complained about not receiving any change, only to find it in his coffee when he stirred it!

The stories about J.A.V. are legion. His lack of pens and pencils, the gunfight he caused in Chicago by losing his brief-case, and his very, very long pauses on the telephone before he answered "Butler here". He was in all ways the epitome of the "absent-minded professor".

We will also remember well his method of crossing the Fulham Road. Head down, deep in thought, shoulders slightly bent, straight across, ignoring all traffic. A screech of car brakes was often the sign that J.A.V. was crossing the road.

He was however, clear and precise about his research. Although he began by applying his knowledge of physical chemistry to the structure of DNA, he soon became interested in the wider aspects of DNA control and his interests lead him into the fields of DNA synthesis, RNA synthesis and the functions of the chromosomal proteins. This was, of course, in the fifties when molecular biology was in its infancy, and most people chose to forget that DNA was associated with an equal amount of protein.

He felt that the best strategic approach to the cancer problem was to understand the fundamental aspects of gene control and he directed his department to this end. However, he was keenly aware that this was a long term

approach and once confided to me that he was sad we could do so little of immediate value.

Apart from his work within the Institute, his scientific interests were wide and varied. He edited *Progress in Biophysics and Molecular Biology* for over 25 years and his publications, apart from his research work, varied from a text book on chemical thermodynamics to a very successful series of books, written for the layman, on various aspects of cell and molecular biology.

He was appointed to the newly-established chair of Physical Chemistry at the Institute in 1952, and was elected a Fellow of the Royal Society in 1956.

After his retirement in 1966 he still took an active interest in the work and was often to be seen at scientific meetings. He frequently called in to see us and to hear about the latest work. His last visit was early this year, when true to form he left his brief-case in my room, fortunately remembering it before he left the building.

He was widely read, keenly interested in the arts and an amateur painter of some merit.

He married Margaret Lois Hope in 1929, and as all his friends who visited his home knew well, he had a very happy and contented domestic life. His three children, two sons and one daughter, all followed his lead and have made careers in various other scientific disciplines. *E. W. Johns*

F. H. Ludlam

F. H. LUDLAM, Professor of Meteorology and Head of the Atmospheric Physics Group at Imperial College, London, died on 3 June 1977 aged 57 years. Frank Ludlam studied clouds. His descriptions of the movement of air and water vapour and rain through clouds were so intense and graphic that one began to suspect that he had some supernatural power to actually identify himself with a cloud.

He saw so much. He would come into a lecture with perhaps 20 slides then spend all his time talking about the first, frequently drawing back from removing it because some new feature had just caught his attention.

Having made fundamental contributions to the microphysics of clouds during the 1940's while working at the Meteorological Office, he moved to Imperial College and away from the study of the details of processes towards the study of the organisation and dynamical structure of clouds. This philosophy culminated in his now

classical description of the motion of air in severe thunderstorms. Like all grand ideas this one could, in retrospect, have been seen coming for years, but Ludlam had it. He discarded what he called 'the haystack theories' of storms. These were illustrated by diagrams, and thinking, with arrows all over the place. He replaced it by a streamline theory showing where the air and water came from and where it went to. He took a certain delight in the fact that, working on a tiny budget, he made a very satisfying advance in a subject of great economic importance.

Subsequently his interest shifted progressively to phenomena of larger scale. For his inaugural lecture in 1966 he showed that weather systems could be seen in a new perspective by examining them relative to axes moving with the system: again almost identifying himself with the phenomenon. This was his way of using the abstract concepts of pattern movement as distinct from pattern development developed by his penetrating contemporary, Eady. But whereas Eady's concepts were based on distinction of mechanism and arose from mathematical elegance and clarity, Ludlam made them lead to pictorial and functional beauty.

No man showed better the false distinction between artist and scientist. To him good science made a pleasing picture. He delighted in the impish gesture. Few present at a lecture to the Royal Meteorological Society (on three-dimensional data analysis) will forget the impact made when he projected a slide of a Rodin nude (very female) in the middle of a masterly description of the techniques of synoptic analysis.

In his magnum opus entitled *Cloud Physics* to be published by the Pennsylvania State University Press, he summarises his views on atmospheric phenomena from a few microns in scale to 40,000 km. He was concerned with the notion that conventional physicists, wedded to the controlled experiment, did not understand the essence of meteorology as he saw it. What he did see was an immensely elaborate system with complex feedback. The essence was the feedback and the uncontrollability of the meteorological experiment. He was the privileged on-looker. He even took lightly some of his beautiful and fundamental work on cloud microphysics and said it did not really matter if it were wrong: the atmosphere would find some way of fulfilling a more over-riding purpose whatever the details of the processes.

Such an integrating philosophy is likely to be fundamental for the study of complex systems like the atmosphere and we are privileged to have had so talented an exponent of it.

John Green

what's new—spectrometers

In addition to the regular Newly on the Market section in *Nature*, special features on a particular type of laboratory equipment or instrumentation will be published in this and forthcoming issues.

The purpose of a special feature is not to give listings of every item in the range of the manufacturers mentioned but to take a variety of equipment of interest in that feature area and give an idea of what kinds of instruments are available and from whom. There is a Reader Enquiry Coupon facing the inside back page.

Anaspec introduce a completely new type of microspectrophotometer, which will record absorption, emission, scattered or fluorescence spectra of any object capable of being viewed in a microscope. This revolutionary system is a miniaturised double monochromator spectrophotometer, that can be attached to a standard trinocular microscope, such that the only limit to sample size is the actual microscope image magnifying. The system is manufactured in the U.S.A. by Nanometrics Inc. and consists of a number of separate modules which can be purchased separately, according to need or application. These modules include microscope, spectrophotometer, wave length programme, recorder and a micro computer for more complex analysis. Applications include almost every area of life sciences, microbiology, geology, forensic science, chemistry and electronics. For example, the system can measure the absorption spectrum of a single chromophore in a cell, or in forensic studies can plot a spectrum from a part of a single fibre. In addition the system is ideally suited to measuring semi-conductor film thickness in an area as small as 0.001 mm.

Although NanoSpec/10 is designed primarily for micro work, it also has application to large area analysis on the same specimen, *in situ*, where before-and-after differences over a wavelength range caused by chemical change, migration, photosynthesis, chromatography or other effects can be computed and recorded. This is not possible with conventional instruments. Any specimen which can fit onto the

microscope stage can be studied in this manner.

Circle No. 45 on Reader Enquiry Card **KeveX** present several new systems. The nuclear fuel analyser is a way of achieving rapid, non-destructive and automatic elemental analysis of reactor fuel elements with a new computerised X-ray energy spectrometer (XES) system. Two solid state silicon detectors are multiplexed to double the spectrometer's count rate capability. 100% element certification is achieved on the order of 10 s per nuclear fuel pellet. The system is completely automatic with the computer selecting and controlling: X-ray tube voltage and current, secondary targets and filters, specimen transport, acquire and analyse data and sample advance. Other applications include: effluent analysis, pollution analysis and accurate accountability of special nuclear materials. Little or no sample preparation is required. The system can be employed as a materials monitor in response to the challenge posed by nuclear proliferation. The Ultra-Trace has the

technology. This new product will provide process control chemists, the pharmaceutical industry and medical researchers with high speed, high dynamic range analytical capabilities. Quantitative element analysis of thin films and surface layers (e.g., oxides) are achieved with a new product and a new surface analysis technique. A new product is the KeveX Alpha-X, and the new surface analysis technique uses 5-MeV α particle induced X-ray emissions. Windowless detector-cryostats and radioisotope source-collimator subsystems are combined to yield heretofore unprecedented analytical performance. Rapid non-destructive quantitative analysis of elements of atomic number 6 (carbon) and up of surface layers are now possible using the KeveX Alpha-X. For example, carbon and oxide layers of less than 15 Å can be detected. Alpha-X spectrometers appear in two configurations: (1) integrated stand-alone systems and (2) attachment or accessory surface analysers. The accessory version of Alpha-X may be attached to existing Auger/ESCA type surface analysis chambers to yield complementary information. The stand-alone system is capable of both surface and bulk material analysis.

Circle No. 46 on Reader Enquiry Card

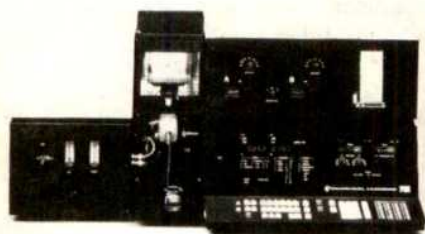
Instrumentation Laboratory (UK) Ltd introduces the IL 751 dual-channel double-beam atomic absorption spectrophotometer with built-in microcomputer. The new IL 751 Atomic Absorption Spectrophotometer is a dual-channel double-beam instrument which includes a microcomputer to make all the required computations. Each channel contains a full-size 330-mm monochromator, and both channels can operate with deuterium arc background correction. The two-channel system can produce great improvements in atomic absorption analysis, when used in either of its two main modes: (1) Doubled analytical speed. The IL 751 can analyse samples for two elements at one time. Conventional instruments can only determine one element at a time. (2) Greatly improved analytical accuracy and simplified sample preparation. In the internal standard mode, the absorbance of the element being determined is compared to that of an element having a known



KeveX Alpha-X system

unique ability to analyse many ultra-trace elements simultaneously, non-destructively and rapidly. The minimum detection limits for elements of atomic number 23 through 92 are on the order of 50–100 pg. Minimum detection limits for elements of atomic number 9 through 22 are somewhat higher. Ultra-Trace uses new and advanced X-ray energy spectrometry

concentration. This technique overcomes many matrix effects, such as sample viscosity or solution temperature, and minimises the effects of errors in pipetting or dilution. The IL 751 includes a microcomputer which performs all the calculations that are useful for atomic absorption data handling. Among these are linearisation of the analytical curves in both channels, using two, three, four, or five standards; readout in concentra-



IL (UK) Ltd 751

tion in single-channel, dual-channel, or internal standard modes; peak height and peak area measurements; and provision of statistics including the mean, standard deviation, and RSD values. There are two special features: one is a keyboard-lock mode, which secures a program from disruption by an unskilled operator or curious passer-by. The other is a standby mode, in which the computer retains its calibration program even when the instrument is switched off. The IL 751 also contains a built-in alpha-numeric printer-sequencer, which identifies the results in addition to printing them out.

Circle No. 47 on Reader Enquiry Card

Techmation announce a new plasma emission spectrometer. A new single element plasma emission spectrometer, Spectraspan IV, will be available at a price normally associated with atomic absorption units. The highly versatile Spectraspan IV, developed by SMI, can perform quantitative and qualitative analyses of trace concentrations including refractories and some non-metals. It has a high sensitivity even in the presence of complex matrix solutions with solids contents as high as 20%. The high intensity excitation source is a d.c. argon plasma which operates at low power but produces temperatures as high as 10,000 K. As with the Spectraspan III Multi-element analyser, an integral microprocessor not only increases the efficiency of the system but allows routine checks of instrument performance. Plasma emission spectrometry has applications to a wide variety of environmental samples of unknown or varying composition; including trace elements in storm drain outfalls, sewage, sediments, soil, animal tissue, oil, plastics, sea water (including

measurements of mercury concentration as low as 0.15 p.p.b.), distilled water, brackish water, waste from plating baths, trace metal impurities in alloys and total phosphorus in storm drains.

Circle No. 48 on Reader Enquiry Card

Perkin-Elmer announces the first microcomputer-based UV-VIS-NIR spectrophotometer. Designed to meet the highest possible standards, the Model 340 offers three important benefits to the user. The first is ease of operation, the second is high performance enabling highly accurate photometric determinations and the third is versatility in sample handling, obtained through convenient instrumental operating conditions and well designed accessories. The heart of the Model 340 is a microprocessor which monitors all instrumental functions. It controls source and filter changes, the stepper motor monochromator drive, the integral X-Y recorder, zero and 100%T calibrations, response times, auto zero and automatic baseline correction, etc. Covering the range 190-260 nm with a resolution of 0.15 nm, the Model 340's double monochromator reduces stray light to 0.0002%T at 300 nm; the microprocessor enables a baseline flatness of $\pm 0.001A$ in the UV/VIS region and 0.002A in the NIR region, to be achieved. A wide variety of sample handling accessories are available. The Model 340 can be equipped with long path cell holders, thermostatted cell holders and flow-cells. A complete line of integrating spheres enable precise measurements on solid and turbid samples over the entire wavelength range. A new addition to the 97 Series of IR Spectrophotometers, the Model 597, has been designed to meet the



Perkin-Elmer Model 340

growing need for instrumentation providing reliability, accuracy, versatility and simplicity of operation at a moderate cost. A high energy double beam optical null spectrophotometer, the Model 597 features an extended wavelength range of 4000 cm^{-1} to 200 cm^{-1} . Four scan speeds are offered,

three slit programmes and a time drive facility allowing transmission changes with time to be recorded on the instruments own Flowchart recorder. It is compatible with Perkin-Elmer's new Infrared Multisampler which allows up to thirty samples to be run completely automatically. For analyses in the region 250 cm^{-1} to 200 cm^{-1} , where dry air or nitrogen purging is recommended, the sample compartment has been fitted with a sliding lid. The unique check gain control allows the gain to be accurately and reproducibly set, even when the lid is closed and a sample is in the beam.



Perkin-Elmer Model 597

The double beam, UV-Vis Spectrophotometer, the Model 556, is ideally suited for applications involving turbid samples, mixture analysis and many other biochemical and industrial applications. Two separate diffraction grating monochromators, with independent selection of wavelength, produce the sample and reference beams which pass along identical paths through the sample cell. To record the spectrum of a turbid sample where no reference solution exists, one monochromator can be fixed and the other set to scan. Alternatively, the two monochromators can be fixed a few nanometers apart and both scanned together to record the first derivative of a spectrum. A simple push button action converts to double beam operation with both sample and reference beams taken from one monochromator. Regular difference spectra can then be recorded with a performance level equal to many dedicated double-beam instruments. The instrument's XY recorder provides recording of the complete spectra on precalibrated chart paper, and allows step-wise expansion and concentration. Charts are conveniently stored on a pull-out roll; automatic pen lift and scan synchronisation are provided. Superimposed repetitive scanning is possible between pre-set limits using controls that read out directly in wavelength. Spectra can be recorded at eight scan speeds (up to $1,200\text{ mm min}^{-1}$) with automatic selection in reverse.

Circle No. 49 on Reader Enquiry Card

The Schoeffel Instrument Corporation introduces a new Universal High Sensitivity Spectrophotometer, HS 870, which accepts both microliter and milliliter type cuvettes to enable the researcher the option of using the instrument as a continuously variable wavelength monitor for HPLC or to do standard spectrophotometric analysis. This two-in-one concept saves the experimenter both money and valuable space. This HS 870 uses a true double-beam approach which assures a superior signal-to-noise ratio and maximum stability. The unit features a digital data display as well as three switchable modes of operation permitting measurement of transmission, absorbance, and concentration. An automatic gain control (over two orders of magnitude) and a zero suppression (total range greater than 2 A.U. or 120%T) are standard. A BCD output for computer interfacing is optionally available. The incorporated continuously variable monochromator with wide wavelength range (190–700 nm) enables the user to select the maximum absorbance of the compound of interest. This unit is modular in design with an emphasis on practicality.

Circle No. 50 on Reader Enquiry Card

Varian announce a new, moderately priced ultraviolet-visible spectrophotometer, featuring a double-pass monochromator and exceptional photometric linearity. The Varian Cary Model 219 UV-Visible Spectrophotometer has a resolution of less than 0.07 nm and a stray light characteristic of less than 0.002% at 220 nm. Photometric linearity is 0.0016 at 1A to 0.03 at 3.0A. Ease of operation is enhanced by the instrument's wavelength coupled recorder, automatic 0%T setting, automatic source changing, automatic baseline correction and automatic repetitive scanning over the entire 185–875 nm wavelength range. Other features are a five-digit absorbance readout for high-precision measurements, expanded recorder ranges for instrument versatility and a large sample compartment preplanned for current or future accessory additions. Modes of operation include single beam for wavelength accuracy checks and double beam for normal absorptiometric applications. Either auto-slit (constant noise) or auto gain (constant resolution) can be selected when operating in the double beam mode. A beam interchange control on the front panel electrically reverses the function of the sample and reference beams.

Circle No. 51 on Reader Enquiry Card

Philips present a variety of spectrometers and ancillary equipment. The PW 1600 is a simultaneous X-ray spectrometer fitted with a 12-position sample changer and fast printer. This versatile system—which permits the analysis of up to 28 elements in under a minute—is designed to meet the demand for high volume quality control analyses in the metal, mining, cement and similar industries. There is a new 3 kW X-ray generator—the PW 1730—together with vertical and horizontal goniometers, a tabletop X-ray generator, two Debye Scherrer cameras with advanced safety features, a radiation alarm monitor specially suitable for monitoring 'soft' X-rays and the latest generation of Philips X-ray tubes. The PW 1730, can be used for single tube and



Philips PW 1730 system

two tube sequential or simultaneous operation. High voltage is variable between 20 and 60 kV and tube current between 10 and 80 mA. Stability of kV and mA is 0.002% per 1% mains voltage variation and temperature drift is 0.002% per 1 °C ambient. Maximum output power is 3 kW. To save space and cost the X-ray programming and control electronics can be housed in the generator cabinet. Where additional modules are required—such as in highly automated systems—these can be housed in the optional PW 1735 Extension Console which also includes provision to mount a second X-ray diffraction tube tower. With the many options offered, the PW 1730 can be easily upgraded as needs change and larger systems are required. For example, a simultaneous control unit allows two X-ray diffraction tubes or one X-ray diffraction and one X-ray spectrometry tube to be used simultaneously. A high voltage switch enables two X-ray tubes to be used sequentially. These accessories

eliminate the need for a second generator in many applications—a very significant cost saving. High voltage, filament supplies, safety devices and cooling water are all automatically controlled for both tubes when either a high voltage switch or the simultaneous control unit is fitted. An additional overload protection unit is also available to protect tube(s) and generator during single or two tube sequential or simultaneous operation. The PV 8350 emission spectrometer is a direct reading vacuum spectrometer providing fast quantitative measurements at a cost that makes it a practical quality control tool for even relatively small steelworks and foundries. Typically it determines up to 20 elements simultaneously, including carbon, sulphur, phosphorus and boron. The EXAM 6 energy-dispersive X-ray fluorescence system is for simultaneous elemental analysis in such fields as metallurgy, ceramics, cement, food and forensic studies.

Circle No. 52 on Reader Enquiry Card

Glen Creston Instruments Ltd present the Spex Fluorolog Spectrofluorometer and the Ramalog 5M/6M Laser Raman Spectrometers. In addition to the standard emission and ratio emission measurement, it is possible to measure transmittance, absorbance and partial fluorescence efficiency without changing the configuration of the Fluorolog. In the ratio emission (or emittance) mode, excitation spectra are corrected automatically by the reference detector. Emission and higher order corrections are possible with the computerised counterpart of the instrument. This incorporates the necessary computer and software. A phosphorimetry attachment is available as well as many other accessories such as sample heater/cooler, variable temperature accessory and polarisation kit. The Spex Fluorolog and its computerised counterpart, the Fluorocomp, are new research systems and any type of luminescence can be measured by the Fluorolog. Its modularity enables change of the source, the detector, the sample compartment, the electronics and the optics. So modified, the system can handle almost any luminescence measurement, at any temperature, in any atmosphere, in a magnetic field and under experimental conditions dictated by the effect sought. A gated detection system of photon counting is one of the Fluorolog features, giving numerous advantages accruing from counting the actual photons impinging on the PMT cathode instead of measuring the resulting direct current including a significantly improved signal

noise ratio. Either a 150 or 450 W xenon arc can be plugged in. Normally operated cw, the lamp can be pulsed with a special phosphorimetry attachment; then the pulse rate can be varied up to 60 Hz and the sampling time from 1 μ s to 10 ms. To maintain focus on a sample regardless of the excitation wavelength, and to assure maximum collection of light from the lamp, a large, off-axis ellipsoidal mirror was selected. The Fluorolog has been designed so that it can operate with a computer controlling both wavelength scanning and data gathering. A system incorporating the necessary computer and software is the Fluorocomp. This system not only acts as a data collection device, but also makes decisions in real time to change such parameters as integration time, scaling, and signal-to-noise (total number of photon counts). Subroutines already developed or being written include: repeat scanning of emission or excitation spectra; storage and retrieval of data in a floppy disc mass storage system. The Ramalog 5M/6M Laser Raman Spectrometers are the latest Spex laser Raman systems available.

Circle No. 53 on Reader Enquiry Card

Oriel introduce a CO₂ laser spectrum analyser which is a unique grating spectroscopy simultaneously displaying all the lasing transitions of a CO₂ laser. It is calibrated both in wavelength and rotational line designation to permit easy identification of 140 possible laser transitions between 9.1 and 11.3 μ m. These transitions are visually displayed through the use of an ultraviolet excited thermal sensitive screen which darkens in the area struck by the IR laser beam. The screen has a response time of 0.25 s and permits the instrument to resolve all the CO₂ rotational lines. The model 16 series is light, portable and can easily be introduced into laboratory setups.

Circle No. 54 on Reader Enquiry Card

Pye Unicam Ltd present a wide variety of spectrometers and associated equipment. The PW 1600 simultaneous X-ray spectrometer is a new multi-channel X-ray spectrometer where the total time for analysis of low or high alloy steel is 20 s, inclusive of loading the sample via the air lock and exclusive print-out of the results. High accuracy is obtained through an integrated computer with advanced software that enables the user to take full advantage of the recent achievement in the field of inter-element corrections. For this purpose tables of influence coefficients can be supplied with the instrument to suit the user's

requirements. In this way the calibration of such instruments to analyse samples with a wide range of compositions has become a matter taking only one or two days and 6 to 30 standards. In the past such wide range calibration programs have sometimes required several weeks of work and up to 200 standards. The instrument has a high stability as is required for routine operation with a minimum of recalibration time needed. A compensation has only to be made for long term drift and this is done once every 8 hs. The drift correction is made for each individual analysing channel by means of a multi-element monitor situated in the 10-position sample turret in the spectrometer. The correction is on operator's command and is computer controlled. A combination of curved and flat crystals together with a new ground cathode end window X-ray tube and advanced measuring electronics gives the PW 1600 extremely short analysis times with high spectral resolution. The PV8350 direct reading emission spectrometer provides rapid analyses at relatively low cost. The concentrations of up to 20 elements, including carbon, sulphur and phosphorus, can be determined simultaneously in materials such as steel, iron, non-ferrous alloys, etc. The instrument is designed to respond reliably to the needs of foundries and steel plants for continuous production control analyses. Outstanding analytical performance and stability are achieved



Pye Unicam SP1050

by a combination of advanced optical and electronic design. The high resolving power, blazed grating optics of one metre radius cover the wavelength range 1,770 Å to 4,100 Å in the first order at a reciprocal dispersion of 4.63 Å mm⁻¹. The instrument's 50 Hz monoalternance source unit generates a special type of discharge of improved reproducibility. Modular electronics and freedom to choose the most appropriate measurement lines in each case, enable a PV8350 spectrometer to be tailor-made to match individual analytical requirements. Running under automatic control of a number of preselected programmes, the system

is simple to operate, and its excitation stand accommodates samples of a wide range of dimensions. The electronics can provide an automatic printout of the measured intensity values by a digital printer. There are alternative electronics for teletype printout and adaptation to a computer controlled emission spectrometer with direct readout in concentration. Other modules which can be incorporated within the standard spectrometer unit include a source unit operating at 500 Hz and offering the ultimate in analysis speed. Infrared spectrometers are being used in every type of industrial and scientific environment. The vast majority of applications require a fast, robust instrument combining flexibility, high performance and simplicity of use. All of these qualities can be found in a newly developed Double Beam infrared Spectrophotometer, the SP1050. The SP1050 has a linear wavenumber scale of 600–4,000 cm⁻¹, nichrome strip source, double beam optics and grating monochromator. It has solid state electronic systems and an integral strip chart recorder. The reliability and sensitivity of the company's IR50 Infrared Detector is now well proven and this successful detector has been incorporated into the design of the SP1050.

Circle No. 55 on Reader Enquiry Card

Zeiss introduces the new DM 4 Double-Beam Spectrophotometer. The Zeiss DM 4 is the latest addition to the Zeiss line of spectrophotometers that meets practically all requirements in absorption spectrophotometry for the ultra violet, visible and near infrared regions. This makes it a very efficient instrument in any analytical laboratory, for research in the material and life sciences, as well as for routine analyses wherever accurate and reproducible results are required. The great advantage of the DM 4 is that it is equally convenient to operate for rapid routine concentration analysis and kinetics as it is when it's used as a scanning ratio recording instrument for qualitative determinations. An exceptionally large and readily accessible sample compartment contains a 6-position sample changer which can be automated. Among its many features are automatic lamp switch-over, fool-proof push-button operation, precision Zeiss grating monochromator, and unique wave-length scan. The DM 4 has a spectral range from 195 to 850 nm, an absorbance range from -0.3 to 2.5 A, a concentration range from 0 to 10,000. It requires no more than 0.3 ml sample volume. Digital and analog outputs are provided.

Circle No. 56 on Reader Enquiry Card

announcements

Appointments

Professor S. T. Butler, to Head of the Nuclear Science and Technology Branch in the Australian Atomic Energy Commission, in charge of the AAEC Research Establishment at Lucas Heights, near Sydney.

Dame Kathleen Ollerenshaw, DBE, to succeed His Royal Highness The Duke of Edinburgh, KG, KT, as President of the Institute of Mathematics and its Applications on 1 January 1978. She is currently a Vice-President.

Dr A. E. Stuart, to Professor of Pathology, University of Newcastle-upon-Tyne, from 1 January 1978, and also as Head of the Department of Pathology for a period of five years from 1 January 1978. Dr Stuart succeeds Professor A. G. Heppleston.

Awards

The Dr W. Elenbaas prize for 1977 has been awarded to the British chemist **A. H. McKeag**, in recognition of his work in the field of luminescent materials.

Two Technology Writers' Awards, sponsored by ITT Business Systems under the auspices of the Association of British Science Writers, will be awarded for work published between 1 January and 31 December 1977. One will be for work published in professional periodicals, trade and technical magazines, or national and regional newspapers, the other for material broadcast on radio or television. Both prizes will be £1,000 cash and £500 for travel or equipment. All entries are by submission (to J. Anstiss, MPR Ltd, 293 Gray's Inn Road, London WC1, UK), and it is hoped to announce the winners in February 1978.

Meetings

24–26 October, **ASTM Symposium on Erosion: Prevention and Useful Applications**, Vail, Colorado (L. E. Burgess, ASTM, 1916 Race Street, Philadelphia, Pennsylvania 19103).

28 November–1 December, **International Conference on Optimisation of Sodium-cooled Fast Reactors**, London (British Nuclear Energy Society, 1–7 Great George Street, London SW1, UK).

29–30 November, **International Conference on the Future of Natural Fibres**, Manchester (J. K. Jackson, Shirley Institute, Didsbury, Manchester, UK).

30 November–2 December, **Conference on New Developments in Automatic Testing**, Brighton (The Institution of Electrical Engineers, Savoy Place, London, UK).

Person to Person

Exchange 2-bedroom furnished house in La Jolla, California, for flat in London, July–September 1978 to September 1979. Contact M. Friedkin, University of California San Diego, M-001, La Jolla, California, 92093.

Exchange 3-bedroom furnished house in San Francisco near UC Medical Center for similar in or near Central London for one year from July 1978. Contact H. E. Varmus, 956 Ashbury Street, San Francisco, California 94117.

Observations welcome from recent work in the field of increasing crop yields using an electrostatic field. The last reference I have, after Professor Lemstrom (1904), and Sir Oliver Lodge, is J. E. Newman, who conducted trials near Pershore, England, and reported increases in wheat yields of up to 20%, and also potato yield increases in experiments in Dumfries. (See Standard Handbook for Electrical Engineers, 5th edn, McGraw Hill). The work ceased about 1917. Contact A. F. Stobart, Manor Farm, Claydon, Banbury, Oxford, UK.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

3 December, **Public Educational Forum, 'The Recombinant DNA Controversy: Public Policy at the Frontier of Knowledge'**, San Francisco (J. Stubbs, Chair, Department of Cell and Molecular Biology, San Francisco State University, 1600 Holloway Avenue, San Francisco, California 94132).

5–7 December, **International Symposium on Mechanisms of Oxidising Enzymes**, La Paz, Mexico (Professor R. N. Ondarza, Consejo Nacional de Ciencia y Tecnología Insurgentes Sur 1677–10° Piso, Mexico 20, DF Mexico). 5–9 December, **1977 Fall Meeting of the American Geophysical Union**, San Francisco (Meetings, AGU, 1909 K Street, NW, Washington, DC 20006, USA).

5–9 December, **International Conference on Radiation Measurement**, Atlanta (J. H. Kane, Special Assistant for Conferences, Energy Research and Development Administration, Washington, DC 20542).

6–8 December, **Symposium on Applications of Electroanalytical Sensors**, London (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

9 December, **21st Meeting of the Peptide and Protein Group of the Chemical and Biochemical Societies**, London (Group Secretary, The Dyson Perrins Laboratory, South Parks Road, Oxford, UK).

12–14 December, **International Conference on the Monitoring of Hazardous Gases in the Working Environment**, London (The Chemical Society, Burlington House, Piccadilly, London W1, UK).

12–15 December, **Conference on Conservation, Management and Development of Recreational Freshwater Fisheries**, Oxford (Water Research Centre, Medmenham Laboratory, Henley Road, Medmenham, PO Box 16, Marlow, Bucks, UK).

13–14 December, **Symposium on Electrocrystallisation, Nucleation and Phase Formation**, Southampton (Faraday Division, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

14 December, **Ion Sputtering and Depth Profiling in Surface Analysis**, London (Institute of Physics, 47 Belgrave Square, London SW1, UK).

20–21 December, **Symposium of the Nucleotide Group, Chemical and Biochemical Societies, on Nucleoside and Polynucleotide Synthesis and Structure**, Birmingham, UK (Please send addressed envelope (stamped if from UK) to Dr R. T. Walker, Chemistry Department, Birmingham University, PO Box 363, Birmingham, UK).

20–22 December, **Cell Motility**, London (M. E. J. Holwill, Department of Physics, Queen Elizabeth College, London W8, UK).

Meetings 1978

3-7 January, **International Astro-nomical Union Colloquium on Proto-stars and Planets**, Tucson (T. Gehrels, Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721).

4-5 January, **Fishfarming and Wastes**, London (Society of Chemical Industry, Water and Environment Group, 135 New London Road, Chelmsford, UK).
4-6 January, **15th Annual Solid State Physics Conference**, Coventry (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

24 January, **Acute and Chronic Effects of Radiation on Man**, London (Professor J. H. Martin, Department of Medical Biophysics, Blackness Laboratory, University of Dundee, Dundee, UK).

25-27 January, **Conference on Muscular Dystrophy and Other Inherited Diseases of Skeletal Muscle in Animals**, New York (Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021).

26 January, **Symposium on Internal Corrosion of Water Systems**, London (Department of Industry, Committee on Corrosion Secretariat, Room 540, Abell House, John Islip Street, London SW1, UK).

23-26 February, **Asian Congress of Parasitology**, Bombay (D. M. Renapurkar, Secretary General, Asian Congress of Parasitology, Haffkine Institute, Bombay, India).

27 February-3 March, **29th Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy**, Cleveland (United States Steel Corporation Research Laboratory, MS 57, Monroeville, Pennsylvania 15146).

6-10 March, **International Colloquium on Earth Observation from Space and Management of Planetary Resources**, Toulouse, France (Secretariat du colloque, OST, BP No. 4130, 31030 Toulouse Cedex, France).

9-11 March, **International Symposium on Quantum Biology and Quantum Pharmacology**, Palm Coast, Florida (Director, Sanibel Symposia, Williamson Hall, University of Florida, Gainesville, Florida 32611).

29 March-1 April, **3rd International Conference on Submillimetre Waves and their Applications**, Guildford (Institute of Physics, 47 Belgrave Square, London SW1, UK).

2-6 April, **2nd Argentine Congress of Palaeontology and Biostratigraphy and the 1st Latinoamerican Congress of Palaeontology**, Buenos Aires (II Congreso Argentino de Paleontología y Biostratigrafía y I Congreso Latino-

americano de Paleontología, Maipú 645, Primer Piso, 1006-Buenos Aires, Republica Argentina).

10-12 April, **International Conference on Geological Information**, London (International Conference on Geological Information, c/o Palaeontology Library, British Museum (Natural History), Cromwell Road, London SW7, UK) (for those resident in N and S America: D. C. Ward, University Libraries, University of Colorado at Boulder, Boulder, Colorado 80309).

10-12 April, **Symposium on Microprocessors in Analytical Instrumentation**, London (Gerda Holt, Scientific Symposia Ltd, 42-43 Gerrard Street, London W1, UK).

10-13 April, **Utilisation of Sewage Sludge on Land**, Oxford (The Conference Organiser, Water Research Centre, Medmenham Laboratory, Henley Road, Medmenham, PO Box 16, Marlow, Bucks, UK).

11-12 April, **Symposium on the Use of Alternatives in the Discovery, Development and Testing of Therapeutic Products**, London (Symposium Organiser, FRAME, 312a Worple Road, Wimbledon, London SW20, UK).

11-13 April, **Colloid Stability**, Lunteren, The Netherlands (Faraday Division, The Chemical Society, Burlington House, London W1, UK).

Reports & Publications

Other countries—August

Energy, Mines and Resources Canada. Geological Survey of Canada, Bulletin 262: The Origin and Migration of Petroleum in the Western Canadian Sedimentary Basin. Alberta—a Geochemical and Thermal Maturation Study. By G. Deroo, T. G. Powell, B. Tissot and R. G. McCrossan. With contributions by P. A. Hacquebard. Pp. xvii + 136, \$12. Bulletin 271: The Use of Pedological Studies in Interpreting the Quaternary History of Central Yukon Territory. By A. E. Foscolos, N. W. Rutter and O. L. Hughes. Pp. 48, \$6. Bulletin 275: Early Paleozoic Ostracoda from Southwestern District of Mackenzie and Yukon Territory. By M. J. Copeland. Pp. 88 (16 plates). (Ottawa: Geological Survey of Canada, 1977.) [48]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Environmental Mechanics—Report for 1975-76. Pp. 37. (Canberra City: CSIRO, 1977.) [59]

Cognitive Therapy and Research, Vol. 1, No. 1, March 1977. Edited by Michael J. Mahoney. Published quarterly. Pp. 1-98. Subscription rates: Vol. 1, 1977 (4 issues) \$36; Individual subscribers \$18. (New York: Plenum Publishing Corporation, 1977.) [59]

CERN—European Organization for Nuclear Research. CERN 77-12: The SPS Auxiliary Magnet Power Supplies. By H. C. Appelo and S. van der Meer. Pp. v + 23. (Geneva: CERN, 1977.) [59]

France. Commissariat à l'Énergie Atomique. Rapport En Annuel 1976. Pp. 110. Activités Scientifiques et Techniques 1976. Pp. 238. (Paris: Commissariat à l'Énergie Atomique, 31-33 Rue de la Fédération, 1977.) [88]

Institut für die Pädagogik der Naturwissenschaften/Institute for Science Education (West Germany). The IPN—Its Structure and Functions. (IPN Report-in-Brief). Pp. 82. IPN-Materialien, Übersichts 76. Pp. 187. (Kiel: Institute for Science Education (FR Germany), Olshausenstrasse 40-60, 1976.) [88]

US Department of Health, Education and Welfare. Public Health Service, National Institutes of Health. Public Accountability and Peer Review in Health Care Delivery in the United States and the United Kingdom. By S. Palmer. Pp. vi + 31. (Washington, DC: US Government Printing Office, 1977.) [88]

Rheinisch-Westfälische Akademie der Wissenschaften. Vorträge N. 169: Cytoplasmatische Actomyosine und ihre Bedeutung für Zellbewegungen. Von Karl Ernst Wohlfarth-Bottermann. Anaerobier Stoffwechsel bei Wirbellosen Tieren. Von Ernsy Zebe. Pp. 77. (Wiesbaden: Westdeutscher Verlag, 1977.) DM 80. [88]

United States Department of the Interior: Geological Survey. Professional Paper 813-G: Summary Appraisals of the Nation's Ground-Water Resources—Great Basin Region. By Thomas E. Eakin, Don Price and J. R. Harrill. Pp. iv + 37 + plate 1. (Washington, DC: US Government Printing Office, 1976.) [88]

Institute of African Studies: Africa Documentation Center. Africa Documentation Service. Series A, No. 14: Drought in Africa: Climatic, Ecological, Socio-Economic Aspects. By Marianne Weiss and Anne Jansen. Pp. 115. (Hamburg: Institut für Afrika-Kunde, Dokumentations-Leitstelle. Neuer Jungfernstieg, 21, 1976.) DM 10. [98]

Center for Disease Control: Abortion Surveillance, 1975. Pp. iv + 49. (Atlanta, Georgia: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Bureau of Epidemiology, Family Planning Evaluation Division, 1977.) [98]

US Department of Health, Education and Welfare. Development and Evaluation of a Hexokinase/Glucose-6-Phosphate Dehydrogenase Procedure for Use as a National Glucose Reference Method. By Jane W. Neese, Patricia Duncan, David Bayse, Mary Robinson, Teresa Cooper and Charles Stewart. Pp. vi + 147. (Atlanta, Georgia: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Bureau of Laboratories, Clinical Chemistry Division, 1976.) [118]

Adi Sankara Advaita Research Centre. Research Bulletin No. 1: Reflections and Speculations on Space, Time and Causality. By Dr T. S. Shankara. Pp. iv + 12. (Madras: Adi Sankara Advaita Research Centre, 28 A.N.H. Road, 1977.) Rs. 3; \$2. [118]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2162: Ground-Water Levels in the United States, 1971-74. Southwestern States. Pp. v + 86. (Arlington, Va.: Branch of Distribution, US Geological Survey, 1200 South Eads Street, 1977.) [128]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15389: Fertilization—The Long-Term Effects of Its Use in Forest Ecosystems, Current Status. Pp. iv + 18. (Ottawa: National Research Council Canada, 1977.) [158]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2163: Ground-Water Levels in the United States, 1972-74. North-Central States. Pp. v + 89. (Washington, DC: US Government Printing Office, 1977. For sale by the Branch of Distribution, US Geological Survey, 1200 South Eads Street, Arlington, VA.) [158]

Food and Agriculture Organization of the United Nations. Report on the Ad Hoc Consultation on a Scheme for Agricultural Credit Development, Rome, 3-4 June 1976. Pp. 24, 97p. FAO Fisheries Technical Paper No. 159: Production, Trade and Utilization of Seaweeds and Seaweed Products. By J. Naylor. Pp. vi + 73, £2.60. (Rome: FAO, London: HMSO, 1976.) [158]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15387: Medicare Data—Its Use in Defining the Effects of the Environment on Health. By C. A. R. Dennis, R. Drope and D. Matz. Pp. 164. (Ottawa: Publications, NRCC, 1977.) \$2.50. [178]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Computing Research, 1975/1976. Pp. iii + 71. Division of Chemical Technology: Research Review 1976. Pp. 151. (Melbourne: CSIRO, 1977.) [188]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1887: Maximum Floodflows in the Conterminous United States. By J. R. Crippen and Conrad D. Bue. Pp. iv + 52. (Washington: US Government Printing Office, 1977.) [198]

Council of Europe. European Information Centre for Nature Conservation. Selective Bibliography on Wetlands. (Documentation Series, No. 2.) Pp. iii + 38. (Strasbourg: Council of Europe, 1977.) [198]

US Department of Health, Education and Welfare. Public Health Service. Alcohol, Drug Abuse, and Mental Health Administration. Drugs and Driving. Edited by Robert E. Lilete. (NIDA Research Monograph, 11.) Pp. xiii + 137. (Washington, DC: US Government Printing Office, 1977.) \$1.70. [228]

International Atomic Energy Agency. The Annual Report for 1976. Pp. 62. The Agency's Budget for 1978. Pp. v + 119. (Vienna: International Atomic Energy Agency, 1977.) [228]

Regional Research Laboratory, Hyderabad. Annual Report, 1975/1976. Pp. xvii + 157. (Hyderabad: Regional Research Laboratory, 1977.) [238]

Hoechst Aktiengesellschaft. Annual Report 1976. Pp. 69. (Frankfurt am Main: Hoechst Aktiengesellschaft, 1977.) [248]

Collections of Research Papers Published in Foreign Countries Selected from Grantees of NSC, Republic of China, 1972-1975. Vol. 3: Mathematics. Pp. 317-604. Vol. 4: Physics. Pp. vi + 605-1152. Vol. 5: Agriculture. Pp. iii + 1153-1335. Vol. 8: Medicine (2). Pp. vii + 1985-2581. Vol. 9: Engineering and Applied Sciences. Pp. vi + 2583-2973 + 57. (Taipei, Taiwan: National Science Council, 2 Canton Street, 1976.) [248]

United States Department of the Interior: Geological Survey. Bulletin 1433: Eocene Rocks in Northeast Washington—Radiometric Ages and Correlation. By R. C. Pearson and J. D. Obradovich. Pp. iv + 41 + plate 1. (Washington, DC: US Government Printing Office, 1977.) [258]

Collections of Research Papers Published in Foreign Countries Selected from Grantees of NSC, Republic of China, 1972-1975. Vol. 1: Chemistry and Geochemistry. Pp. xxvi + 1-235. Vol. 2: Geology. Pp. 236-316. Vol. 6: Biology. Pp. iii + 1336-1556. Vol. 7: Medicine (1). Pp. v + 1557-1983. (Taipei, Taiwan: National Science Council, 2 Canton Street, 1976.) [258]

The Bushmen. Pp. 38. (Cape Town: Trustees of the South African Museum, 1976.) [268]

Electric Power Systems Research, Vol. 1, No. 1, September 1977. Pp. 1-86. 1977 subscription: volume 1, 4 issues. Institutional subscription: SFr. 145; \$60. Personal subscription: SFr. 50; \$20. (Lausanne: Elsevier Sequoia, S.A., 1977.) [268]

nature

27 October 1977

How much further can the pendulum swing?

IN THE past few years the tide of inflation has run so steadily in Britain, and the economy has been so sluggish, that we have rather lost sight of former landmarks and tended to measure performance mainly on the outcome of individual battles and on our ability to survive from year to year. Viewed in a longer time perspective, however, how has science fared in these years? In the Third Leverhulme Lecture in Liverpool last week, the biochemist Hans Kornberg pulled together a number of scattered statistics to show that although we may have survived the battles in some way or another, we are still in danger of losing the war. These matters are of particular interest now that North Sea oil is bringing some relief to a beleaguered economy and everyone who has been asked to make sacrifices is wondering when the sacrificing is going to stop.

Most striking of Kornberg's examples is the way in which expenditure on civil research and development in five European countries moved in the years 1969-74. West Germany, France and the United Kingdom spent respectively 1.5, 1.2 and 0.9 billion (10⁹) Eurodollars in 1969; by 1974 Germany was up to 3.6 billion, France to 2.0 billion . . . and the UK almost static at 1.0 billion. Expenditures in the Netherlands and Belgium, previously down at the 0.2-0.3 billion level, are steadily rising.

The figures do, of course, need extensive qualification. No one who has travelled on the Continent will be unaware of the discrepancies in cost of living which exchange rates seem poorly to reflect. And there are complications between countries in the allowance that is made for social security payments. So a better measure might well be how many people are employed in research and development per thousand of population in each country. But the general shape of the conclusion would be the same: that in Europe, Britain is lagging very seriously in its investment in civil R&D, though still spending heavily in the defence field in its urge to keep in touch with the United States.

Even within the term 'civil R&D', however, there needs to be some attention to detail. In recent years

the Rothschild reorganisation of government R&D and the more recent enthusiasm for engineering has ensured a moderate degree of health for the applied side of things. But with a static total budget this has inevitably meant harder times for the more basic side. In many ways the approach to these harder times has been too subtle to cause individual comment. One such way is the upwards creep of costs, necessary just to stand still. As staff become more senior, they are entitled to higher wages; as research proceeds, more complicated equipment is generally needed. Such costs outstrip cost-of-living scales normally used to adjust authorisations from year to year. As Kornberg puts it, "The pendulum has now swung too far [from the more affluent days of the 1960s] and its action must be reversed if it is not to cause possibly irreversible damage".

Few scientists would need to be reminded of one perfectly valid argument for supporting basic research—that you can never tell what it will turn up, and the occasional discovery, be it Newton's Laws or Mendel's peas or whatever, is infinitely more valuable in the long run than any amount of basic research. But there are two more arguments which should be deployed more frequently. They are, first, that support of basic research is support of a cultural activity, which government ought to have a genuine pride in fostering; and, second, that basic research and indeed basic researchers recognise no national frontiers, and so if support wanes in one country whilst waxing elsewhere British researchers will first have to depend more and more on the kindness of their foreign colleagues and ultimately will move themselves, or recommend their students to move to more fertile pastures.

The erosion is slow and there is no psychologically 'right' time at which to start protesting; besides, scientists recognise as well as anyone that there have been some genuine national needs for economy. But maybe Kornberg's speech, together with the Secretary of State for Education and Science's call a few months ago for a science lobby, will start something. □

The Nobel prizes (1): Physics

Rewarding solid state

Sam Edwards on Nevill Mott,
Philip Anderson and John van Vleck

IT is a pleasure to contribute a note on these awards, as I have had two of the recipients, Nevill Mott and Phil Anderson, as colleagues and have worked with them, and have attended the inspiring lectures of the third, John van Vleck, when a graduate student at Harvard.

Nevill Mott started research in physics when quantum mechanics was recently invented. His first great contribution was to show that quantum mechanics affected the most basic of scattering experiments, that of α -particle-helium collisions. Mott relates that, starting his first research appointment, he went to explain to Rutherford that his famous scattering formula needed amendment and that Chadwick, who came along, had found it was so. Rutherford listened with interest but without comment until Mott rose to leave, when the great man called after him, "If you think of anything else like this, please come and tell me".

This interest of Mott extended to atomic collisions and led to the collaboration with Sir Harrie Massey in their famous text on collision theory; it extended into nuclear scattering and very notably into the theory of internal conversion of gamma rays. His interests in the 1930s, after a move to Bristol, turned to the solid state, and there were many notable contributions to the theory of semiconductors, for example, to the understanding of rectification. This latter problem led him naturally to the study of oxides where he realised that the quantum theory of metals, semiconductors, and insulators, as developed by physicists in the 1930s, seemed quite inadequate to explain why under certain conditions oxides were conductors, whilst in other conditions they were insulators. People with a chemistry background found these facts explainable on structural grounds, but the basic interpretation of the solid state by quantum theory had now reached a level that anything other than a complete *ab initio* explanation of behaviour was inadequate.

It should be remarked that Mott had left Cambridge first for Manchester and then for Bristol where he played a major part in establishing the great eminence of the Bristol physics department, and made great contributions to the theory of dislocations, the theory of fracture and other aspects of the structure of solids, which has long

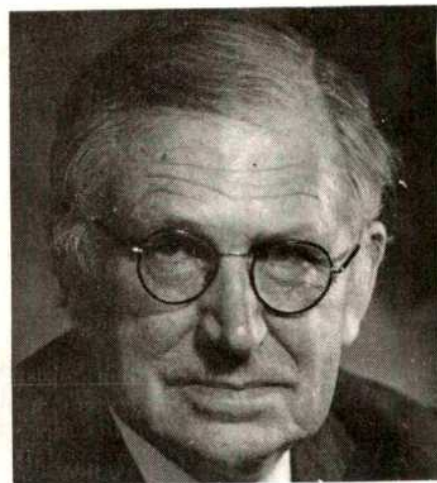
been a speciality of Bristol, and also to the theory of ionic crystals, a key to the photographic process which led to the famous book written with Gurney. He also wrote another famous book on metals in collaboration with Professor Jones, recently retired from Imperial College. But to return to the events which led to the Nobel Prize, Mott realised that external circumstances could lead a solid to make transitions between metal and insulator configurations, and the Mott transition was born. This study explained in a much deeper way than ever before why a material took up the metallic or non-metallic condition in the crystalline state, and is developed in detail in his book *Metal-Insulator Transitions*.

Back in Cambridge, he realised similar problems must exist in the amorphous state, which also shows metallic, insulator and semiconductor behaviour, and does so in a way which contradicts any straightforward approach. About this time Anderson published his paper 'Absence of diffusion in certain random lattices', which gave a rigorous basis to the phenomena of the localisation of electron wave functions by disorder. A lifetime of experience enabled Mott to see what the physical consequences of localisation would be, and a torrent of new physics resulted. Again this work formed the basis of a major book in collaboration with Dr E. A. Davis who has been Sir Nevill's experimental collaborator. Dr Davis tells me that no sooner does he prepare a new edition of the book than the number of papers in the literature justifies starting again. The phenomena flaring from the new theory include semiconductor switches and the prospect of much cheaper materials for, say, the conversion of sunlight to electricity, a field where Professor Spear has been quick to grasp the significance of the revolution and produce remarkable new amorphous materials.

The stature of Mott's work from the very beginning has been of the highest calibre, but it is perhaps most remarkable that the Nobel citation particularly referred to work done in his sixties, when most people are coasting to rest.

Anderson on magnetism

Phil Anderson started his research career studying aspects of magnetism with Van Vleck at Harvard, and early in his published papers is a notable study of the antiferromagnetic state, where he shows how quantum mechanics introduces great complexity into a problem which is classically so simple. His many early papers on magnetism led him to great facility with the application of quantum mechanical ideas in particular with spin systems into which he always tries to translate problems, since this way of thinking demands a quantal rather than classical intuition. It was natural therefore for him to think of the



Van Vleck, Anderson, Mott

diffusion of spins when he studied the problem of disorder in quantum mechanics in his famous 1958 paper referred to above. The same framework of thought produced impressive contributions to the theory of superconductivity which had been opened up around that time by the BCS theory.

When Anderson took a joint appointment in Cambridge and at Bell Telephones, one of the great fruits of his time in Cambridge was his interaction with Sir Brian Pippard and with Brian Josephson, when Josephson was developing new ideas on superconducting tunnelling which led to his Nobel prize. The other big interaction in Cambridge was with Mott and many other British physicists which has produced many new ideas both in Cambridge and elsewhere in Britain, notably with Professor Thouless in Birmingham.

Anderson also did basic work on helium 3 over this period, and even the application of solid state ideas to astrophysics, but it is his work on the electronic structure of disordered systems and on magnetism which has earned him the Nobel prize. Other notable work on glasses has been on the low temperature thermal properties, and on disordered magnets, the 'spin glasses' where it has been my privilege to collaborate with him.

I have noted Anderson's early and continued interest in magnetism and how it has influenced his other interests. That interest was kindled by J. H. Van Vleck, who brought

magnetism into the realm of quantum mechanics. It is astonishing to realise the rate of progress in understanding in the late 1920s and early 1930s when, compared to today, a small number of scientists revolutionised physics. Van Vleck published the first edition of his *Theory of Electric and Magnetic Susceptibilities* in 1932 and with this virtually the whole of the modern theory of magnetism was set up: not just solved because there are still deep and difficult problems remaining, but just how the classical theory could be absorbed into quantum theory, what the interactions had to be, how the Schrödinger equation was to be studied when crystal fields are present—it is all there, written out in a completeness and clarity which quite belie the date.

Subsequently Van Vleck developed and clarified many aspects of the electromagnetic nature of crystals, whilst also making major contributions to microwave and resonance spectroscopy, fields which have taken on new importance with the discovery of cosmic hydroxyl maser effects, and microwave communications. His work also extended to clarification of the nature of the chemical bond unifying the different valence bond and orbital pictures, but most importantly to the unification of the different models of magnetism. His Nobel award is something which his many advisers have been expecting for some years and gives just recognition for his achievement. □

The Nobel prizes (2): Chemistry

Thermodynamicist

J. S. Rowlinson on Ilya Prigogine

THE Swedish Academy of Sciences has awarded this year's Nobel prize for chemistry to Professor Ilya Prigogine of the Université Libre of Brussels. He was born in Moscow sixty years ago but went to Belgium at an early age, and has spent his whole career there, although for the past ten years he has also held an appointment at the Center for Statistical Mechanics and Thermodynamics at the University of Texas.

Brussels has had a long tradition of research in classical or equilibrium thermodynamics, and although Prigogine has contributed to this field he has done his most important work in two other branches, statistical thermodynamics, which is based on molecular descriptions, and non-equilibrium thermodynamics. He started in the first with some war-time papers which developed the cell theories of liquid structure put forward in the 1930s by Lennard-Jones, and developed them to cover liquid mixtures also. Such theories suppose that a molecule spends most of its time in one of a lattice of cells to which it is confined by its neighbours. To be applicable to liquid mixtures such theories require that each species can be accommodated equally well in each cell.

This work, and parallel work in England and America in the 1950s, was a real advance, but was restricted in its useful application to mixtures of molecules of the same size. His book *Molecular Theory of Solutions* (1957) was influential in its day but is not much used now. The theory of liquids has developed along other lines which eschew the restrictive concepts of lattices or cells.

Meanwhile Prigogine was putting more of his effort into non-equilibrium thermodynamics, a subject which has had a long but not always very productive history. It started in the last century with the study of apparently simple transport processes such as the flow of electric current caused by a temperature difference between two junctions of different metals (Seebeck effect), or the inverse, the evolu-

tion of heat produced by an electric current across a metallic junction (Peltier effect).

Similar coupled flows of matter and energy occur when two fluids mix by diffusion, or when chemical reactions occur in inhomogeneous systems. Kelvin put forward quasi-thermodynamic explanations of some of these phenomena but it was not until 1931 that Onsager brought order to the whole subject by describing exactly how such forces and flows are coupled—his famous 'reciprocal relations' for which he was awarded the Nobel Prize for Chemistry in 1968. An interesting consequence of Onsager's work was Prigogine's discovery in 1945 that such systems are characterised by minima in the rates of entropy production.

These results are, however, limited to systems which are very close to equilibrium so that the flows are always proportional to the gradients of potential which generate them, such as temperature, or electric or chemical potential. They are, therefore, restricted in their applications, particularly if the systems are chemically reactive. One critic of this stage of development of the subject, believing that the useful results were not commensurate with the effort expended, said in 1966 that we should "ask not what we can do for irreversible thermodynamics; ask what irreversible thermodynamics can do for us". It was from this impasse that Prigogine sought to escape.

Within the regime of linear laws he undertook the development of molecular or statistical theories of non-equilibrium thermodynamics. Until the 1950s such theories had been confined to dilute gases and had reached their climax with the work of S. Chapman and D. Enskog during the First World War. The methods they used, however, and even the name of the subject—the kinetic theory of gases—had little in common with equilibrium



Prigogine

statistical thermodynamics. Prigogine, Résibois, and others of the Brussels school have played a leading part in developing kinetic theories that are applicable to all fluids. One of the principal tools of this research has been to exploit the analogies between the Liouville equation, which describes how a molecular system evolves, and the time-dependent Schrödinger equation of quantum mechanics.

A second line of attack has been to push the macroscopic treatment of Onsager beyond the narrow range of situations in which flows depend linearly on forces. If this could be done then a whole range of new phenomena would come within the scope of non-equilibrium thermodynamics, such as the turbulent flow and convective instabilities of hydrodynamics, and chemically reactive systems that oscillate in space or time. However a macroscopic description implies that we can define such statistical properties as pressure, temperature and entropy even if the system is far from equilibrium. Prigogine showed that such an assumption is a sufficient condition for the use of thermodynamic methods and justified it in certain cases, for example, by showing that even in fast gas reactions the molecular distribution of velocities shows little departure from a local Maxwellian distribution.

The behaviour of fluctuations of these local properties distinguishes stable from unstable systems. Spontaneous fluctuations are always occurring but, except at a critical point, are usually unimportant. In a system with weak potential gradients each fluctuation quickly regresses to the steady-state behaviour, but when gradients are large then the fluctuations can grow until a new pattern of flow is established, for example the Bénard cells of rotating elements of fluid in a liquid heated strongly from below. Prigogine has sought to marry the classical theories of

stability of structures with a generalised thermodynamics which would encompass such dissipative systems. He does this by introducing a 'local potential', which is a generalisation of the more familiar chemical potential, and then by using a variational principle to calculate the flows. The boundary between the stable or steady states and the dissipative structures is described by an 'excess entropy production' which is positive for the former and negative for the latter.

These new methods encompass many of the traditional ones used in discussing hydrodynamic instabilities, but their more interesting applications lie in the field of chemical reactions. This is, first, because reaction rates are, in practice, never linear in differences of chemical potential, and secondly because of the biological occurrence of systems in which complex chemical reactions are coupled to flows of matter and energy. He and his colleague, G. Nicolis, have used these methods to investigate the conditions under which reaction systems can oscillate in space and time with the aim of developing a thermodynamics that is applicable to biochemical systems.

Whatever new results emerge it is clear that Prigogine, both by his writings and by his enthusiastic advocacy, has changed the ways in which we can discuss a variety of difficult problems. He is, above all, a man of original ideas. In his book (with P. Glansdorff) *Thermodynamic Theory of Structure, Stability and Fluctuations* (1971), he quotes Bergson's saying of 1907 that the second law of thermodynamics is the most metaphysical of all laws of nature. He adds, "Whether a compliment or a criticism, this applies also to the 'generalised thermodynamics' we develop in this monograph". □

The Nobel prizes (3): Physiology and Medicine

Starting a revolution

Jesse Roth and J. E. Rall on Rosalyn Yalow

DR Rosalyn Yalow was awarded a Nobel prize for medicine and physiology in 1977 for the development of the radioimmunoassay. The introduction of the radioimmunoassay for plasma insulin in humans (R. S. Yalow and S. A. Berson, *Nature* **184**, 1648; 1959; *J. Clin. Invest.* **39**, 1157; 1960) started a revolution in endocrinology which spread from insulin to other peptide hormones including substances previously thought not to be antigenic, to steroid and thyroid hormones and to the cyclic nucleotides. Like other great revolutions it spread rapidly to encompass other fields including clinical pharmacology, enzymology, oncology, virology, immunology and haematology. The recent explosion of studies of cellular receptors for hormones, neurotransmitters, lipoproteins and drugs has its technical and intellectual roots in the radioimmunoassay.

Missing from the Nobel citation is the name of Dr Solomon A. Berson who died in 1972. Nobel prizes are not awarded posthumously, but historians and scientists everywhere recall the hundreds of joint publications of Yalow and Berson in the two decades that flank the publication of the seminal papers that introduced the radioimmunoassay.

To appreciate the revolution that the radioimmunoassay wrought it is necessary to recall the incredible state of endocrinology in 1960. The peptide hormones, which constitute about 80% of all hormones, could not, with rare exception, be measured in blood or other biological fluids. If one wished to study the dynamics of insulin *in vivo*, one could only guess from measurements of the blood glucose, or

some other very distant component in the complex of reactions that regulate the blood glucose. Biological assays were available to measure the high concentrations of hormones in extracts of glands but these assays were orders of magnitude too insensitive and lacked the necessary specificity for measurements of hormones in blood.

How was it possible to measure peptide hormones at concentrations of 10^{-10} M in a sea of contaminating proteins at 10^{-3} M, when these hormones were virtually indistinguishable from thousands of other peptides and proteins in the blood? To solve this problem Berson and Yalow used antibodies to insulin, which they had recently characterised and had shown were capable of binding insulin but no other substances. Insulin that they labelled with radioactive iodine was reacted with antibody that had the desired specificity and the then unheard of affinity constant 10^{10} L/M. Conditions were set so that minute concentrations of unlabelled insulin competed with the labelled insulin for binding to the antibody. By measuring the ratio of radioactively-labelled insulin that was bound to antibodies to that which was free, the precise concentration of unlabelled insulin in the system could be determined.

This simple method of measurement of hormones in the blood was made possible by a series of conceptual leaps and technical innovations by Yalow and Berson in the preceding five years. They discovered that all insulin-treated patients generated antibodies to the hormone and recognised the problem of species specificity among insulins. They recognised the need to find antibodies with affinities so high as to be undreamed of by immunochemists of those days. They introduced methods to protect and purify proteins that were being labelled at high specific radioactivity. They devised a rapid precise method for separating antibody-bound hormone from free hormone and characterised in detail the kinetics and thermodynamics of insulin antibody interaction.

With precise and specific measurements for plasma hormone, it became clear that many of the earlier guesses were incorrect. For example, the radioimmunoassay proved that only a minority of diabetics have an absolute deficiency of insulin. Most diabetics have normal or supernormal amounts of insulin which is biologically intact but the cells of these diabetics are subnormally responsive to insulin. Yalow and Berson not only introduced the radioimmunoassay for insulin but applied the method to study the physiology of many other peptide hormones, including growth hormone, parathyroid hormone, adrenocorticotropin and gastrin. They also used this approach to devise a very sensitive method for detecting hepatitis virus and its antibodies in blood, which is widely used in blood banks in the United States and elsewhere. Other applications to problems of infectious diseases are now emerging. Although just beginning, the extension of radioimmunoassay to measure blood levels of drugs, including cardiac glycosides, anticonvulsants and antibiotics, has made it much more feasible for physicians to achieve therapeutic effects without sustaining serious untoward effects of these agents. The principle of competitive binding assays has been extended beyond antibodies to other binding substances of appropriate specificity and affinity.

In the light of these extraordinary scientific accomplishments, it is interesting to recall Dr Yalow's own modest personal and academic background. Born in 1921, she was raised in the South Bronx, the area that is now recognised as the most devastated in the urban United States. She was the first physics major at Hunter College, and although she graduated *magna cum laude* and *phi beta kappa*, her future was clouded by the fact that she was both Jewish and female, two traits which were not considered to favour success in physics in those days. In part because the military draft was depleting the ranks of eligible males, Dr Yalow was accepted by the University of Illinois, and after receiving her Ph.D in physics she returned to New York, where she worked until the end of the Second World War. In 1947, when applications of radioactive isotopes to clinical medicine were in their infancy, Dr Yalow joined the newly formed radioisotope unit of the

Veterans Administration Hospital in the Bronx where she has worked ever since.

Dr Berson, from a similar background, joined the radioisotope unit in 1950. His participation in the research was much less direct after 1968 when he assumed the chairmanship of the Department of Medicine of the Mount Sinai School of Medicine. In spite of his sudden death four years later, there has continued a flood of exciting new studies from the laboratory (renamed the Solomon A. Berson Research Laboratory) at the Bronx Veterans Administration Hospital under Dr Yalow's



Yalow

solo leadership. It has become clear that each peptide hormone in the blood is not a single substance but is rather a family of related peptides that include the active hormone, modifications of the active hormone, precursors and degradation products, as well as phylogenetically related peptides. Dr Yalow has been the major contributor to this area over the last decade and has extended these observations to numerous biological problems *in vivo*.

Her achievements are the subject of editorials in *The Daily News* and *The New York Post* as well as the subject of hundreds of sermons in churches and synagogues throughout the area. Dr Yalow is being viewed as an example of how talented and determined people of modest background can become world champions even in today's America. Women's groups all over North America have turned to her as a symbol. The Urban Crisis Task Force, which has its headquarters in the South Bronx, sees her as an ideal and has decided to name one of their new housing projects in her honour. □

Inadvertent collaboration

George Fink on Roger Guillemin and Andrew Schally

DR Roger Guillemin of the Salk Institute and Dr Andrew Victor Schally of the Veterans Administration Hospital, New Orleans, were awarded the Nobel prize in physiology and medicine for isolating, characterising and synthesising three polypeptides which mediate the neural control of the anterior pituitary gland. The discoveries of Schally and Guillemin have already proved significant for clinical and basic medical science and are likely to offer new and safer methods for the control of population size.

The delight of neuroendocrinologists will only be dampened by the fact that the untimely death in November 1971 of Geoffrey Wingfield Harris, FRS, prevented him from sharing the fruits of his labours. As a medical student at Cambridge, Harris was the first (1937) to provide experimental proof for the then tentative view that the anterior pituitary gland was controlled by the central nervous system. The elegant studies carried out by Harris in the 1940s and early 1950s, alone and in collaboration with Dora Jacobsohn and the late John Green, established beyond doubt that this control was mediated by a neurohumoral mechanism involving the transport by hypophysial portal

vessel blood of chemical substances from the hypothalamus to the anterior pituitary.

The three polypeptides and their aminoacid sequences are: thyrotrophin releasing factor (TRF) pyro Glu-His-Pro-NH₂; gonadotrophin releasing factor (GnRF) pyro Gly-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂; and somatostatin (or somatotrophin release inhibiting factor, SRIF) H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH. Synthetic TRF and GnRF are now widely used in the investigation of thyroid dysfunction and infertility, respectively. Because TRF releases prolactin as well as thyrotrophin, the tripeptide is also used for investigating patients in whom prolactin secretion appears to be abnormal. Conceivably, antagonists of TRF may prove useful for controlling hyperprolactinaemia, a condition frequently associated with infertility in women, and for the control of hormone dependent tumours of the breast.

Superactive analogues of GnRF, made by substituting a D-aminoacid (for example D-Trp, D-Ala or D-Leu) for Gly⁶ and an ethylamide group for Gly¹⁰, are being investigated mainly by Schally's group with a view to improving the precision of ovulation in women who prefer to or must use the rhythm method of fertility control. The parent decapeptide has already been used successfully for the treatment of certain types of infertility in men and women. Because GnRF will only discharge the amount of luteinising hormone (LH, the hormone which triggers ovulation) normally available for release, the risk in terms of pro-

ducing multiple pregnancy is negligible compared with that of administering exogenous gonadotrophins. Concern about the safety of the steroid contraceptive 'pill' has added impetus to the search for other chemical contraceptives.

As well as inhibiting the release of growth hormone, somatostatin inhibits the release of thyrotrophin, prolactin, insulin, glucagon and gastrin. In spite of these widespread effects, early trials suggested that the tetradecapeptide may prove useful for the control of certain aspects of diabetes mellitus. An inhibitory effect of somatostatin on platelet aggregation has, however, made it mandatory for clinicians to proceed with caution.

As expected, access to large amounts of the three peptides, which are relatively easily synthesised, has proved invaluable for investigating the physiology and biochemistry of the hypothalamic-pituitary system. Perhaps of greater importance, however, is the fact that the peptides have reawakened interest in extra-hypothalamic peptidergic neurons, a field which has largely remained dormant since the studies of Gaddum and Von Euler on substance P (1931). Using antisera to TRF, GnRF and somatostatin, immunoassay and immunohistochemical studies have shown that relatively large amounts of these peptides are present in areas outside the hypothalamus, including the cerebral cortex and spinal cord.

The small amount of polypeptide in the hypothalamus (nanogram quantities), the protected N and C terminals of TRF and GnRF, and the fact that the hypothalamus contains more pharmacologically active substances than any other tissue, made the isolation of the three peptides exceedingly difficult. Both Schally with Murray Saffran at McGill, and Guillemin in Houston, started their isolation studies on corticotrophin releasing factor (CRF). Subsequently, at Baylor University, they collaborated on this project, but, by the early 1960s, they realised that for various technical reasons, notably the lack of a specific assay for CRF, it would have been better to leave the investigation of this potentially important factor until last.

After Schally became chief of the Veterans Administration Endocrine and Polypeptide Laboratory in New Orleans (1963), the alarming rivalry which soon developed between the two Laureates provided the necessary stimulus for the completion of their Herculean task. Almost every result obtained by one was checked by the other, and, although separated more by passion than distance, much of the work proved inadvertently to be collaborative. Thus, for example, Guillemin and his co-workers were the first to show that hypothalamic extracts could release thyrotrophin (*C.R. Acad. Sci. Paris* **255**, 1018, 1962). Schally's group revealed that His, Glu and Pro were the aminoacids present in porcine TRF (*Biochem. Biophys. Res. Commun.* **25**, 165, 1966). On the basis of the activity of various synthetic tripeptide isomers of these acids, both groups reached the conclusion in 1969 that TRF was likely to be pyro Glu-His-Pro-NH₂.

Shortly afterwards, Guillemin reported that the structure of natural ovine TRF was identical to that of the active isomer as assessed by infrared, mass and nuclear-magnetic resonance spectrometry (*C.R. Acad. Sci. Paris* **269**, 226, 1969; *Nature* **226**, 321, 1970). Schally found that natural porcine TRF was also identical to pyro Glu-His-Pro-NH₂ as assessed by identical Rf values in various chromatographic systems (*Biochem. Biophys. Res. Commun.* **37**, 705, 1969) and by spectral analysis (*Biochemistry* **9**, 1103, 1970).

Samuel (Don) McCann in 1960 and Geoffrey Harris in 1961, using respectively the ovarian ascorbic acid depletion method of Albert Parlow (an assay used extensively by Guillemin and Schally) and the rabbit ovulation test, reported that hypothalamic extracts released LH. After ten years of intense work carried out by several groups, Schally was able to announce in 1971 that his team had determined

the structure of porcine GnRF (*Biochem. Biophys. Res. Commun.* **43**, 393 and 1334). The amino-acid sequence was confirmed by Guillemin's group who used ovine tissues (*Proc. Natn. Acad. Sci.* **69**, 278, 1972). Both groups agreed that the decapeptide releases LH and follicle-stimulating hormone (FSH) (hence Schally's preferred term, LH-RH/FSH-RH). Earlier evidence for the existence of a separate releasing factor for FSH appeared to be due to the non-specific effects of polyamine contaminants. So far there is no convincing biochemical evidence for the existence of a FSH-RF distinct from the decapeptide, and this is disappointing since it makes remote the possibility of developing an acceptable male contraceptive 'pill'.

McCann and his co-workers (*Endocrinology* **83**, 783, 1968) first demonstrated the presence in fractions of sheep and rat hypothalamic extract of a growth hormone inhibitory factor and a growth hormone releasing factor. The structure of ovine growth hormone inhibitory factor (somatostatin) was reported by Guillemin's group in 1973 (*Science* **179**, 77). In spite of extensive studies the structure of growth hormone releasing factor remains unpublished.

Since the isolation of the three polypeptides, Schally's team have focused attention on the development of potent agonists and antagonists of GnRF, as well as on the isolation of prolactin-inhibitory, corticotrophin-releasing and growth-hormone-releasing factor. As well as developing analogues of the peptides, Guillemin has concentrated his efforts on the endorphins, his most significant recent contribution being the isolation of a pituitary protein (MW 31000) which appears to be a common precursor of β -endorphin and ACTH.

Apart from their expertise in chemistry and physiology, and their tenacity and perseverance, the success of the two Laureates depended on the recognition in the early sixties that the isolation of each hypothalamic factor required an all or none approach. They had the capacity to attract research funds amounting to many millions of dollars which enabled them to assemble large teams of competent chemists and biologists, and as well, purchase the many hundreds of thousands of hypothalamic fragments necessary for successful isolation.

Since the late 1950s, when he began to test ovine hypothalamic fragments for LH-releasing activity, Geoffrey Harris was determined to isolate LH-RF. He managed to secure only one senior chemist at a time: first Peter Fawcett at Mill Hill and then (1966) Harry Gregory at ICI. In the light of the resources available to the American teams, it is perhaps remarkable that, using only a few thousand fragments, Gregory had by 1970 isolated six of the aminoacids in GnRF and had shown that this factor had no free α amino groups (*Control of gonadal secretion*, eds. Baird and Strong, page 15). Is there a lesson here for the funding of British biomedical research?



Guillemin, Schally

JET

Permission to land

The delays experienced by the EEC's Joint European Torus (JET) fusion project are over. Chris Sherwell reports

A FULL two years after the matter came before it, the EEC Council of Research Ministers has agreed on the research laboratories at Culham near Oxford as the location for the next stage of the Community's fusion project, JET. The choice, made at a Council meeting on Tuesday, ends a controversial wrangle that has damaged the Community as well as the project. The decision also paves the way for the establishment of a JET Council, the release of funds to boost a stalling research effort and the appointment of a project head.

Political rather than scientific disputes have persistently hamstrung the £100-million project. Contracts for the team based at Culham have had to be extended at the last moment many times, and continuing uncertainties have driven some members away. Only last week Paul Rebut, the present project leader and a strong candidate to head the next phase, warned that rebuilding the team would take at least six months. Over the past 18 months JET has come up at five Research Ministers' meetings, two Foreign Ministers' meetings and one heads of government meeting; there have been six elections, and seven research ministers have changed; so have six foreign ministers and four heads of government.

The site of the project has sparked most controversy. National interest arguments prevented agreement on any of the four sites originally pro-

posed (Ispra in Italy, Cadarache in France, Garching in Germany and Culham in Britain), even when they were eventually whittled down to Garching and Culham. A widely expected decision failed to materialise at a Research Council meeting in March this year, and EEC heads of government passed the matter three months later to the Foreign Ministers. When they too failed to agree, everything seemed to hang on Anglo-German exchanges. But the Schleyer kidnapping intervened, no exchanges took place and JET was not discussed at a Foreign Ministers' meeting in September.

The breakthrough finally came last week when the West German Chancellor, Helmut Schmidt, and the UK Prime Minister, James Callaghan, met in the aftermath of the successful Mogadishu raid. Its euphoric effects, and Britain's supporting role in it, may have helped the two men submerge differences on matters like Community budget contributions. They agreed that a site for JET should be decided by a consensus—in other words, that neither country would veto whatever preference the majority expressed. This was conveyed to the Foreign Ministers' meeting in Luxembourg the same day, and though Germany did not waive her claim for Garching, the immediate presumption, based on past voting intentions, was that JET would go to Culham when the Research Ministers met in Luxembourg. In the event, five countries voted for Culham and two for Garching. One of the two abstaining countries went with the majority. Earlier fears of an Irish veto proved unfounded.

Reports that two JET projects were being discussed gained credence at a press conference in Brussels on Mon-

day given by Guido Brunner, the EEC's Energy and Research Commissioner. Culham and UK Foreign Office spokesmen last week discounted the idea, the former pointing out that even if this referred to the possibility of siting 'Super JET' or 'Jumbo JET' (as the next phase is known) in the country which failed to win JET, such a decision could not be taken bilaterally. The impression nevertheless remained on Monday that the Research Ministers would take a consensus view on locating the next phase at the losing site, and that ancillary fusion research might also be used as compensation.

The removal of other potential obstacles at a meeting of high-level officials a week earlier also assisted agreement on a JET site. Helpful concessions from France, which originally proposed a JET Council to manage the project and saw it become a stumbling block, produced agreement that the member states, along with other participating countries (Sweden and Switzerland), would each have two members on it, one a scientific expert. Voting will be weighted, 26 out of 37 constituting a majority. The Council, which will have a scientific council to consult at its convenience, will meet as and when necessary. Once it is established (initially in interim form) and funds become available, the senior scientific appointment for the project will be made. But that could prove as politically contentious as anything that has befallen JET so far.

The aim behind the £100-million project—often forgotten by the politicians, often simplified as "cheap, safe and abundant energy supplies"—is essentially to construct a third-generation tokamak device. The equivalent device in the USSR is known as T-20, in the USA as TFTR, and in Japan as T-60. □

DIOXIN

Informing on 2,4,5-T

Alastair Hay looks at two recent reports on the controversial herbicide 2,4,5-T

MANUFACTURERS and users of the herbicide 2,4,5-T are not letting attacks on it pass without comment, if two recent reports that attempt to vouch for its safety are anything to go by. The first, a Dow Chemical Company report to the US Environmental Protection Agency (EPA), insists that there is no "significant hazard" to humans asso-

ciated with its use. The second, from the UK Forestry Commission, claims that if foodstuffs or water supplies should become contaminated with 2,4,5-T, "serious consequences [for humans or animals] are unlikely" to occur.

The use of 2,4,5-T has aroused concern since the late 1960s when formulations of the herbicide in use in Vietnam were shown to be contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin), which is highly toxic and a known teratogen. The public outcry,

together with scientific criticism of the use of 2,4,5-T, led the EPA to prohibit its use around homes, recreational sites, aquatic areas and on human food crops. In 1975 an EPA study reported that dioxin had been found in beef fat taken from cattle in areas where 2,4,5-T was known to have been used.

The Dow study was undertaken to assess these EPA findings. In a two-year study Dow scientists fed rats diets containing 0.1, 0.01 and 0.001 $\mu\text{g kg}^{-1}$ of dioxin a day. The animals receiving the highest dose experienced the known clinical and pathological symptoms of dioxin exposure—decreased weight gain, decreased red blood cell and

haemoglobin levels, elevation of some serum enzymes, and increased mortality. Discernible increases in the incidence of liver cancer and carcinomas of the lung, hard palate and tongue were also observed. But the report also speaks of a remarkable decrease in the "incidences of tumours of the pituitary, uterus, mammary glands, pancreas and adrenal gland" in these animals. In the rats receiving the lower doses of dioxin a lesser degree of toxicity was observed but there was no increase in tumours. On the basis of these results the report states that if the EPA measurements are used then the average person would need to consume fifty times his "body weight in beef every day to receive the daily dose level" of dioxin linked with cancer incidence in Dow's studies. Thus, in Dow's view it appears that 2,4,5-T is safe if used properly.

The same conclusion is drawn by the Forestry Commission report, 'The Safety of Herbicides 2,4-D and 2,4,5-T'. The author, D. J. Turner, describes many of the criticisms of the use of 2,4,5-T as unfounded and ill-informed. He points out that 2,4,5-T, still enjoying widespread use in Europe and South-East Asia, remains the most effective herbicide for defoliating rubber trees, which is the way to contain and isolate the South American

leaf blight disease *Dothidella ulei*. But he also says that "if reaction conditions [for converting tetrachlorobenzene to trichlorophenol—the precursor of 2,4,5-T] are properly regulated dioxins are not formed". Chemists knowledgeable in reaction mechanisms say this is not so, arguing that the reaction makes dioxin contamination unavoidable and that control of the reaction conditions will only limit the amount of dioxin produced, not eliminate it entirely. In their view the manufacturing stage presents greater risks of dioxin contamination than the use of the herbicide in silviculture.

In spite of the now considerable volume of literature relating to dioxin's effects, the report claims there is no evidence that the "defoliation spraying programmes in Vietnam had any direct effects on humans". This is flatly contradicted in the fifteenth report of the International Agency for Research on Cancer (IARC). In a chapter on chlorinated dibenzodioxins that cites the same National Academy of Sciences report referred to in the Forestry Commission publication, this states that the Montagnard people of South Vietnam were badly affected by exposure to the herbicide 'Agent Orange', a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T,

containing up to 30 mg kg⁻¹ of dioxin. The consequences included diarrhoea, vomiting, skin rashes, fever, abdominal pain and the deaths of some children. The IARC publication, one of the most comprehensive reports yet produced on the dioxins, aimed to assess carcinogenicity of chlorinated dibenzodioxins, but concluded that this evaluation could not be made with the data available to it at the time of publication.

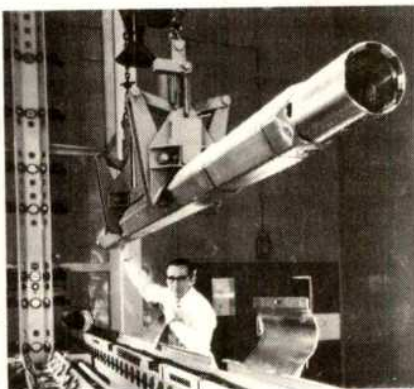
At Séveso in Italy—where the authorities are now satisfied that decontamination following last year's release of dioxin has made it safe for people to return to their homes (last week the first 24 families were allowed back)—135 children have chloracne as a result of dioxin exposure. A total of thirty-three adults but no children have neurological disorders, yet if (as some argue) chloracne is an obligate sign of dioxin poisoning, children might have been expected to have neurological problems. No eye damage has been reported in the 400 people examined by an oculist. Sixteen cases of children born with malformations were reported by August this year, of whom two died, but the diversity in the type of malformation and the fact that the number was no greater than the statistical norm suggests that dioxin was probably not the causative agent.

THE development of a new chelating agent does not usually make headlines in the national press, but this week saw the widely publicised announcement of a chelating agent with two important differences. Its scientific interest lies in the fact that it can enter the cells of the body and will therefore be much more efficient at removing heavy metal contamination. The reason it hit the headlines is that it has been developed (at the Harwell laboratories of the UK National Radiological Protection Board) to remove plutonium from the body in the event of an accident.

One of the problems in devising effective treatments for the rare cases of accidental contamination with plutonium has been the difficulty of removing the plutonium which becomes locked up in the cells of the body, mostly in liver and bone, where over the years there is the risk that it might induce cancers. The only available treatment up to now aimed to inactivate the plutonium when it was freely accessible in the blood before it became deposited inside tissue cells. The chelating agent calcium diethylene - triamine - pentaacetic acid (CaDTPA) can pick up plutonium from the bloodstream and the resulting complex is excreted in the urine.

But CaDTPA has one great drawback—it cannot enter cells and so is only effective against plutonium circulating in the blood. In real life the

Fighting plutonium



Handling plutonium fuel assembly (BNFL)

BACKGROUND

contaminant may well be 'insoluble' plutonium, such as plutonium dioxide particles, which leach very slowly into the bloodstream from the lungs; in this case DTPA is not particularly efficient in reducing the total body burden.

The NRPB's new drug, which it calls Puchel, is a derivative of DTPA

with one important modification. Fatty acid chains have been added to the molecule enabling it to penetrate the lipid cell membrane and pick up plutonium from inside the cell. The complex is then excreted, mostly in the faeces. The NRPB is not yet divulging the structure of its new molecule, pending a possible patent application, but the secret apparently lies in the length of the fatty acid chains.

So far, of course, the drug has only been tried out in animals, where it has proved essentially non-toxic when given as the calcium salt. Injected intraperitoneally into hamsters it released most of the plutonium deposited in the liver after injection of a soluble plutonium salt, and given as an aerosol removed plutonium from the lung after inhalation of plutonium particles. It will also probably be effective in removing intracellular metal if given orally. Puchel has proved less effective at releasing plutonium from bone than from liver.

Puchel has been developed as a contribution towards reducing the risks of a nuclear accident, but if it proves itself it may be useful in cases of poisoning with non-radioactive heavy metals such as lead and mercury.

IN BRIEF

ISEE OK

The first stage of the joint European-American International Sun-Earth Explorer (ISEE) satellite mission was launched successfully at the weekend. Lift-off was postponed from 13 to 19 October and then suffered two further delays. This was to allow NASA to adjust the Thor Delta vehicle, launching the ISEE A and B satellites in tandem. The second part of the mission, ISEE C, will be launched in nine months' time.

Whale decision

A Federal Court of Appeal has rejected the Alaskan Eskimos' plea that the US Administration be required to file an objection against the International Whaling Commission's (IWC) decision

to bar the hunting of bowhead whales next year. The Court upheld the US decision not to object to the ban. It did so on the grounds that a US refusal to abide by IWC recommendations would give other countries licence to follow suit and would seriously threaten the bowhead with extinction, whose numbers are estimated at between 500 and 2,000.

Washington meeting

An idea to curb the spread of nuclear weapons by setting up an International Fuel Bank was suggested by President Carter last week at the opening of the 40-nation international conference on nuclear fuel disposal. The Bank would ensure the US and other countries of an adequate supply of uranium for

peaceful purposes. The US government also has a plan for storing all spent nuclear fuel produced by reactors both at home and abroad, which would further reduce the Administration's fears of unchecked proliferation.

Med meeting

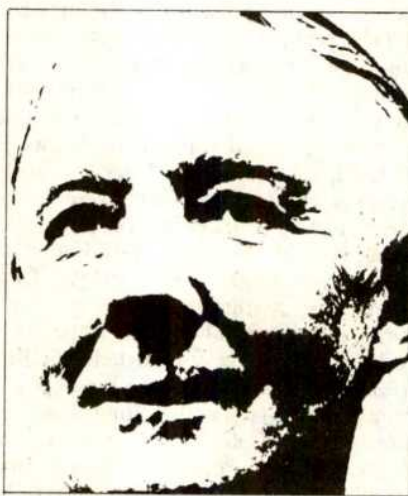
A five-day meeting of 13 Mediterranean states and the EEC to discuss the draft of a treaty to fight Mediterranean pollution, ended this week with agreement on limiting or banning the discharge of certain materials. The United Nations Environment Programme (UNEP) estimates that the cost of implementing the treaty, which will be submitted to Mediterranean states at a major conference next January, at \$5 billion.

CONSERVATIONISTS, anxious to preserve the native animals and plants of the countryside, generally assume that modern agricultural techniques are increasingly damaging to wildlife. The wholesale removal of hedges in the cereal growing areas of eastern England has, locally, left few wild habitats, spray drift from carelessly used weedkillers has denuded roadside verges of many attractive flowers, and some insecticides have slaughtered thousands of songbirds. However, these abuses are being generally controlled today, and in Britain there is growing cooperation between farmers, the makers of agricultural chemicals and conservationists to make at least the second best of both worlds, of agricultural productivity and wildlife conservation.

It is also encouraging to find that some modern farming methods do less damage than those which they are replacing. Ploughing, the traditional means of cultivation, is a drastic process, with profound effects on soil structure and chemistry and on all subterranean living organisms. It is done to produce a tilth for planting, and to control weeds. Work at Rothamsted Experimental Station in England over thirty years ago showed the surface-casting earthworms, which produced Charles Darwin's 'vegetable mould' (the stone-free well-textured surface layer in old pasture), were eliminated, and their populations might not recover for 70 years. Where cereals have been grown continuously for many years using only chemical manures, levels of organic matter have fallen and soil structure has suffered. Ploughing also uses a lot of expensive and scarce fuel.

Some farmers are now ceasing to plough, and are turning to direct drilling. A field of wheat is harvested, and the straw and stubble is burned. This burning, if improperly controlled, can do immense damage to the countryside, to hedges, woods and verges, and their wildlife, and bodies

Drill to till



KENNETH MELLANBY

like the National Farmers' Union are trying to discipline their members to accept a safety code. But burning has many agricultural advantages. It gives some control of diseases, weeds and pests, and returns a substantial amount of immediately-available nutrient to the soil. A burned field, with little trash on the surface, is easily cultivated, and rain does not produce a quagmire as it does after ploughing.

The field is usually left for some

weeks after burning, to allow weeds and shed grain to germinate. During this period it is a rich feeding ground for birds which obtain little food where stubble is ploughed straight after harvest. The surface is next sprayed with the herbicide paraquat, which kills the growth, but which is immediately immobilised. The sprouting grain is unharmed, and thus it is possible to drill the seed at the same time. This saves a lot of fuel. There has in the past been controversy about the effectiveness of direct drilling, as some farmers have obtained higher and some lower yields than with conventional ploughing. It seems now that good results are obtained by those who persist even after initial disappointment. After three or four years, even on heavy clay soils, the organic matter level increases (as several tonnes per hectare of roots rot down to produce humus) and the soil structure improves markedly. The soil fauna, including the delicate worms, also flourishes, and makes its contribution. The amount of herbicide used may be less than on ploughed fields, for some weeds are killed by the paraquat and the rapidly-germinating crop may smother others, including the usually troublesome couch grass.

Direct drilling is done to help the farmer to grow the most profitable crops, not to please the conservationist. It will not bring back our flower-rich meadows or the hedgerows once full of singing birds. But it may make the soil healthier and support a richer soil fauna. This is the basis of healthy agriculture and a healthy countryside. We, the conservationists, must now find how to encourage wildlife in this milieu.

news and views

Gene derepression in tumours

from Robert Shields

MANY non-endocrine tumours produce a wide variety of peptide hormones; perhaps the best known example is ACTH, (adrenocorticotropin) secreted by oat cell carcinoma of the lung (Rees *J. Endocr.* **67**, 143; 1975). The favoured explanation for this ectopic hormone production is that it results from 'gene derepression' which is claimed to be a frequent occurrence in neoplasia (Odell *New Engl. J. Med.* **297**, 609; 1977). This idea goes some way towards explaining many of the features of ectopic hormone synthesis. Quite often these hormones are larger than the normally occurring peptides (as happens with ACTH). These larger proteins are probably the prohormone precursor to the normal mature protein. Other ectopic hormones lack the modifications normally made before secretion; for instance the subunits of human chorionic gonadotropin (hCG) secreted ectopically by a variety of tumours lack the normal complement of carbohydrate. The gene derepression hypothesis could also explain why ectopic insulin production (which requires the tumour to execute the correct cleavage of proinsulin) is seen so rarely and why ectopic synthesis of non-peptide hormones such as steroids or thyroxine (which would involve the derepression of an entire enzyme pathway) has not been found.

But a number of observations are not fully explained by a simple gene derepression hypothesis. First, if gene derepression is common in neoplasia why do cells plump so often for hormones when there are 1×10^4 – 5×10^4 other genes to choose from? A partial answer may be that most of these genes code for housekeeping functions, over-production of these might be lethal. However, one might still expect luxury proteins to be derepressed with roughly equal frequency. Ectopic production of non-hormone proteins is in fact seen quite often, the best known examples are carcinoembryonic antigen (CEA) or α foetal globulin and

placental alkaline phosphatase. It is probable that many other proteins are synthesised ectopically, and that only the lack of clinical symptoms or suitable assays prevents their detection (Odell & Wolfson in *Cancer*, 81, (ed. Becker F.) Plenum, New York, 1975).

In spite of this range of tumour products specific tumours tend to be associated with particular proteins: squamous carcinoma of the bronchus often produces parathyroid hormone (PTH) but seldom makes ACTH or MSH (melanocyte-stimulating hormone) whereas these hormones are relatively common in oat cell carcinoma (*Br. Med. J.* **1**, 1300; 1976). Why 'gene derepression' (if that is the explanation) occurs so non-randomly has not been satisfactorily explained.

The relative frequency of ectopic hormone production makes it worth asking whether ectopic protein production is unique to neoplasia and whether gene derepression is widespread in tumour cells. A recent report presents evidence for the presence of the β subunit of hCG in liver and colon from normal subjects (Yoshimoto *et al. Science* **197**, 575; 1977). Like ectopically synthesised hCG the protein lacked carbohydrate. Other experimenters have reported finding ACTH in non-involved lung tissue from patients with lung cancer (Bloomfield *et al. Clin. Endocr.* **6**, 95; 1977). These results suggest that ectopic hormone production may be widespread and occur in normal tissue as well. These findings also cast some doubt on the usefulness of ectopic hormones as tumour markers.

mRNA changes

As the techniques of nucleic acid hybridisation become more refined it is possible to ask more directly whether gene derepression occurs in tumour cells. If cDNA to total mRNA from transformed cells is cross-hybridised to normal cell mRNA any unhybridised cDNA should represent sequences present only in the transformed cell. If this remaining cDNA

is then back hybridised to transformed mRNA an estimate of the abundance and complexity of the new mRNA species can be obtained. When such experiments were done with human fibroblasts transformed by SV40 virus it was found that the great majority of mRNA species in transformed cells were present in the normal cells as well (Williams *et al. Cell* **11**, 901; 1977). At most around 3% of the mRNA in tumour cells was new (and part of this was probably virus coded) so vast host gene derepression did not occur. The estimate of how many new host cell sequences were derepressed can only be approximate but the authors calculate that around 35 new species may be involved. Since there are around 10^4 different mRNAs in the cell anyway 35 sequences does not represent large amounts of new information.

In these experiments care was taken to maintain normal and transformed cells under identical conditions to minimise the possibility that the differences seen were unconnected with transformation. Real tumours do not bother with these precautions and it might be expected that their mRNA patterns would diverge widely from normal. Surprisingly when mRNAs from HeLa cells was compared with those from normal cells there was very little difference once again suggesting that few new mRNA sequences were present in this abnormal cell. This result is particularly interesting as some clones of HeLa produce ectopic hCG (Lieblich *et al. Nature* **260**, 532; 1976; Ghosh *et al. Biochem. J.* **166**, 265; 1977). Whether hCG was produced in the clone examined by Williams is not known (Williams, personal communication), but in any event the number of new sequences in HeLa cells may be rather small.

These hybridisation experiments are only accurate in detecting entirely new mRNA transcripts. They are relatively insensitive to switching of mRNAs from low to moderate abundance classes. In other words, some mRNAs that are present in only a

few copies in normal cells may be present in tenfold greater amounts in transformed cells (and *vice versa*) and this would not be picked up. Viral transformation is known to affect the levels of mRNA for host proteins such as LETS and collagen (Adams *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3399; 1977) and it would be surprising if other mRNAs remained unaltered.

Protein changes

Many experiments suggest a close relationship between the amount of mRNA and the amount of protein produced, so one indirect way to detect alterations in the levels of mRNA species is to examine the spectrum of proteins produced by normal and transformed cells by 2-D electrophoresis. There are relatively few differences in freshly transformed cells but when cells have been in culture for some time the differences are remarkable. Examination of the proteins from established lines of 3T3 cells, or 3T3 cells transformed with SV40 (DNA virus) or Kirsten sarcoma virus (RNA virus) revealed that although both transformed cell lines were similar to each other they differed from their untransformed counterparts in that around 30% of the proteins detectable in the transformed cells were not detected in untransformed cells (Strand & August *Proc. natn. Acad. Sci. U.S.A.* **74**, 2729; 1977).

Many of these 'new' proteins may not be new at all but represent increases in protein that were below

the level of detection in untransformed cells. Also some of these 'new' species may result from different patterns of glycosylation and other modifications in transformed cells which would alter the electrophoretic mobility of pre-existing proteins. Still other proteins may be induced because of differences in the utilisation of the culture medium by transformed and untransformed cells (Shill *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 3840; 1977). Nevertheless, the results show that these cells differ widely in the relative amounts of different protein produced and by inference in the relative amounts of many abundant mRNAs. If one can extrapolate from the experience with human cells (Williams *op. cit.*) (where few new abundant mRNAs were detected) it seems likely that the differing patterns of gene expression in normal and transformed cells may be due more to modulation of pre-existing mRNA sequences than introduction of new genes by oncogenic viruses or the derepression of pre-existing genes.

Whether this is generally true or not must await the preparation of cDNA probes to large numbers of different messages so that specific mRNA levels can be monitored directly after transformation and during evolution in cell culture. Perhaps a good way to start would be to prepare probes specific for ectopically produced proteins to see if mRNAs for these proteins are always present in normal cells (as the data of Yoshimoto *et al. op. cit.* might suggest) or whether these genes are really derepressed in tumour cells. □

then being made by comparing the rate of the drops' fall under gravity with the rise when an electric field is superimposed.

Millikan's original work involved studies of droplets of water or alcohol. On page 220 of his article in the *Philosophical Magazine* of February, 1910 he notes 'I have discarded one uncertain and unduplicated observation apparently upon a singly charged drop which gave a value of the charge . . . some 30% lower than the final value of e '.

This observation was remembered by many people recently when possible evidence for fractional charge was reported in the literature (La Rue *et al. op. cit.*). However the probable cause of Millikan's anomalous result is given by him only two paragraphs later in his paper: singly charged drops were so small that they evaporated rapidly during observation, hence a poor value of e was obtained. To avoid this evaporation problem Millikan ' . . . replaced the droplet of water or alcohol by one of oil, mercury or some other non-volatile substance . . . ' (*Phys. Rev.* **32**, 351; 1911). Never again did he see a fractional charge and one can safely conclude that evaporation was indeed the source of his spurious result.

If one intends to mount a search for quarks, whose occurrence as isolated particles sitting in lumps of matter is at best extremely rare, then one will want to study as much material as possible. The Millikan procedure is fine for very small drops where air friction can be used as a gauge for measuring the small forces on a charge in an electrical field. To study drops with masses of order 10^{-6} – 10^{-7} g an alternative technique is required. Hence studies turned to levitation of ferromagnetic or diamagnetic and superconducting materials by applying external fields. As an example of these experiments one can cite Gallinaro and Morpurgo (*Phys. Lett.* **23**, 609; 1966) who find no evidence for fractional charge in an experiment where more than 10^{-6} g of graphite were studied in a magnetic levitation spectrometer.

Possible evidence for fractional charge on a ferromagnetic material appeared in an experiment where twelve steel balls of 0.2 mm diameter were suspended in turn in a magnetic field. An electric field of $3,000 \text{ Vcm}^{-1}$ was then applied and the charge on the ball deduced. Of the 12 balls 10(!) had charges consistent with $\pm 1/3e$ and only one was consistent with integral charge. However, the authors (Garris & Zioc Nucl. Inst. Meth. **117**, 467; 1974) noted a source of systematic error, namely that due to

What price free quarks?

from F. E. Close

ALTHOUGH the concept of quarks as basic constituents of matter is almost universally accepted no evidence for isolated quarks has yet stood the test of time. However, interest in the search for 'free' quarks was heightened recently in the light of reports of new measurements of e , the magnitude of the element of electric charge, using variations of the Millikan oil drop technique (La Rue *et al. Phys. Rev. Lett.* **38**, 1011; 1977; Gallinari *et al. Phys. Rev. Lett.* **38**, 1255; 1977; Bland *et al. Phys. Rev. Lett.* **39**, 369; 1977). Quarks are widely believed to have fractional electric charge ($2/3$ or $-1/3$ of a proton's charge) and if they can exist as free particles then this property would make them easily

recognisable; hence the interest in the above experiments.

Measurements of e

In advance of Millikan, Ehrenhaft and others (refs in Millikan *Phys. Rev.* **32**, 349; 1911) had deduced the magnitude of the elementary charge from the average behaviour of charged particle swarms in electric and gravitational fields. Millikan appears to have been the first person to make a measurement by studying a single charged carrier, and the result quoted in his 1917 paper (*Phil. Mag.* **34** No. 199, July 1917) is within 1% of that accepted today. Every physics student has met the ideas behind this experiment whose essential feature is that extremely small drops of oil ($\approx 10^{-12}$ g) are observed in an electric field; an accurate determination of the charge

crystal structure and impurities metal surfaces are not equipotential surfaces. These 'patch effects' cause the potential of the metal surface to vary by 50 to 100 mV from place to place hence inducing a field gradient which can act on dipole moments induced in the balls by the applied electrical field. Reinserting several of the balls four months later the deduced charges had altered by as much as $0.45e$ showing that there is a long term change in the patch effect. The authors note that time-dependent changes in the patch effect could have caused a spread in the observed charge covering the measured range and so one cannot conclude that this experiment provides positive evidence for fractional charge.

Recent excitement was generated by reports of evidence for fractional charges on superconducting niobium (La Rue *et al. op. cit.*). Eight niobium balls each of mass $\approx 10^{-4}$ g were levitated in turn in a magnetic field. The charge on a ball can be changed at will by β^+ or β^- emitters adding positive or negative units of electronic charge to the ball. By studying the oscillations of a ball in the field the total force on the ball is deduced. The experimental problem which is crucial to interpreting the results is to decide the extent to which dipole forces might mimic a charge. Much of the paper is devoted to discussing these effects. The conclusion is that five niobium balls heated on a niobium substrate had charges compatible with zero whereas three balls heated on a tungsten substrate had charges of $(+0.337 \pm 0.009)e$, $(-0.001 \pm 0.025)e$, $(-0.331 \pm 0.070)e$ which, if genuine charge effects, are remarkably consistent with quark expectations.

If correct, it has been suggested that the earlier failures to find quarks might be understood if isolated quarks had some preference for existing on heavy nuclei. This does not seem to be borne out however as Bland and coworkers (*op. cit.*) have recently suspended tungsten particles in an electric field. A radioactive source can change the charge on a particle and the new electric field that balances the ball against gravity is determined. Sixty-nine balls were studied and no evidence for fractional charge on a ball was found. Over 10^{12} atoms of tungsten have been studied in this experiment and the authors plan to extend their study to other heavy elements.

Another null result has recently been reported by Gallinaro *et al. (op. cit. 1977)*. These authors studied iron balls of masses $\approx 10^{-4}$ g each and find no evidence for isolated quarks in over 10^{21} nucleons.

Ironically the observation of an isolated fractionally charged quark would

now cause more turmoil to theorists than its non-observation even though all are convinced that the quark concept is correct. Much effort is being devoted to see whether quarks can theoretically be confined inside hadrons in groups of three or as quark-antiquark pairs since these are what seem to be the rules in Nature.

A fifth quark, but no quarks

A recent discovery is that of a new particle, the upsilon (see *News and Views* 268, 297; 1977), which provides the first evidence for a possible fifth flavour of quark. The upsilon has

similar metastability to the ψ (*News and Views* 257, 535; 1975) but is approximately three times as massive (the ψ discovery in 1974 provided the first clear evidence for the existence of the fourth flavour—the charmed quark). It seems that the upsilon is a bound state of a new quark and its antiquark in analogy to the ψ being a bound state of a charmed quark and charmed antiquark.

Hence we have now evidence for five types (or flavours) of quark, yet the intensified search for an isolated quark reveals no sign of one. The quark paradox continues to deepen. \square

Rapid RNA sequencing

from Maria Szekeley

1977 has proved a fruitful year for nucleic acid sequencing. After the outstanding results with DNA achieved with new methods which shorten the time required for sequencing from years to weeks, these new principles have now been introduced to the temporarily neglected field of RNA sequence determination.

Several new developments have been reported at the same time. An RNA sequencing method based on the same principles as Maxam and Gilbert's DNA sequencing technique has been worked out simultaneously by Donis-Keller, Maxam and Gilbert (*Nucleic Acids Res.* 4, 2527; 1977) and by Brownlee's group in Cambridge (Simoncsits *et al.* (See this issue of *Nature*, page 833). This is also a rapid gel sequencing technique in which RNA fragments all ending at a given base are fractionated according to size so that the positions of this base in the RNA sequence can be read off the gel electrophoretic pattern. There is some difficulty involved in the production of these fragments from RNA. Splitting by base-specific endonucleases is used in place of the chemical reactions used for DNA. Although specific cleavages at purine residues are easy to achieve (RNase T_1 is specific for G, RNase U_2 for A in appropriate conditions), there are no enzymes available which split only at C or only at U residues. Simoncsits *et al.*, overcome this difficulty by using pancreatic RNase and RNase I from *Physarum polycephalum*. The former cleaves at C and U, the latter at A, G and U. By comparing the patterns obtained from the four enzymic digests it is therefore possible to deduce the positions of all four nucleotides, although with not quite 100% accuracy. The method may be improved; at its present stage the sequences obtained still need some

further confirmation. But it is rapid, sensitive and widely applicable and thus represents a very important new approach in RNA sequencing.

The other highly efficient gel sequencing technique—Sanger's plus and minus method (See *News and Views*, 267, 104; 1977) has also been adapted to RNA by Brownlee and Cartwright (*J. molec. Biol.* 114, 93; 1977) and has been successfully applied to three eukaryotic messengers: ovalbumin, globin and immunoglobulin mRNAs. The principle is the same in DNA and RNA sequencing, but different enzymes are used. cDNA is synthesised on the RNA template by reverse transcriptase and the same enzyme is used in the minus systems to extend these DNA fragments. Degradation of the DNA fragments in the plus systems is achieved by making use of a hitherto unknown property of DNA polymerase I: in the presence of Mn^{2+} ions this enzyme shows a 3'-exonuclease activity towards the DNA strand of a DNA:RNA hybrid.

There are still some difficulties: interference by RNase activity of reverse transcriptase, anomalies in copying regions of the template where long hairpin loops are present. Ambiguities could, however, be corrected and the above anomalies even proved useful in some cases to locate hairpin loops in the RNA. The accuracy of the technique is not always perfect, occasional artefacts may cause minor mistakes, as is the case also with Sanger's technique. The sequences obtained should therefore be confirmed by other sequencing methods. Still, the possibility of working out a 200-residue long sequence in one experiment, to at least 90 to 95% precision is a development which will greatly accelerate studies on RNA structure.

It is predictable that progress

will be fastest in the study of eukaryotic messengers, not only because these molecules are at the centre of interest, but also because Brownlee's method is especially suitable for messenger RNA sequencing. Reverse transcriptase requires a primer to synthesise cDNA. This is perhaps the only drawback of the method, as the synthesis of oligonucleotide primers is time-consuming and presupposes some knowledge of the RNA sequence. But the poly(A) tail of eukaryotic mRNAs facilitates this task: a 10-residue long oligo(dT) or more suitably an oligo(dT) to which two nucleotides, complementary to the 3' terminal dinucleotide of the mRNA, are attached, can anneal to the beginning of the poly(A) tail and enable the reverse transcriptase to start copying the mRNA exactly from the 3' terminus. Brownlee and Cartwright worked out in this way a 172-nucleotide long sequence from the 3' terminus of ovalbumin mRNA. Although they had to use 13 gels to find the best conditions and to correct the ambiguities, the sequence of the major part could be read off a single gel.

The most interesting recent result of the rapid gel sequencing techniques is the completion of the nucleotide sequence of a eukaryotic messenger: rabbit β -globin mRNA. The structure of this RNA species has attracted keen interest since the isolation of the first pure mRNA. The first attempt to determine the nucleotide sequence of globin mRNA was made years ago and since then practically all sequencing techniques have been applied to this RNA at one stage or another to elucidate some part of its structure. Progress was initially rather slow: the classical techniques had only limited application to large molecules which could not be labelled *in vivo*. Copying techniques were introduced; in dif-

ferent laboratories different enzymes were used to produce highly labelled complementary strands to mRNA which could be subjected to fingerprint analysis. In Cambridge, Proudfoot and Brownlee synthesised cDNA from globin mRNA with the aid of DNA polymerase I (*Nature* **252**, 356; 1974; *J. molec. Biol.* **107**, 491; 1976). At Harvard and Yale, Forget *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **72**, 3614; 1975) and Marrotta *et al.*, (*Progr. Nucl. Acid Res. molec. Biol.* **19**, 165; 1976) applied a double transcription procedure, using reverse transcriptase on globin mRNA to obtain a cDNA transcript which in turn was copied into cRNA with *Escherichia coli* RNA polymerase. Salser's group at the University of California followed similar principles, sequencing parts of the cDNA and parts of the cRNA (*Progr. Nucl. Acid Res. molec. Biol.* **19**, 177; 1976). Lockard and RajBhandary at Massachusetts Institute of Technology used the 'wandering spot' procedure to determine the 5'-terminal capped structure (*Cell* **9**, 747; 1976). As a result of these different approaches major parts of the primary structures of rabbit globin and human globin mRNAs have been established.

In the April issue of *Cell* the long-awaited complete sequence of this mRNA has been published. Baralle (*Cell* **10**, 549; 1977) and Proudfoot (page 559) describe the 5' and 3' noncoding regions, respectively, applying the rapid gel sequencing technique of Brownlee and Cartwright. At the same time, Efstratiadis, Kafatos and Maniatis, using the gel sequencing technique of Maxam and Gilbert, determined the nucleotide sequence of their synthetic globin gene and thus also derived indirectly the sequence of globin mRNA (page 571). They present a 576-nucleotide long sequence almost complete in itself. The perfect agreement of this sequence with partial information available on the mRNA structure and

with the amino acid sequence of the protein proves that genes produced by *in vitro* transcription of mRNA and cloning of the double-stranded DNA product are faithful copies. □

Poland's 'fifth problem'

from I. J. Smalley

THE Polish government has defined nine major problems which are to be investigated by the country's scientists and engineers. These include such basic topics as developing a national electronics industry, finding a cure for cancer and tackling the difficult problem of water supply in a country with only one major river system. The fifth problem is housing—trying to find ways of overcoming the shortage of housing units in Poland. Such was the destruction caused in Poland during the Second World War that the housing balance has still not been restored.

Within project 5 perhaps the most urgent need is to find alternative sources of building materials, and this is closely followed by a need to improve the organisation of the building industry. The modern building material is concrete and for general purpose construction there is no real alternative. In a normal concrete only about 25% of the volume is occupied by the cement paste while the remaining 75% is occupied by inert space filling aggregate—and Poland is very short of aggregate.

Various approaches to the building material problem are being tried out at the Technical University in Warsaw. A. Degler is attempting to produce aggregates from pulverised fuel ash (PFA). Poland's coal-fired power stations (like those in the UK and USA) produce vast quantities of PFA and although some of this can be used as a pozzolanic cement substitute it would be best to convert it into aggregate. Degler's investigations on pelleting and extrusion processes have shown that it is possible—but there are delays in designing and constructing the necessary industrial capacity for the actual production. One of the nine major problems is that of environmental protection, so that if ways of using waste as aggregate can be found, two of the major problems will be alleviated.

Halina Badowska at the Technical University is attempting to find ways of utilising waste material from fertiliser

Maria Szekeley is a lecturer in the Department of Biochemistry at Imperial College, London.

THE rabbit globin mRNA sequence differs in various respects from the known nucleotide sequences of prokaryotic messengers. The latter contain a purine-rich tract near the initiation site, about 10 nucleotides towards the 5' end, which can form several base pairs with a pyrimidine-rich region at the 3' end of bacterial 16S RNA. No stable base-pairing is indicated between the 5' non-coding sequence of globin mRNA. A possible site for interaction of these RNAs was found around the initiation codon: here 6 base pairs could be formed. This, however, would imply that the initiator tRNA and the ribosomal RNA compete for the same

sequence in the mRNA.

The 53-nucleotide long non-coding sequence at the 5' end does not suggest formation of stable hairpin loops, but may allow base pairing with a stretch in the 3' noncoding region. A secondary structure which brings together the 5' and 3' ends of mRNA may be relevant to the control functions of the untranslated regions.

The 3' noncoding region is 95 nucleotides long. It shows extensive homology with the human β -globin mRNA. The human sequence has, however, an addition of 39 nucleotides which may have arisen by gene duplication.

I. J. Smalley is a lecturer in the Department of Civil Engineering at the University of Leeds.

production to make building blocks. Gypsum is the critical raw material and blocks using a form of gypsum cement and a polystyrene filler have been produced. A complex programme is also under way to develop resin materials as cements. There is in fact quite a severe shortage of Portland type cement in Poland although ironically enough Polish cement is exported. The need for foreign exchange is deemed greater than the need for cement.

G. Chrabczynski has been particularly concerned with the production of cement building units in which the hydration is accelerated by heating. This is often done under pressure in an autoclave but Chrabczynski has successfully developed methods which do not need the slow and expensive autoclave stage. Concrete railway sleepers with adequate strengths have been produced using much less cement than normal. Success, however, has been at the pilot plant level. There remains the difficult problem of organising factory production of the new items. Research is going on in Chrabczynski's section on organisation and management at all levels in the prefabricated building

block industry and it seems that, unless the persistent organisational problems are solved, the technical advances made in the laboratories will not benefit those who need project 5 to succeed.

It is ironic and regrettable that the new showpiece hotels in Warsaw have had to be built by Swedish contractors. These are certainly impressive buildings but it is a pity that desperately-needed foreign exchange had to be used to build hotels to give foreign businessmen and tourists the opportunity of returning the money to Poland. Project 5 is paradoxical in many ways. The population has recently passed 34 million—a level it reached for the first time just before the war, so that the colossal population losses have been replaced but the housing stock lags. Should family apartments have absolute priority or should the national construction industry be used to stimulate overseas trade? Major economic decisions will affect project 5: it is to be hoped that they will not have too adverse an effect. The 'fifth problem' does not have the scientific glamour of a possible cure for cancer but the Polish people would welcome its solution. □

New calculation of the nucleon optical potential

from P. E. Hodgson

THE differential cross section and polarisation for the elastic scattering of nucleons by nuclei can be very well described by a complex potential: the real part refracts the incident waves and the imaginary part absorbs them, just as a light wave is refracted and absorbed by a medium of complex refractive index. The absorbing potential takes account in a global way of all the reactions that remove flux from the elastic channel.

Unfortunately the potential describing the scattering is not unique: it is frequently possible to find several different potentials that fit the same data. This is unsatisfactory because these potentials give different wavefunctions, and unless we know which is correct it cannot be safely used to calculate the cross sections of non-elastic processes such as inelastic scattering and nucleon transfer reactions.

In principle the ambiguities between the potentials can be resolved by calculating the potential from more fundamental data, in particular from the nucleon-nucleon interaction,

together with the structure of the target nucleus. In practice, however, this is difficult due to the many-body nature of the problem.

Over the past few years there have been many attempts to calculate the optical model potential. These differ in the choice of approximations made to simplify the calculations and they give results in fair agreement with the experimental data. Particularly encouraging work has been carried out by Jeukenne, Lejeune and Mahaux of the University of Liège, who have been able to account for the volume integrals of the optical potentials (*Nature* **253**, 163; 1975).

Recently a new approach to this problem has been made by Brieva and Rook of the University of Oxford, and some preliminary results were reported at the Conference on Nuclear Structure at Tokyo in September. They start from the Hamada-Johnston potential that gives an accurate fit to the nucleon-nucleon scattering data, and then solve the Bethe-Goldstone equation for the motion of nucleons in infinite nuclear matter subject to this potential. This gives a quantity called an averaged *t*-matrix that is used to obtain the

optical potential by folding with the nuclear density distribution, using

$$V(r) = \int p(r') t(|r-r'|) dr'$$

with addition of exchange terms.

In this calculation the approximations were carefully chosen to facilitate the calculation without too much loss in accuracy. It differs from most previous work in giving both the real and imaginary parts of the optical potential, and allows the radically-dependent *t*-matrix to be non-local and energy-dependent. The spin-orbit potential is also calculated and is found to be very similar to the phenomenological spin-orbit potential.

The optical potential they calculated for ^{40}Ca is shown in Fig. 1 and compared with the phenomenological potential that gives the best fit to the differential scattering and polarisation data. It is particularly notable that the calculated real potential does not have the same radial shape as the phenomenological Saxon-Woods form. This indicates that this very frequently-used analytical form of the potential may not always be adequate. The calculated imaginary potential shows considerable structure, partly due to the form of the nuclear density distribution and partly due to the self-consistency requirement imposed on the potential. The differential cross section and polarisation calculated from these potentials are shown in Fig. 2. The agreement is strikingly good, indicating that it is now possible to calculate optical model potentials that give fits to the data that are comparable in quality to those found with phenomenological poten-

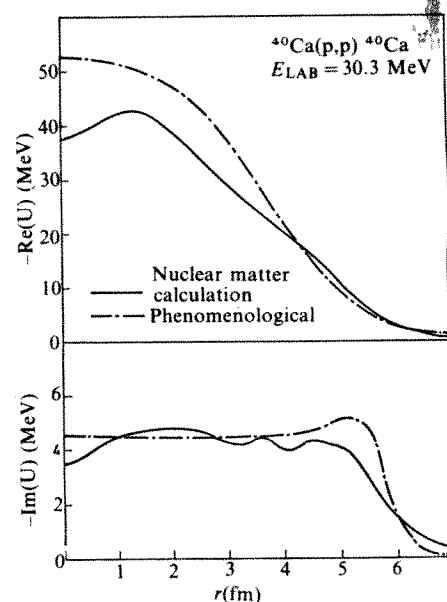


Fig. 1 Real and imaginary parts of the optical potential for protons on ^{40}Ca calculated from the nucleon-nucleon interaction compared with the corresponding phenomenological potentials.

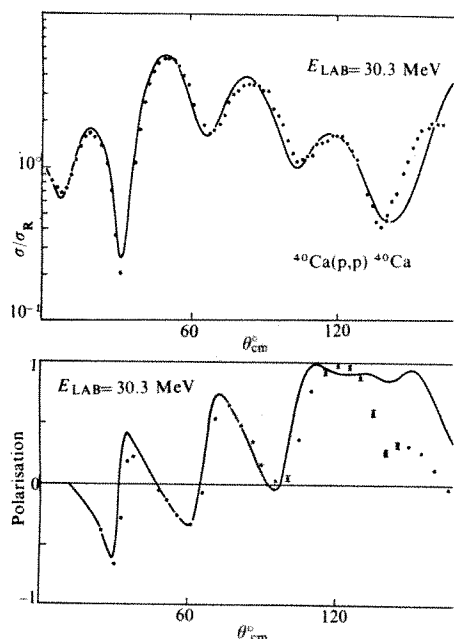


Fig. 2 Differential cross section and polarisation for the elastic scattering of 30.3 MeV protons by ^{40}Ca compared with optical model calculations using the calculated potential.

tials with many adjustable parameters. In some respects they are even superior as they can give features of the cross sections that cannot be explained by phenomenological potentials.

This work is very promising, and needs to be extended and applied to a range of nuclei to enable its validity to be explored. It should also prove possible to extend the method to the calculation of the optical potentials appropriate to composite particles such as deuterons and alpha particles, and perhaps also to heavy ions. The availability of optical potentials that can be reliably calculated from fundamental data instead of being obtained by phenomenological analyses of elastic scattering data will be of the greatest value in nuclear structure studies. □



A hundred years ago

THE question has been discussed of late whether the ancient Greeks had an acute and true sense of colour. I remember once to have seen the remark that Sophocles shows his want of colour-sense by speaking of wine-coloured ivy. Now this really shows how true his perception of colour was. I enclose two ivy leaves which I have gathered to-day off a wall; I could have gathered plenty of the same colour, which, as you see, is claret colour.

JOSEPH JOHN MURPHY

Old Forge, Dunmurry,
co. Antrim, October 21
From *Nature* 16, 25 October, 551; 1877.

Modulation of hormone receptors

from J. R. Tata

It is now widely accepted that receptors for hormones and other cellular signals, whether located on the cell's surface or intracellular, turn over rapidly so that the modulation of receptor level can be an important element regulating a cell's activity. About two years ago an account of the first reports on the possibility that some hormones themselves may control the levels of their own receptors or receptors for other hormones appeared in these columns (*News and Views* 257, 741; 1975). Whereas autoregulation of hormonal receptors is still a matter of some controversy, many investigators now accept that one hormone can regulate the level of receptor for another. In some instances it is hard to see the physiological relevance of such interplay among hormones but a recent paper suggests that it may be of clinical significance.

Endocrinologists and cardiologists have for long been aware of the tachycardia accompanying hyperthyroidism or thyrotoxicosis and that cardiac function in such patients was abnormally sensitive to small changes in adrenaline levels. Williams *et al.* (*J. biol. Chem.* 252, 2787; 1977) now explain this well-known syndrome by suggesting that thyroid hormones regulate the number of adrenergic receptor molecules in cardiac cells. In a carefully designed experiment they used the binding of the potent β -adrenergic antagonist (—)-dihydroalprenolol, to compare the number and affinity of β -adrenergic receptors in membranes isolated from myocardium of normal rats and animals made hyperthyroid by injections of the two thyroid hormones, L-thyroxine and triiodo-L-thyronine. Within 3–7 days, this treatment caused an increase in the binding capacity of cardiac membranes from 89 fmol of (—)- ^3H -dihydroalprenolol per mg protein in normal (euthyroid) rats to 196 fmol per mg protein. On the other hand, there was no difference in the affinity of the receptor for dihydroalprenolol or the β -adrenergic agonist isoproterenol (K_D at equilibrium of 2–15 nM dihydroalprenolol) between membrane preparations from euthyroid and hyperthyroid animals. Williams *et al.* therefore conclude that thyroid hormones control the number but not the nature of β -adrenergic receptors in myocardial cells. This would explain the tachycardia and increased myocardial contractility in clinical hyperthyroidism. Their observations would

J. R. Tata is at the National Institute of Medical Research, London.

also explain an earlier report that cultured foetal mouse hearts were rendered more sensitive by triiodothyronine to the chronotropic effect of β -adrenergic receptor stimulation (Widenthal *J. Pharmacol. exp. Therap.* 190, 272; 1974). If the same were true of other cells, then these findings could also explain skeletal muscle tremor or the enhanced rate of glycogenolysis and lipolysis in hyperthyroid subjects or experimental animals.

Are the observations of Williams *et al.* merely a peculiarity of thyroid hormones or are they part of a general pattern of an interhormonal regulatory device? Several recent publications point to the latter possibility. Such evidence comes mainly from studies on the interaction between hormones regulating reproductive processes which are known to be under multiple hormonal control. To cite but a few examples, Charreau *et al.* (*Molec. Cell Endocrinol.* 7, 1; 1977) have put forward a novel explanation why androgen, which like other steroids is not thought to act on adenylate cyclase, causes an increase in cyclic AMP levels in the rat prostate. These workers found that dihydrotestosterone (the active form of testosterone in target cells) increases the number of receptors for prolactin, a hormone known to influence male sexual development and to have a marked effect on adenylate cyclase in other tissues such as the mammary gland. Any interpretation of long-term studies of this kind in whole animals suffers from the drawback that one may not be measuring direct effects of hormones on receptor levels. For this reason, the demonstration that FSH (follicle stimulating hormone) induced receptors for hCG (human chorionic gonadotropin) directly *in vitro* in rat granulosa cells (Nimrod *et al. Nature* 267, 632; 1977) is significant. Hormonal interplay can of course operate in both directions, namely that one hormone can also dampen the sensitivity of a cell to another. Thus, it is interesting to note a recent report by Bhakoo and Katzenellenbogen (*Molec. Cell Endocrinol.* 8, 121; 1977) in which progesterone is shown to suppress the synthesis of oestrogen receptor molecules in the uterus. The resulting suppression of the replenishment of receptor molecules would thus explain the lowered uterine sensitivity to oestrogen that is known to be provoked by progesterone.

It is not difficult to predict that we shall be seeing many more examples of hormonal modulation of hormone receptors, particularly in reproductive tissues, mammary gland and the neuro-endocrine complex, where multiple hormones are involved in the regulation of their development or adult

functions. The question then arises as to whether modulation of receptor levels is restricted to interhormonal regulation or whether this phenomenon is even more widespread. Raff (*News and Views* 259, 265; 1976) has reviewed the general topic of regulation of receptors, particularly where it concerns immunological sensitivity and lymphocyte maturation. A recent paper from Munck's group (Smith *et al.* *Nature* 267, 523; 1977) is therefore significant in this context since it suggests that non-hormonal signals could also modulate hormone receptors. These workers studied glucocorticoid receptors during mitogen (concanavalin A) stimulation of human lymphocytes and noted that blast transformation and mitosis is associated with a striking increase in the number but not nature of receptor sites per cell. How such a modulation of receptor numbers is brought about remains unknown, but their observations explain quite nicely the increased sensitivity to glucocorticoid hormones known to be brought about by mitogenic stimulation of lymphocytes and may also be relevant to the general question of how steroid hormones control the immune response. □

Pollen analysis at INQUA

from Paul Colinvaux

The X INQUA Congress was held in August, 1977 at the University of Birmingham. Pollen analysis was one of the many topics bearing on Quaternary research that were discussed.

FOR most of its existence, pollen analysis has been a tool of other disciplines; being used to age strata, to supply the temperatures of past times, to serve phytosociologists as they mapped the supposed movements of plant associations through millennia.

Time stratigraphy is now provided by Quaternary geology and isotope chemistry. Oceanic sediments now provide maps of past global climates. The press of contemporary ecological theory is to question strongly the validity of many former ideas about the integrity of plant communities. These developments allow pollen analysis to be used in more exhilarating ways, and the new ways are now taking hold.

One group of pollen analysts is interested in the ancestry of the remarkable complexity of the deciduous forests of Eastern North America. For several years this school has been

growing more confident in its insistence that the forest associations of Eastern America are comparatively recent assemblages of plants without the integrity, or persistence as communities, required by classical plant sociology. This school has used the pollen record to infer the responses of plant populations to known climatic change, borrowing climatic data from other disciplines. At the conference these analysts were confident that their case is made. Pollen analysis shows that plant associations beyond the front of the Wisconsin ice were different from all the associations we know today; and the tundras, prairies, and forests that replaced each other on the lands once held by ice were always critically different from the modern assemblages.

However, INQUA also saw reports of the refined use of pollen data to infer past climates. Fossil pollen assemblages are being compared with many surface samples, and the data are then being analysed with multivariate statistical techniques. It sounds like using principal components and canonical series to do what was once done by eyeball fits, but the approach is vitally different. The old way was to seek modern plant associations that were homologous with past communities and to graft on to those past communities the climate under which the supposed modern homologue was living. The new way is to select from ancient pollen spectra only those taxa found in comparable numerical abundance in surface samples, and then to compare the distributions of this select array with distributions of the same plants in historic times. There is in this no dubious assumption about the integrities or environmental requirements of whole plant communities; the only biological assumption is the respectable Darwinian one that niche requirements of each species do not change very much. Climatic reconstructions of this kind follow the methods that have been used by students of foraminifera in deep sea cores to reconstruct ancient water temperatures. INQUA saw several elegant papers using this approach. At INQUA, therefore, pollen analysis was used both to refute the existence of permanent plant communities and to improve the resolution of the climatic inferences that can be drawn from pollen.

All pollen analysts at INQUA drew comfort from the certainty that the oxygen isotope record of deep sea cores yields a chronology of the volume of global ice. The isotope record is not of ocean temperature, as was thought only a short while ago, but of glacial ice. It tells us nothing about world climate, other than the existence of ice itself, but it is a global time stratigraphy and

it must loosely relate to the particular climate of any one place. All pollen analysts have found themselves asking 'Are the local developments of vegetation that I see in step with the curve defining relative ice volume?' INQUA saw the first data relating isotope measurements to pollen in the same samples of offshore cores, revealing a gratifyingly close synchrony between changes in coastal forests and the volume of distant glaciers. A climatic linking mechanism is inescapable.

There were the expected developments in longer records and explorations in lesser-known parts of the world. Those with the long cores will look to the ice-volume curves to help with their time-stratigraphies and the first attempt of this kind to date long cores with the help of the ice volume curve was reported—a splendid long record from Searls Lake in California. The still very uncertain possibilities of remanent magnetism are also being explored to date pollen records back to the postulated Blake Event and beyond at Japanese Biwa, in Alaskan Imuruk Lake, and for the superb section from Grande Pille in France. The next INQUA may well see some ambitious reconstructions from these places describing the shufflings of plant species on single sites for more than 100,000 years.

The number of pollen records from the rest of the world grows slowly but steadily. Mapping the past in the Soviet Union continues as pollen analysts follow the completed map of the Quaternary deposits of that vast country, taking particular note of regions where archaeologists are active. But in lower latitudes where we need to know most there is still least to report. From the Indo-Pacific there are a few scattered records but from the South American equator near silence, particularly from the Amazonian basin. A member of the CLIMAP team at INQUA was heard to remark that he had had to infer the Wisconsin age vegetation of Amazonia from a paper on the modern distribution of bird species.

Yet in Africa there is progress, as papers come from Ethiopia and Chad, the lakes of the Rift, the dry basins of Sahara, and an array of stations in the South. But this African work shows how formidable are the difficulties of pollen analysis in tropical regions where the crucial species leave little pollen and where the species list is huge. As D. A. Livingstone remarked, deep in Tanganyika and the other lakes of the Rift lies a detailed history of the vegetation of this cradle of humanity since the Pliocene and beyond, but he despaired of seeing it read in his lifetime. □

review article

Claims and accomplishments of applied catastrophe theory

Raphael S. Zahler* & Hector J. Sussmann†

Several representative attempts to apply catastrophe theory to biological and social science problems turn out on close analysis to be characterised by incorrect reasoning, far-fetched assumptions, erroneous consequences, and exaggerated claims. Catastrophe theory seems to have made no significant contributions to biology and the social sciences, and to have no advantage over other better-established mathematical tools which have been used to better effect.

EMBRYOLOGY, ethology, ecology, and geology; physics, economics, dynamics, and linguistics; prison riots, literary symbolism, and the Vietnam war—these are some of the subjects to which catastrophe theory is said to be applicable. Its novel mathematical apparatus seems to be a near-universal tool, according to its proponents: "Properly understood and exploited, this ever-expanding web of concepts promises mankind a unique weapon against ignorance and a profound insight into the universe"¹.

We disagree. And because we feel that the many researchers now being attracted to catastrophe theory stand to gain nothing but disappointment and wasted time, we have written a critical study of applied catastrophe theory². Our conclusion is that the claims made for the theory are greatly exaggerated and that its accomplishments, at least in the biological and social sciences, are insignificant.

This is because catastrophe theorists have misused the basic mathematics in ways that lead to incorrect reasoning; they have offered models which are based on unreasonable assumptions and which lead to erroneous conclusions; and they have made predictions which are either vacuous, tautologous, vague or impossible to test experimentally. We do not say that catastrophe theory cannot possibly be applied. There may be legitimate uses in areas of physics and engineering, and some have suggested that the use of topological methods will be conceptually stimulating to researchers. So far, however, its record is poor.

We stress that we are not discussing the correctness or importance of catastrophe theory as a purely mathematical subject: we are sceptical only about its usefulness as a tool for extramathematical applications. We present this critical picture because we are excited about the prospect of new applications of mathematics, and concerned that many will be disenchanted with all of modern mathematics when they discover, as we have, that catastrophe theory is a blind alley.

This article is organised round a list of ten main kinds of defect found in catastrophe theory models in biology and social science. Each is illustrated briefly by one or two examples. For more detail, the reader should consult our longer paper². We are grateful for the advice of R. FitzHugh, J. C. Scanlon, H. Othmer, C. S. Hui, B. Goodwin, J. Sturtevant, G. Velicelebi,

S. Mabrey, J. F. Chlebowski, E. S. Crelin, J. F. G. Auchmuty, N. Van Arkel whose comments have been of great help in the preparation of this paper.

The cusp catastrophe

Most applied catastrophe theory is based on the 'cusp catastrophe' (Fig. 1). In this picture, the horizontal plane represents the possible values of two control parameters a and b , and the behaviour of the system is plotted on the vertical x -axis. For example, suppose we place a globular protein in solution and study its denaturation in terms of the concentration a of a denaturant and the temperature b . The extent of denaturation x_0 (as measured by the negative of the optical rotatory dispersion) as a function of denaturant concentration a_0 and temperature b_0 can be plotted as a point in the vertical line through (a_0, b_0) ; all such points form the curved 'cusp' surface shown, according to Kozak and Benham³.

So far this is just standard analytical geometry. The alert reader, however, may have noticed that above each point (a_0, b_0) in the shaded region R of the control plane lie not one but three different points of the behaviour surface; which one represents the actual behaviour at (a_0, b_0) ? The answer, given by the delay rule, is that it depends on how (a_0, b_0) is approached. As a and b vary continuously, x is to vary continuously in the cusp surface as long as possible. When continuous variation of x is not possible, then x is to jump to another sheet of the surface. For example, suppose the control parameters vary in such a way that the point (a, b) moves from Q_1 to Q_2 along the path P_1 , shown in Fig. 1. Initially, there is only one point in the cusp surface lying above each point in P_1 , so we follow the line shown from Q_1 towards J . Even when we pass point J the delay rule keeps us on the bottom sheet. But, when we reach point K , we must jump to K_2 on the upper sheet as shown. This is how sudden changes of behaviour in response to smooth changes of controls occur in catastrophe theory models.

Why the cusp? Catastrophe theorists argue that, according to a "deep mathematical theorem" of René Thom, essentially any system where sudden changes occur and where two control parameters appear can be described by the cusp; it is an inevitable, universal paradigm. According to Kozak and Benham, in fact, Fig. 1 correctly predicts the denaturation behaviour of RNase or collagen subject to the influence of temperature and CaCl_2 concentration. For example, following path P_1 from Q_2 to Q_1 , or P_3 from Q_3 to Q_4 gives a sigmoid curve for x , which corresponds to the appropriate experimental data. By distorting the cusp M somewhat (which is permissible

*104 Hubinger Street, New Haven, Connecticut 06511.

†Department of Mathematics, Rutgers College, Hill Centre for the Mathematical Sciences, Busch Campus, New Brunswick—New Jersey 08903.

the data or (2) purely unverified hopes, or (3) independent of CT or (4) just wrong.

Consider, for example, Zeeman's studies of how homogeneous tissue of an embryo differentiates into two types separated by a frontier, a phenomenon he calls divergence or differentiation². The 'main theorem' of this paper is worth quoting in full: "Homeostasis, continuity, differentiation, and repeatability imply the existence of a primary wave. In other words a frontier forms, moves and deepens, then slows up and stabilises, and finally deepens further".

It is hard to imagine what we could learn from such a breath-takingly vague statement, but we need not ponder this very long, since its proof is wrong as well.

Misuse of genericity

For one thing, the surface eventually chosen to model the cellular differentiation is not dictated by Thom's theorem but is obtained by a series of arbitrary choices which are justified—if at all—by appealing to 'genericity'. This mathematical concept is a way of excluding 'exceptional' or 'degenerate' cases. (For instance: 'generically, the axes of an ellipse are not equal' means that circles are an exceptional class of ellipse or, more intuitively, that a 'randomly chosen ellipse' is quite unlikely to be a circle.) In the course of his proof, Zeeman arbitrarily translates the vague word 'repeatability' into the precise concept of genericity. But then, all his theorem says is that if nothing exceptional happens, then the frontier moves.

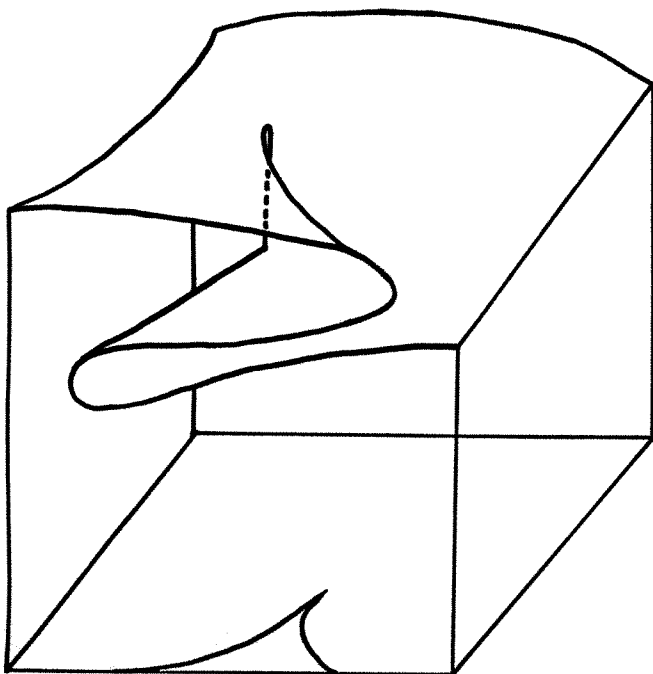


Fig. 2 Distorted cusp catastrophe; see ref. 3, Fig. 2.

Zeeman's 'proof' consists of no more than the observation that, if the frontier did not move, that would be quite exceptional. To evaluate this kind of reasoning properly, notice that the same logic, if correct, would apply equally well not just to frontiers of tissues but to anything whatsoever. So, Zeeman's reasoning 'proves' that everything moves except for those exceptional objects that do not. (For details, see ref. 2 §12 a).

Later in the same paper, Zeeman 'proves' that the frontier moves initially at constant speed. His proof is as follows: the frontier can be replaced initially, to first order, by its tangent, QED. Again, there is nothing in this reasoning that restricts its validity to frontiers of tissues, or to the time when the frontier forms. So Zeeman is really proving that everything moves

at constant velocity. But let us apply the same logic once again. Why not use the second degree Taylor approximation? And why not argue about the acceleration as Zeeman does about the velocity, and conclude that the acceleration is non-zero, because it would be exceptional if it were zero? This gives us the conclusion that everything moves with non-constant velocity.

Finally, why can we not take the zeroth order approximation? The conclusion then is that nothing moves at all.

We see that using these methods, everything, no matter how absurd, can be proved.

More than two thousand years ago, Zeno formulated his celebrated paradox of the arrow. Consider an arrow in its flight. 'At each instant of its flight the tip of the arrow occupies a definite position. At that instant the arrow cannot move, for an instant has no duration. Hence, at each instant the arrow is at rest. Since this is true at each instant, the moving arrow is always at rest. This paradox is almost startling. It seems to defy logic itself.'

The development of Calculus has resolved this paradox, and taught us how it is possible for things to change and, at the same time, be what they are at each instant. The position of the tip of the arrow is what it is at each time, but it is different at different times. Hence the arrow can have a definite position at each time, and not be at rest. What is true of the position is also true of velocity. The velocity has a value at each point, but this does not mean that it is constant. Zeeman, a Twentieth Century Zeno, is reformulating the paradox as a corollary, thus ignoring more than two millennia of mathematics.

But Zeeman's main theorem is wrong for other reasons as well: it is easy to show by means of counter examples that Zeeman's conclusions that the frontier does not stabilise or deepen do not follow from his assumptions. When these counter-examples were brought to the attention of catastrophe theorists, the theorem was defended by disclosing new interpretations of the terminology and the hypotheses. For instance, the hypothesis of 'differentiation' is now taken to mean that after some time two different types of tissue are found and no more changes occur. If so, then after some time the frontier no longer moves, so the frontier does indeed stabilise. But of course this 'proof' of stabilisation is transparently circular.

In general, the hypotheses on which many catastrophe-theory arguments are based are often vague. When terms like 'differentiation' and 'repeatability' are used without any precise translation, the correctness of the 'proofs' is hard to assess. Catastrophe theory papers consistently violate one of the most basic rules of the scientific method: state clearly what you mean and do not change definitions in the middle of your reasoning.

Misleading use of mathematics

The most basic criticism of Zeeman's catastrophe theory of embryology, however, is the way the mathematical foundations are used. Others have pointed out that the 'deduction' that differentiation will occur as a pattern of primary and secondary waves arises not from catastrophe theory itself but from the conditions assumed to exist before the primary differentiation process begins (for example a gradient of cell states). In fact, then, most of the results of this paper, regardless of their merit, have nothing to do with catastrophe theory at all.

To see why this is important, we must remind the reader of what catastrophe theory is and is not. First, events in which apparently 'catastrophic' changes occur are not necessarily 'catastrophes' in the technical mathematical sense. Catastrophe theorists agree that the term 'catastrophe' is reserved for certain kinds of singularity of smooth maps, seven of which have been described and classified elegantly by Thom. The keystone of catastrophe theory, in fact, is Thom's profound theorem. It is what is supposed to give catastrophe theory its deductive powers, because it allows one to conclude that a given situation must be represented by a cusp, or one of the other elementary catastrophes.

As we have illustrated, however, most catastrophe theory models make no use of Thom's theorem. Thus the cusp is no longer inevitable or unique.

How, then, does the cusp arise? Often, as in the embryology models, the cusp arose because the hypotheses were carefully chosen to make sure that it would. In other cases, however, experimental data are plotted and found to resemble a cusp^{3,8}. This is not surprising: if we plot an ordinary hysteresis loop in

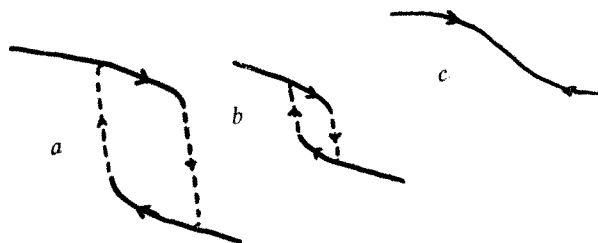


Fig. 3 Hysteresis loops of decreasing size.

two dimensions (Fig. 3a), in which fast vertical jumps alternate with slower horizontal movements, and then imagine a continuum of such loops getting smaller (Fig. 3b) and finally vanishing to form a continuous curve (Fig. 3c), we get a surface resembling the cusp of Fig. 1. Thus we can regard the cusp as a nice way of picturing a system with variable degrees of hysteresis. But it is certainly not unique in this: a surface with a double fold whose projection was any smooth curve (a parabola, for example: see Fig. 4) would do as well. So we must ask again: what is special about a model that looks like a cusp? Although some have found such pictures aesthetically satisfying, or a source of insight, we can only state that the fact that certain data happen to form a cusp-like shape tells us nothing new about the system.

Careless discussion of evidence

Catastrophe theorists have asserted that there is experimental evidence for some of their models. For instance, Zeeman⁴ writes, "I have constructed CT models of the heart beat, the nerve impulses and the formation of gastrula and of somites in the embryo. Recent experiments by J. Cooke and T. Elsdale appear to confirm some of my predictions". The facts are as follows. (1) No experiments have been made to test the predictions of Zeeman's nerve impulse models. As for the heart-beat, Zeeman is said to have made experiments in 1972, but the results have not been published (Zeeman, personal communication). (2) The catastrophe theory nerve impulse models disagree with experimental voltage-clamp data⁹ in several important aspects, deny the universally accepted concepts of the sodium leak and the independence of sodium and potassium channels, and lead to the wrong propagation speed for the action potential. (3) Zeeman's embryology paper⁵, besides being mathematically wrong, betrays the author's inexperience in embryology. For example (p. 27), Zeeman likens the embryonic neural tube to a roll of stiff paper which tries to maintain its curl. But experiment shows that cut neural tube persistently tries to unroll¹⁰. (4) T. Elsdale *et al.*¹¹ write: "... we do not yet conclude that the observations here presented have confirmed Cooke and Zeeman's model to the exclusion of others". (5) J. Cooke (personal communication) writes: "I, at least, do not regard any of the predictions of the model in which I am involved as being deeply distinctive to catastrophe theory".

Stewart¹ repeats the untrue assertion that Zeeman's embryology predictions have been "recently verified by experiment". Regarding the use of catastrophe theory in social sciences, Stewart writes: "Although most such models still lack precise data, an interesting exception is a study by Zeeman and several collaborators of how tension and alienation among prison inmates influence disorder. A cusp catastrophe fits the data

very well, the sudden jumps being riots and truces". In fact, when Zeeman and his coworkers plot points in a plane, and look for a cusp curve which fits them, they do not succeed in fitting one curve, so they use two, and claim that the cusp must have moved during the process. Finally, they do not use any statistical techniques to determine whether or not their pair of cusp curves gives a good fit (compare Fig. 5).

Dixon Jones⁸ has made a catastrophe theory model for budworm infestation. His model relies on a 'cusp' which is really not a cusp in the correct mathematical sense. His model predicts a fast rise of a variable, but the data rise slowly. Jones then rescues his model by making a logarithmic change of scale!

At this point the reader may suspect that we have chosen the weakest catastrophe theory papers as targets for our criticism. The contrary is true: the best biological applications are contained in the papers just cited. Others^{12,13} consist of long expositions of the mathematics of catastrophe theory, copious explanations of elementary biology, and vague speculations on how the two could be brought together.

Unreasonable or ambiguous hypotheses

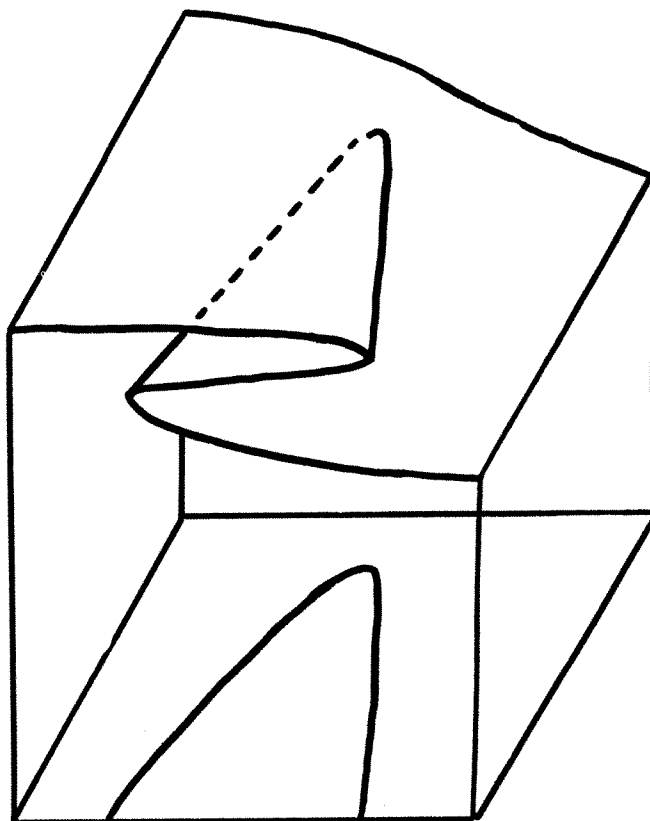
Isnard and Zeeman¹⁴ have a model for how a country makes decisions about going to war. It is assumed that public opinion depends on the 'cost' of the war, and the (perceived) 'threat' posed by the enemy nation.

Among the five hypotheses of the war model are: Hypothesis 2: If the cost of the war is low, then opinion will be unified, and the greater the threat, the greater will be the level of military action called for.

Hypothesis 3: If the cost is high, and the threat moderate, then opinion will be split between doves and hawks.

Now in a given conflict situation, no sensible person would advocate a level of military action so low that the enemy is sure to win an easy victory. Either one wants no war at all, or

Fig. 4 A folded surface which is not a cusp since the projection of the fold curve on the control plane does not have a sharp point.



one wants to use as much military force as he thinks is needed to win. (War will follow when at least one side miscalculates, and both sides think they can win.) So, the dichotomy war—no war is a discrete one, and this is so whether the cost is high or low. But, if this is taken into account, we are left with a better theory which, however, does not involve a cusp.

Not only are the hypotheses of the war model unreasonable but they do not lead to the conclusions reached by the authors. Their main claim—that the hypotheses imply, via Thom's theorem, that the behaviour set is a cusp surface—is once again dubious, since the proof, which is not given, is presumably

ingless. The same reasoning applies to the 'level of military action' in the war model, as we have shown.

Better alternatives

Defenders of catastrophe theory claim that it provides, for the first time in history, a mathematical method for modelling discontinuous phenomena. This is false because, as we have shown, catastrophe theory does not lead to satisfactory models. But it is also false because it ignores the whole body of discrete mathematics, the study of shock waves, bifurcation theory, and the mathematics of quantum mechanics. Better alternatives certainly exist.

Besides, no mathematical theory, no matter how elegant it may be, can serve as a substitute for the hard work of learning the facts about the world. Catastrophe theory is one of many attempts that have been made to deduce the world by thought alone. It offers to mathematicians "the hope of applying mathematics without having to know anything but mathematics"¹⁶. An appealing dream for mathematicians, but a dream that cannot come true.

Appeal of the theory

Why has catastrophe theory acquired such widespread popularity? One possible reason may be its impressive claims of universality and usefulness, backed up by a large number of (mostly unrefereed) publications praising each other extravagantly; another might be the unusual nature of the mathematics used, combining concepts that are completely inaccessible to anyone who is not a professional mathematician with the use of some pictures of amazing simplicity, so that the result is simple to grasp intuitively but difficult to criticise.

It may be said that catastrophe theory is a new theory and that, while all the applications proposed so far have serious flaws, each one has benefits that make it worthwhile. Our criteria for judging a theory (or method) are rather generous. Even if a theory rests on questionable assumptions, or is based on faulty reasoning, or leads to false conclusions, or deals with ambiguous concepts, or does not make true testable predictions, we are ready to accept that it may be valuable, so long as it does not have all these faults at once.

The catastrophe theory models that we have examined, however, combine all these faults. The assumptions on which they are based are unreasonable and/or vague. The reasoning used to draw conclusions from the hypotheses is mathematically incorrect. The conclusions are either trivial (for example "a frightened dog, if angered, may attack"⁴) or false.

The possibility that, in the future, catastrophe theory may produce solid applications, cannot be dismissed *a priori*. However, its spectacular failures should suffice to raise serious doubts. For a method that has been said to have "the potential for describing the evolution of forms in all aspects of nature", existing evidence is indeed disappointing. The scientific community must remain sceptical until the proponents of catastrophe theory succeed in substantiating their claims. The burden of the proof is on them.

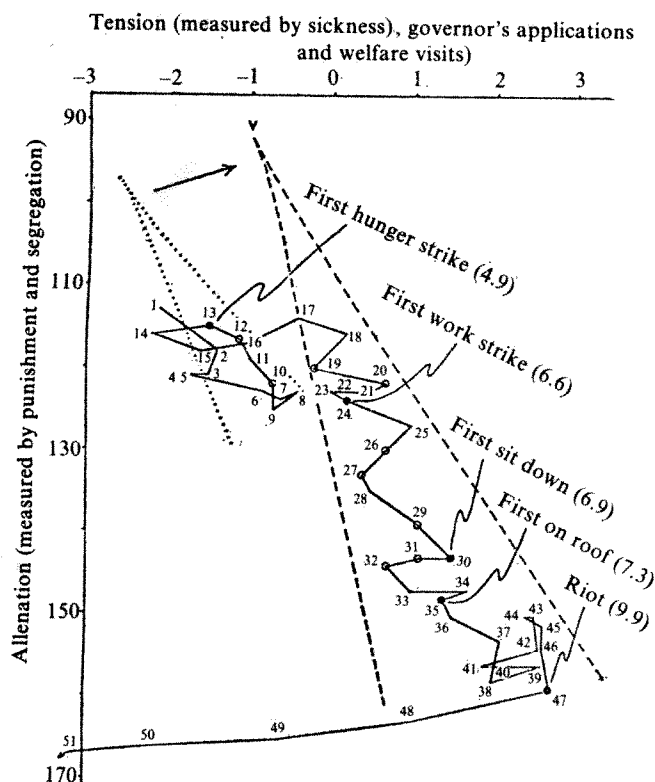


Fig. 5 The 'analysis of the data' of the paper by Zeeman *et al.* on prison riots¹⁷. These data are supposed to show that a cusp catastrophe gives a good fit.

based on some translation (also not given) of the vague hypotheses into precise language. It is not hard to show, however, that depending on one's interpretation of the hypotheses, either the conclusion does not follow; or the result does not depend on Thom's theorem, and is essentially contained in the hypotheses.

In fact, the only possible part Thom's theorem might play is to exclude a rounded bifurcation curve (such as the parabola of Fig. 4) in favour of a cusp (Fig. 1). But since either Fig. 1 or Fig. 4 would lead to the conclusions stated by Isnard and Zeeman, there is really no need to use Thom's theorem after all.

Spurious quantification

Catastrophe theorists often attempt to make a discrete variable into a continuous one so that CT can be applied. In Zeeman's dog aggression model⁴, for example, the level of aggression of a dog is considered as a continuous variable x , ranging "from outright retreat through cowering, avoidance, neutrality, and growling and snarling to attacking". He also states, however, that an 'attack catastrophe' occurs when the value of x jumps upwards from one sheet of the behaviour surface to another. Despite this ambiguity, it can be shown that with either interpretation the attack is embedded in a continuous family of behaviours. But this is absurd: the idea of a 'semi-attack' by a dog, or of a snake gradually attacking a person, is utterly mean-

1. Stewart, I. N. in *Encyclopedia Britannica Book of the Year* (Encyclopedia Britannica, New York, 1977).
2. Sussman, H. J. & Zahler, R. S. *Synthese* (in the press).
3. Kozak, J. J. & Benham, C. J. *Proc. natn. Acad. Sci., U.S.A.* **71**, 1977 (1974).
4. Zeeman, E. C. *Sci. Amer.*, **234**, 65 (1976).
5. Zeeman, E. C., in *Lectures on Mathematics in the Life Sciences* **7**, 69 (1974).
6. Zeeman, E. C. in *Towards A Theoretical Biology* **4**, 8 (Edinburgh University Press, Edinburgh, 1972).
7. Kline, M. *Mathematics in Western Culture* (Oxford University Press, New York, 1974).
8. Jones, D. D. *The Application of Catastrophe Theory to Ecological Systems* (International Institute for Applied Systems Analysis, Schloss Laxenburg, Austria, 1975).
9. Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **117**, 500 (1952).
10. Crelin, E. S. *J. exp. Zool.* **120**, 547 (1952).
11. Elsdale, T., Pearson, M. & Whitehead, M., *J. Embryol. exp. Morph.* **35**, 625 (1976).
12. Woodcock, A. E. R. *Ann. N.Y. Acad. Sci.* **231**, 60 (1974).
13. Dodson, M. M. *Math. Biosci.* **28**, 243 (1976).
14. Isnard, C. A., Zeeman, E. C., in *Use of Models in The Social Sciences* (Tavistock, London, 1974).
15. Blainey, G. *The Causes of War* (Macmillan, New York, 1973).
16. Kolata, G. *Science*, **196**, 287 (1977).
17. Zeeman, E. C., *et al. Br. J. math. stat. Psych.* **29**, 73 (1976).

articles

Radio structure of 3C147 determined by multi-element very long baseline interferometry

P. N. Wilkinson & A. C. S. Readhead

Owens Valley Radio Observatory, California Institute of Technology, Pasadena, California

G. H. Purcell*

National Radio Astronomy Observatory, Charlottesville, Virginia

B. Anderson

University of Manchester, Nuffield Radio Astronomy Laboratories, Jodrell Bank, UK

We have determined the radio structure of the quasar 3C147 from multi-baseline VLBI data at 609 MHz using both a conventional method and a technique which uses the 'closure' phase information to produce a good approximation to a synthesis map of the source. The structure is similar to the central part of M87, with a bright core and a linear 'jet' of projected length ~ 1.5 kpc which is concentrated in bright 'knots'.

THE quasar 3C147 ($z = 0.545$, $V \simeq 17$ m) is one of the more intense radio sources in the sky at decimetre wavelengths. Previous interferometer and interplanetary scintillation observations¹⁻⁶ have shown that its radio emission is confined to a region $\lesssim 1.0''$ in extent. At high frequencies most of the radiation comes from a complex region of the source $\lesssim 0.2''$ in size, while at low frequencies ($\lesssim 100$ MHz) the size of the predominant emission region is $\sim 0.7''$. The interferometer observations, in which only fringe amplitudes on single baselines were measured, were inadequate to determine in any detail the morphology of the compact region. We report here the results of more extensive observations which do reveal this structure in considerable detail. They constitute the most reliable complex radio structure determined by VLBI techniques.

The observations

The data were obtained in two independent observing runs, both at 609 MHz, using circularly polarised primary feeds on each antenna. In each case the data were recorded with NRAO MkII VLB equipment and the video tapes correlated on the NRAO processor⁷. The details of the telescopes used and their rf performance are given in Table 1. The six interferometer baselines are shown schematically in Fig. 1a, and the resolution achieved by each interferometer pair as a function of hour angle is shown in Fig. 1b.

The first observations, made in December 1973, involved only the three US stations (giving the three inner tracks in Fig. 1b) and were analysed solely by G. H. Purcell in 1974. The plots of fringe amplitude as a function of interferometer hour angle are shown in Fig. 2a. Further observations of 3C147 were made, using all four telescopes, in March 1975. We observed the source for nearly all the time for which it was jointly visible at each station in order to obtain the maximum possible 'closure' phase information, as well as visibility amplitudes.

A closure phase, C , is formed by the addition of the observed interferometer phases around a closed loop of baselines at a given time. The simplest loop is a triangle. Four triangles can be formed from our four station network (see Fig. 1a), but only three of these yield independent information. On each baseline we measured the fringe phase, ϕ_i , given by

$$\phi_i = \psi_i + \theta_i$$

where ψ_i is the phase of the visibility function and θ_i is a

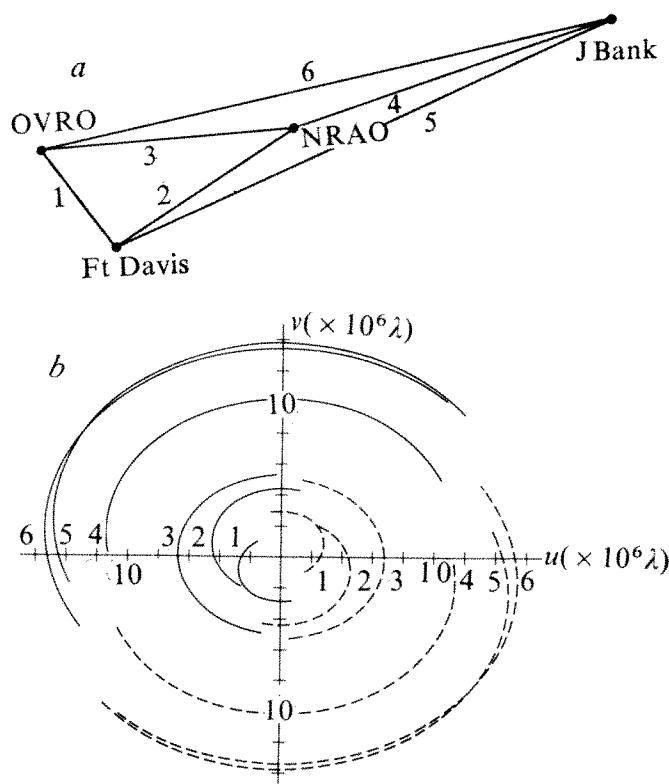


Fig. 1 a, Schematic representation of the six interferometer baselines. We formed closure phases around all four triangles but used mainly C_{123} , C_{245} and C_{346} (see text). b, The baseline of each interferometer as a function of hour angle. The source was observed continuously along each track. The baselines are as follows: 1, Ft Davis-OVRO; 2, NRAO-Ft Davis; 3, NRAO-OVRO; 4, J. Bank-NRAO; 5, J. Bank-Ft Davis; 6, J. Bank-OVRO.

*Present address: Jet Propulsion Laboratory, Pasadena, California

perturbation due to changes in the path delay to each telescope through the atmosphere and ionosphere, to phase drifts in the independent local oscillators and to uncertainties in the baseline and source position. A combination of these effects, with the ionosphere having a dominant role at this relatively low frequency, precludes the direct use of the six interferometer phases. It has been shown^{8,9} that in the closure phase the perturbations cancel, and thus the three closure phases we form

$$C_{123} = \phi_1 + \phi_2 - \phi_3$$

$$C_{245} = \phi_2 + \phi_4 - \phi_5$$

$$C_{346} = \phi_3 + \phi_4 - \phi_6$$

are equivalent to

$$C_{123} = \psi_1 + \psi_2 - \psi_3$$

$$C_{245} = \psi_2 + \psi_4 - \psi_5$$

$$C_{346} = \psi_3 + \psi_4 - \psi_6$$

respectively. The three closure phase curves we obtained are shown together with the six fringe amplitude curves in Fig. 3a, b and c. Unfortunately, because of a malfunction in the NRAO processor, the signal-to-noise on all baselines except NRAO-Ft Davis was reduced by a factor of two from the normal value.

The agreement between the independently measured fringe amplitude curves for the US baselines at the two epochs is within the expected uncertainty due to receiver noise and calibration errors (~5%). This agreement shows that there have been no significant changes in the structure of the source during an interval of 15 months¹⁰.

The brightness distribution

The brightness distribution of the source was inferred by Purcell from the 1973 amplitude data alone, using a model fitting method in which the parameters of elliptical Gaussian components are adjusted by an iterative procedure to produce a best fit to the data in the least squares sense. The complexity of this source is such that 13 gaussian components were required to produce the solid lines in Fig. 2a, which are the fringe amplitudes calculated from the final model. We do not list the parameters of this model, but rather display the contours of radio brightness which they represent; this 'picture' of 3C147 is shown in Fig. 2b. Note that because no phase information was included there is a 180° ambiguity in its orientation.

To analyse the more extensive 1975 data we devised a new approach which is an extension of the following scheme proposed by M. H. Cohen: beginning with a model of the source based on amplitude data alone, calculate the visibility phase of this model on all baselines and use this phase, with the observed amplitudes, to make a 'map'. Using the CLEAN technique¹¹⁻¹³ reduce the sidelobe level on this 'map' and thereby produce a new model of the source. The phases predicted by this new model are different from the original phases and thus if we repeat the process using these new phases the 'map' will be different from the original one. Iterate, using the new model to predict the phases each time and see whether the method converges to a stable 'map'. This method is essentially that proposed by Fort and Yee¹⁴, except that where we use the CLEAN technique, they set all negative regions of the 'map' to zero at each iteration. We decided to extend the procedure proposed by M. H. Cohen by including the closure phase data, and thus to make use of all the information available in present-day VLBI observations (that is fringe amplitude, closure phase and extent of (u,v) coverage).

Table 1 Interferometer elements

Location	Institution	Diameter (m)	System Noise (K)	Sensitivity (kJy ⁻¹)
Jodrell Bank, Cheshire, UK	Manchester Univ.	76	140	0.95
Green Bank, W. Va., USA	NRAO	43	160	0.21
Ft. Davis, Texas, USA	Harvard Univ.	26	300	0.08
Big Pine, Calif., USA	Caltech	40	260	0.18

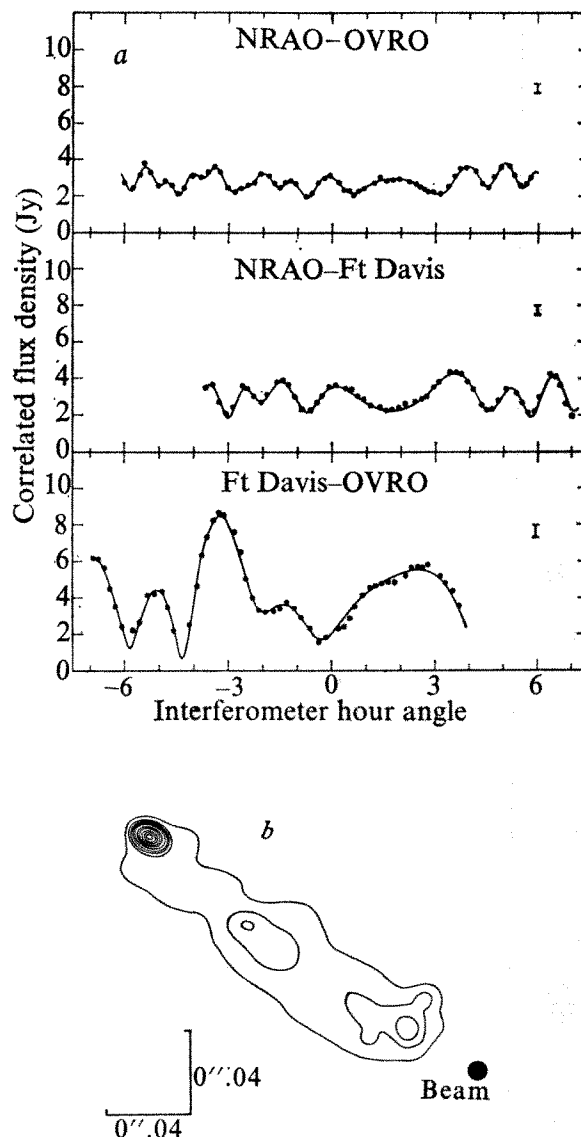


Fig. 2 a, Fringe amplitude data on the US baselines in December 1973. Mean error bars shown include a contribution from the uncertainty of the flux scale on the different baselines. The solid lines are the predicted amplitudes from the model in Fig. 2b. b, Contours of radio brightness derived from the 13-component model fitted to the fringe amplitudes in Fig. 2a. The contour levels were chosen to correspond with those in Fig. 4. The original Gaussian components have also been convolved with a circular Gaussian (FWHM = 0.009") to simulate the effect of the 'clean beam' in Fig. 4.

This combination of the closure phase with an iterative 'cleaning' technique has subsequently proved to be a powerful method for determining source structure provided the (u,v) plane is sufficiently well sampled. The method will be described in more detail elsewhere, but the analysis of the 3C147 data provides a simple and illuminating example of its use. It is clear from the fringe amplitude and closure phase plots that this source contains a compact region which is barely resolved on the three longer baselines. It is reasonable to assume that the phase of the visibility function is almost constant and close to zero on these baselines (that is, $\psi_4 \approx \psi_5 \approx \psi_6 \approx 0^\circ$) irrespective of the detailed structure of this region. For each UT interval we can therefore solve the three independent closure phase relationships and determine unambiguously, within the accuracy of our assumption, the true visibility phase on the shorter baselines (that is, ψ_1, ψ_2 , and ψ_3). In this case we are using the compact region as a phase reference.

Note that the phase closure information has enabled us to

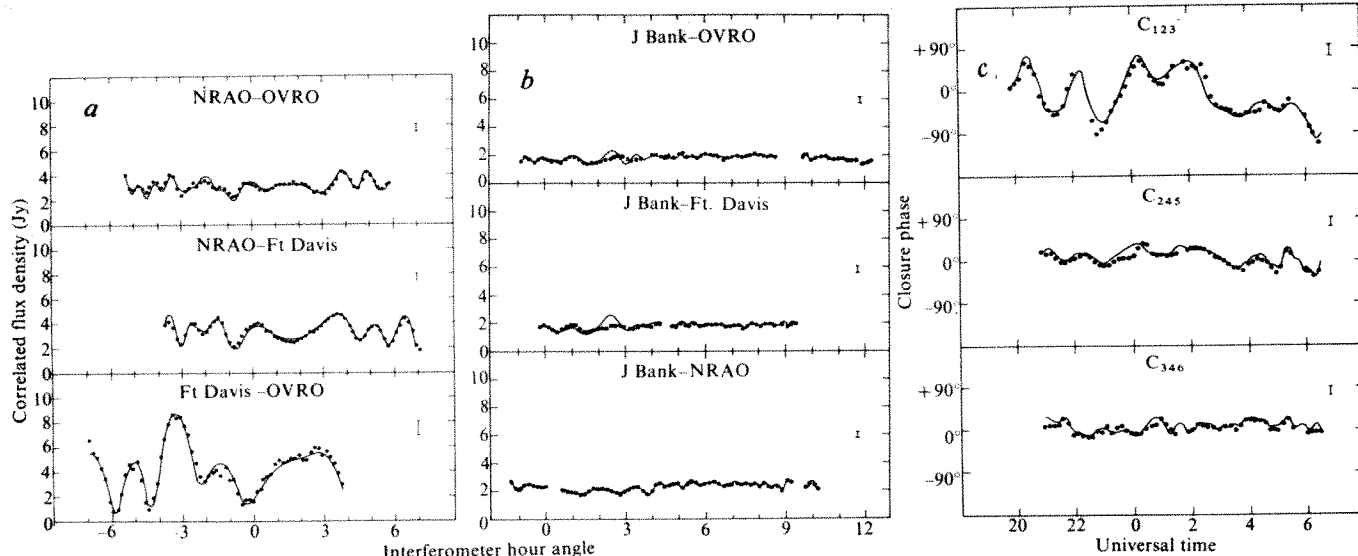


Fig. 3 *a, b*, Fringe amplitude data from March 1975. The signal-to-noise ratio on all baselines, except NRAO-Ft Davis, is only one half the expected value (see text). The solid lines are the predicted amplitudes from the brightness distribution shown in Fig. 4 *a, c*. Three independent closure phase relations from the March 1975 observations. The solid lines are predicted phases from the brightness distribution shown in Fig. 4 *a*.

resolve the usual 180° ambiguity in the position angle of the source, but it does not allow us to locate the absolute position of the radio source on the sky⁸. Apart from the absolute position of the source, we have derived all the complex visibility information accessible to these interferometers. In other words, the data can be regarded as synthesis observations of 3C147 with (u, v) coverage limited to the three US baselines.

The measured fringe amplitudes and the constructed phases were convolved on to a regular grid in (u, v) space using a Gaussian convolution function. Zero values were assigned to grid points not filled by this procedure. This array was then transformed into the sky plane resulting in the so-called 'dirty map' of the source, which is badly disturbed by sidelobes resulting from the incomplete u, v coverage. The effect of these sidelobes was reduced by means of a version of the CLEAN technique developed by D. Rogstad. The primary output of this program is an array of point sources in the sky plane which approximates the true source brightness distribution. This first 'map' can be improved slightly because of the small extent to which the extended structure does influence the observed fringe amplitude on baselines 4, 5, and 6. The corresponding phases can therefore be expected to show minor deviations from the assumed value of zero. To take account of this, the major features of the first 'map' were used to re-predict the phases on the long baselines and the analysis was repeated to produce a second 'map'. This process was continued and, while no significant changes were noted after the second or third iteration, five iterations were performed to check the stability of the derived brightness distribution.

The array of point sources produced by CLEAN has been smoothed with a Gaussian 'clean beam' whose FWHM is the same as that of the central maximum of the dirty beam. Fig. 4*a* shows the final composite clean map, the result of the fifth iteration, in which the clean beam is circular and has a FWHM of $0.009''$. In Fig. 3*a-c* the solid lines are the amplitudes and closure phases obtained by Fourier inversion of the final array of point sources only. The agreement with the data indicates that we have subtracted most of the real features of the dirty map.

We have also checked the sensitivity of this process to our original supposition of zero phases on the longer baselines. To do this, we repeated the above procedure exactly, except that initially we used phases predicted by the model of the source

shown in Fig. 2*b*. The result is shown in Fig. 4*b*; the brightness distributions in Fig. 4 are very similar and this gives us confidence that they accurately represent the compact features in the source.

The similarity between the brightness distributions shown in Figs 2*b* and 4, obtained from independent data by entirely different methods, is remarkable. The distribution of Fig. 2*b* predicts amplitudes lower and more smoothly varying than those actually observed on baselines 4, 5 and 6, indicating that the true distribution contains more compact features than those of the Gaussian model. Nevertheless, the agreement supports the conclusion of Pauliny-Toth *et al.*¹⁵ that the main features of a complex brightness distribution can usually be reconstructed reliably even without phase data. The objective nature of our method, however, the speed with which a complicated brightness distribution can be determined, and the avoidance of the 180° position angle ambiguity make this approach a significant advance on conventional model-fitting methods.

As well as the compact structure discussed above, 3C147 also has an extended component which has been observed both at low frequencies, by interplanetary scintillation observations^{6,16}, and at 2,695 MHz by single baseline, conventional interferometric observations⁴. At low frequencies ($\lesssim 100$ MHz) the extended component dominates, and $\gtrsim 80\%$ of the radiation must come from a region $\sim 0.7''$ in extent. At the higher frequency the compact features dominate, and only $\sim 25\%$ of the radiation originates in the extended region. In November 1976, 3C147 was observed twice with the Cambridge 5 km telescope at 15 GHz (M. Ryle, personal communication), and these observations show that the source is 30% resolved in p.a. $30^\circ \pm 5^\circ$ on the longest baselines, indicating a size $\sim 0.7''$. The source is unresolved in p.a. $\sim 120^\circ$. The position angle and the size are consistent with the model-fitting results of Donaldson and Smith⁴, and in Fig. 5 we show schematically a composite of our VLBI results with their preferred interpretation. The exact details of the extended structure are not known, but it is clear that the components are not colinear, and it seems that the outer lobe does not form a smooth extension of the jet. Note however, that only one contour of a Gaussian is drawn to indicate this outer lobe and it would not be surprising if future observations, with baselines of a few hundred thousand to about one million wavelengths, do

Table 2

Component	S_{peak} (Jy)	ν_{peak} (MHz)	θ_{eq} (arc s)	θ_{obs} (arc s)	Total energy (erg)	Pressure (dyn cm ⁻²)
Outer lobe	70 ± 10	120 ± 30	$0''.35$	$\sim 0''.6 \times 0''.15$	5×10^{57}	$\sim 10^{-8}$
Jet	20 ± 8	420 ± 100	$0''.05$	$\sim 0''.2 \times 0''.025$	5×10^{56}	$\gtrsim 3 \times 10^{-7}$
Core	3.2 ± 0.5	$500 \pm 200?$	$0''.012$	$0''.006$		
*Core (if on straight part of spectrum)	3.2	609	$\gtrsim 0''.0055$	$\sim 0''.006$		

* See text.

in fact show a bridge of emission between the jet and outer lobe. In this case the source would resemble 3C66B (ref. 17). The minimum resolution of our observations ($\sim 2 \text{ M}\lambda$) is too great to study the outer lobes, although they are probably the cause of the systematic deviations of the expected fringe amplitudes from the data on the shortest (OVRO-Ft Davis) baseline. The flux density of these lobes must be relatively greater at 609 MHz than at 2,695 MHz because the compact features shown in Figs 2b and 4 only account for about half the total flux density of 35 Jy from the source at 609 MHz.

The radio spectrum of 3C147

The total spectrum of 3C147 has a maximum at $140 \pm 20 \text{ MHz}$, and a marked low frequency cutoff, which is thought to be due

to synchrotron self absorption¹⁸. We have constructed three-point spectra for the individual components using the total spectrum and high resolution observations at 609 MHz (present data), 1,660 MHz (refs 5, 19), and 2,695 MHz. The 2,695 MHz points are taken from a re-analysis⁵ of the data of Donaldson and Smith⁴. In all cases, except 609 MHz, the core was taken to be unresolved ($\lesssim 0.01''$).

The spectra of the individual components cannot be determined with great accuracy; however, it is clear from the total spectrum that the spectra of both the outer lobe and the jet must have low frequency cut-offs. We have fitted to the data a series of three-component spectra, which are consistent with the total spectrum (Fig. 5). The parameters of the different components derived from these spectra are shown in the first two columns of Table 2.

The spectrum of the core is the least well determined of the three components. There is no indication of a low frequency cutoff and it is not possible to infer, even approximately, the frequency at which this occurs. The value of ν_{peak} in Table 2 of $500 \pm 200 \text{ MHz}$ has therefore been included merely as an indication of what might be reasonable (see later).

The energy balance in the different components

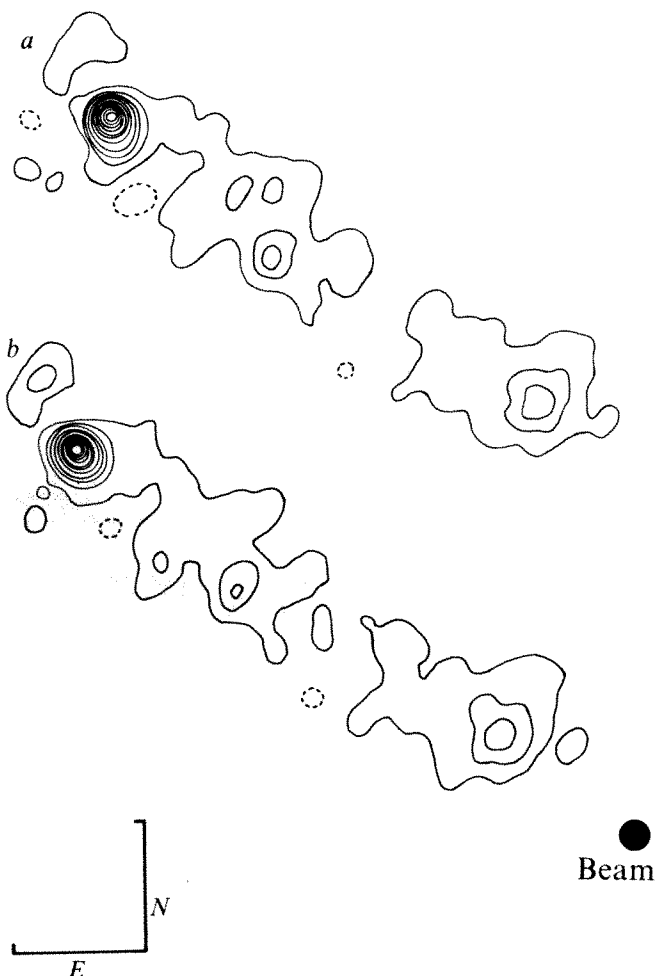
We now discuss the possible physical conditions which obtain in the different components.

The jet and outer lobe. A number of different mechanisms could be responsible for the low frequency cut-offs which we observe in the jet and outer lobes of 3C147. Of these, synchrotron self-absorption seems to be by far the most likely explanation since both free-free absorption and the Tsytovich-Razin effect would require too much thermal plasma in the components (~ 2 and $\sim 3,000 \text{ electrons cm}^{-3}$ respectively). If there were such a high density of thermal plasma present, it would cause considerably more depolarisation within the source than is observed²⁰. Scott and Readhead¹⁸ have recently shown that the turnover in the total spectrum is almost certainly due to synchrotron self-absorption (their work is based on low frequency measurements and therefore refers to the outer lobe), because the measured diameter at 81.5 MHz is within a factor 1.5 of the equipartition diameter, θ_{eq} , assuming that synchrotron self-absorption is responsible for the low frequency cut-off. Following the treatment of Scott and Readhead for a simple slab source we have calculated θ_{eq} for the three components of 3C147, and these are given in Table 2. It is clear that the observed angular sizes of both jet and outer lobe are consistent with equipartition.

The total energies and the pressures in the outer lobe and jet, calculated assuming equipartition between the particle and magnetic field energy densities are also shown in Table 2.

The compact core. The spectrum of the core is apparently flat (spectral index $\gtrsim 0.2$) down to $\sim 600 \text{ MHz}$. Since the cut-off has not been observed, we calculate a firm lower limit to θ_{eq} by assuming that the $S \propto \nu^{5/2}$ line of the self-absorbed spectrum passes through the 609 MHz point (see Table 2). The observations are only just consistent with equipartition ($\theta_{\text{eq}} \gtrsim 0.0055''$) if the core is a single homogeneous source. If the cut-off in the spectrum occurs at a frequency substantially lower than 609 MHz then there must be considerably more energy in

Fig. 4 Brightness distributions determined from the March 1975 amplitude and closure phase data using the method described in text. The 'clean beam' is a circular Gaussian with FWHM = $0''.009$. Contours are plotted at $-25, 25, 75, 125, 175, 275, 375, 475, 575, 675, 775, 875 \times 10^8 \text{ K}$. ($25 \times 10^8 \text{ K} \equiv 62 \text{ mJy}$ per unit beam area). The arms of the L each represent $0.040'' \text{ arc}$. a, Initial phases on baselines 4, 5, and 6 predicted from a point source. b, Initial phases predicted from the model shown in Fig. 2b.



the particles than in the magnetic field. A flat spectrum can also be produced by several individually self-absorbed components whose spectra peak at different frequencies. The characteristic undulations can easily be missed if the individual flux density measurements are not determined to sufficiently high accuracy. Preuss *et al.*²¹ have recently detected at least two weak (maximum correlated flux density ≈ 0.26 Jy) components in 3C147 at 5,000 MHz with a baseline of $\sim 10^8$ wavelengths. Their

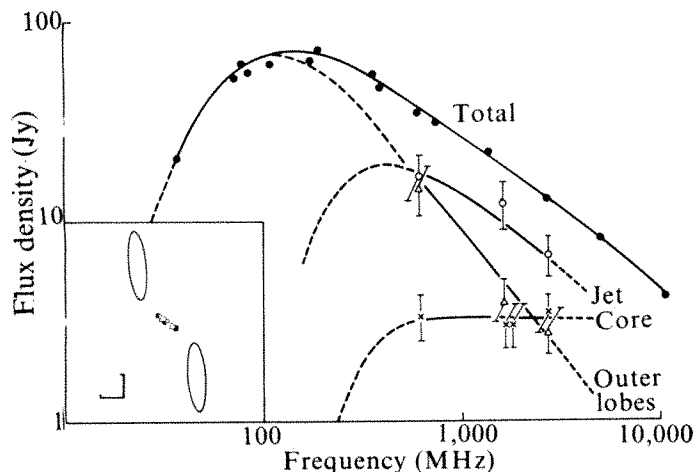


Fig. 5 Total source and individual component spectra. Errors of $\pm 20\%$ have been assigned to the component flux densities to take account of model fitting uncertainties. (See text for references.) (Inset: composite brightness distribution showing schematically the relationship between our results and the preferred interpretation of Donaldson and Smith⁴ at 2,695 MHz. The contour level in the outer components is arbitrary and the contour is drawn at the FWHM of their Gaussian model. Scales represent $0.15''$.)

much greater resolution and lack of knowledge of the location of these components makes it difficult to compare these results with ours. The characteristic size they derive, however ($0.004''$, that is, ≈ 30 pc) suggests that the results refer to the core and that our measured size may not refer to a single component.

Discussion

The observations and analysis described above constitute the most reliable determination of radio structure on the scale $0.01''$ to $0.25''$ for any source. The 'core', 'jet' and 'outer lobes' morphology is very similar to 3C66 (ref. 17), M87 (ref. 22) and 3C219 (ref. 23), and suggests that in trying to explain 3C147 one should concentrate on theories involving the generation of energy in a central highly active region, and the transfer of the energy to outlying regions via a collimated beam of waves and/or particles²⁴.

The angular extent of the jet ($\sim 0.20''$) corresponds to a projected physical length of ~ 1.5 kpc at the distance of 3C147. This is similar to the projected length of the M87 jet. Another similarity is that the 3C147 jet also contains discrete 'knots'. The two knots in 3C147 have linear diameters ~ 150 pc, somewhat larger than the radio knots in M87 (~ 50 pc) (ref. 25). Turland²³ has shown that there is a wide range in both the luminosity and size of radio jets. Our results on 3C147 are remarkable if the same physical processes are responsible for producing all jets, since the radio luminosity of the 3C147 jet is four orders of magnitude greater than that of the M87 jet, and yet the physical sizes of both jet and knots are very similar in the two cases. The difficulties associated with the confinement of the knots in M87 have been discussed by Okoye²⁶ and by Turland²³, who suggested that the optical extent of the knots is determined by the lifetime (~ 20 yr) of the electrons responsible for the optical emission assuming it to be synchrotron radiation.

The possibility, however, that these knots are confined should be seriously considered for the following reasons. Wilkinson²⁵ has shown that the sizes of the radio and optical knots in M87 are very similar, yet the radio emitting electrons have a lifetime $\gtrsim 10^3$ years, much greater than the light travel time across a knot. Similarly in 3C147, the synchrotron lifetime of the radio-emitting electrons ($\gtrsim 3 \times 10^3$ yr) is much greater than the light travel time across a knot, and therefore they must be confined. The pressure in the 3C147 knots is, however, at least an order of magnitude greater than in the optical knots of M87, and therefore it should be easier to confine the M87 knots than those in 3C147.

Our knowledge of the outer lobes is very limited but we do know that the overall position angle is different from the jet. Further VLBI observations with resolutions in the range $0.1''$ – $2.0''$ could determine the structure on this scale if the closure phase were used. The uncertainty about the exact locations of the lobes illustrates one limitation of the 'closure phase' technique—namely that we cannot use it to determine absolute positions. If we knew the position of the core it would be a simple matter to subtract the core and jet from the 15-GHz map of the source made with the 5-km telescope, and this would then show clearly the disposition of the more extended features.

We have shown that it is possible to use VLBI observations to make a reliable determination of the structure of compact features in a fairly complex source. To determine that structure on other scales we have also made three station observations at 327 MHz and 5 station observations at 1,667 MHz (in preparation). These should enable us to determine both the structure on a scale $\sim 0.002''$ to $\sim 0.5''$ and the spectra of the different components, since the inclusion of the closure phase information allows us to study the components at different frequencies without ambiguity. Thus these observations should provide us, for the first time, with a complete picture of the structure of a high redshift radio source on a scale of parsecs to kiloparsecs.

We thank Professor M. H. Cohen for useful discussions and support. We also thank Dr D. Rogstad for his help in using the CLEAN procedure, and H. Yee for computing assistance. We are grateful to the staff of each observatory for their help in making these observations, and we thank Professor Sir Martin Ryle for allowing us the use of 5-km telescope data before publication. A.C.S.R. gratefully acknowledges the support of the Royal Society Weir Research Fellowship. The Owens Valley Radio Observatory is supported by NSF grant AST 73-74660. The National Radio Astronomy Observatory is operated by Associated Universities Inc., under contract with NSF.

Received 17 June; accepted 9 August 1977.

- Anderson, B. A. & Donaldson, W. *Mon. Not. R. astr. Soc.* **17**, 81–94 (1967).
- Clark, B. G., Kellermann, K. I., Bare, C. C., Cohen, M. H. & Jauncey, D. L. *Astrophys. J.* **153**, 705–714 (1968).
- Clark, R. W., Broten, N. W., Legg, T. H., Locke, J. L. & Yen, J. L. *Mon. Not. R. astr. Soc.* **146**, 381–397 (1969).
- Donaldson, W. & Smith, H. *Mon. Not. R. astr. Soc.* **151**, 253–258 (1971).
- Fort, D. N. thesis, Univ. Manchester (1970).
- Readhead, A. C. S. & Hewish, A. *Memoirs R. astr. Soc.* **78**, 1–49 (1974).
- Clark, B. G. *Proc. I.E.E.E.* **61**, 1242–1248 (1973).
- Jennison, R. C. *Mon. Not. R. astr. Soc.* **118**, 276–284 (1958).
- Rogers, A. E. E. *et al. Astrophys. J.* **193**, 293–301 (1974).
- Readhead, A. C. S., Wilkinson, P. N. & Purcell, G. H. *Astrophys. J.* **215**, L13–L16 (1977).
- Hogbom, J. A. *Astron. Astrophys. Suppl.* **15**, 417–426 (1974).
- Schwarz, U. *Astron. Astrophys.* (in the press).
- Schwarz, U. *Astron. Astrophys.* (in the press).
- Fort, D. N. & Yee, H. K. C. *Astron. Astrophys.* **50**, 19–22 (1976).
- Pauliny-Toth, I. I. K. *et al. Nature* **259**, 17–20 (1976).
- Duffett-Smith, P. J. & Readhead, A. C. S. *Mon. Not. R. astr. Soc.* **174**, 7–17 (1976).
- Northover, K. J. E. *Mon. Not. R. astr. Soc.* **165**, 369–379 (1973).
- Scott, M. A. & Readhead, A. C. S. *Mon. Not. R. astr. Soc.* **180**, 539–550 (1977).
- Kellermann, K. I. *et al. Astrophys. J.* **169**, 1–24 (1971).
- Conway, R. G. *et al. Mon. Not. R. astr. Soc.* **168**, 137–162 (1974).
- Preuss, E., Pauliny-Toth, I. I. K., Kellermann, K. I. & Shaffer, D. B. *Astron. Astrophys.* **54**, 297–300 (1977).
- Turland, B. D. *Mon. Not. R. astr. Soc.* **170**, 281–296 (1975).
- Turland, B. D. *Mon. Not. R. astr. Soc.* **172**, 181–189 (1975).
- Blandford, R. D. & Rees, M. H. *Mon. Not. R. astr. Soc.* **169**, 395–415 (1974).
- Wilkinson, P. N. *Nature* **252**, 661–663 (1974).
- Okoye, S. E. *Mon. Not. R. astr. Soc.* **165**, 393–402 (1973).

Secular changes in marine inundation of USSR and North America through the Phanerozoic

A. Hallam

Department of Geological Sciences, University of Birmingham, P.O. Box 363, Birmingham, UK

Analysis of palaeogeographic and facies maps of the USSR and North America confirms an overall trend towards marine regression through the Phanerozoic with a major deviation in the late Mesozoic. These are related to world-wide changes of sea level attributable primarily to a combination of continental thickening controlled by subduction processes, and changes in the overall length and hence volume of the oceanic ridge system.

AN apparent secular withdrawal of the sea from the continents during the course of the Phanerozoic, superimposed on shorter-term oscillations of sea level, has been inferred from world palaeogeographic maps^{1,2} and held to support the notion of slow earth expansion of $\sim 0.5 \text{ mm yr}^{-1}$ during that time³⁻⁶. This interpretation has been challenged by Armstrong⁷ and Hallam⁸, who argue that the secular withdrawal can be adequately explained without invoking a change in the Earth's radius, and by Wise⁹, who disputes the very fact of secular change. The problem is re-examined here using the analysis of palaeogeographic and facies maps of the USSR and North America, which leads to a discussion on the relative merits of the various hypotheses that can be put forward to account for Phanerozoic sea-level changes. Attention is devoted to the longer-term secular changes rather than the shorter-term transgressions and regressions.

Soviet Union

The series of palaeogeographic and facies maps of the Soviet Union published in the 1960s¹⁰, as yet unmatched for any other extensive region, allow a detailed analysis of changes in the areal extent of sea through the whole Phanerozoic by a technique outlined elsewhere¹¹. All palaeogeographic maps involve, of course, a degree of inference, but there can be little doubt from the abundant data provided that the Russian maps give a close approximation to the area of continent flooded at several intervals for each successive geological period, although areas of present continental shelf cannot be included because of lack of data.

As shown in Fig. 1, the sea spread slowly in the early part of the Palaeozoic to cover approximately 60% of the

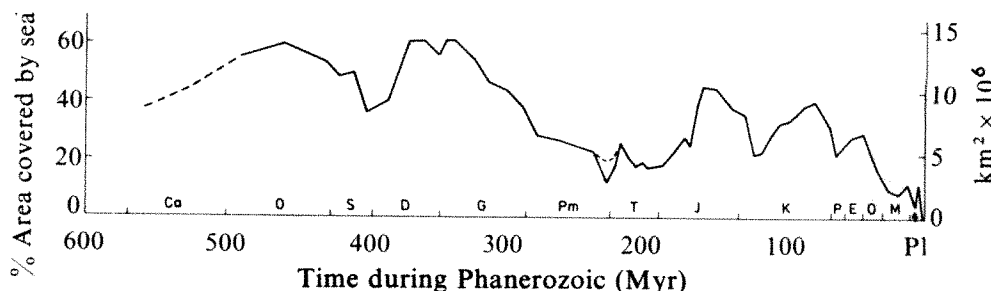
USSR by middle Ordovician times. Thereafter there was a faster withdrawal to reach a minimum value at the Silurian-Devonian boundary, followed by a relatively rapid restoration to middle Ordovician values in the late Devonian and early Carboniferous. Subsequently a progressive withdrawal culminated in a minimum at the Permian-Triassic boundary. During the Mesozoic, there were two transgressive peaks, in the late Jurassic and late Cretaceous, interrupted by an early Cretaceous minimum and followed by a sharp Cainozoic decline interrupted by a small peak in the middle to late Eocene, and a further, more dubious, peak in the Pliocene. None of the Mesozoic and Cainozoic peaks attained the values of the Palaeozoic maxima and in fact barely extended beyond the Silurian-Devonian trough.

The close spacing of data points, back at least to the Devonian, rules out a systematic temporal bias, with the length of time intervals chosen increasing the probability of overestimating the extent of sea the earlier the period⁹. Indeed, the probability of losing the stratigraphic record through subsequent deep burial, metamorphism and erosion must increase with time, and so the maps for older periods are more likely to underestimate than overestimate the former extent of marine cover. Furthermore, cratonic areas such as the Russian Platform and margins of the Siberian Shield, in fact almost everywhere outside eugeo-synclinal regions, contain much more substantial proportions of carbonates and evaporites to terrigenous clastics in the Palaeozoic than Mesozoic or Cainozoic, implying more areally restricted and topographically subdued sediment sources (mechanical denudation rates on the present continents show a tendency towards an exponential increase with increasing topographic elevation¹²). Hence the Russian data support strongly the notion of Phanerozoic secular withdrawal of sea.

North America

The only comprehensive series of palaeogeographic maps available for North America is that published in the atlas of Schuchert¹³, which was used by Wise⁹ to demonstrate a condition of essentially constant continental freeboard throughout the Phanerozoic, interrupted by short-term oscillations of sea level, 80% of which remained within about 60 m of a normal freeboard level $\sim 20 \text{ m}$ above the present level. These maps were drawn, however, several decades before the atlas was published, and therefore do not take

Fig. 1 Area of the Soviet Union covered by sea at different times during the Phanerozoic, expressed in both relative and absolute terms. Dating based on Geological Society of London Phanerozoic time scale. Ca, Cambrian; O, Ordovician; S, Silurian; D, Devonian; C, Carboniferous; Pm, Permian; T, Triassic; J, Jurassic; K, Cretaceous; P, Palaeocene; E, Eocene; O, Oligocene; M, Miocene; Pl, Plio-Pleistocene. Broken line signifies extent of non-marine Permo-Triassic.



into account extensive modern discoveries of strata, especially in the Arctic. Moreover, Schuchert left large areas of the cordilleran region blank for the Palaeozoic, but facies changes from areas further east clearly indicate a marine eugeosynclinal regime¹⁴. As with the USSR, Palaeozoic rocks of the North American central craton are much richer in carbonates than younger rocks, and testify to a correspondingly greater spread of sea, so that Schuchert's maps, based on erosional remnants, tend to be cautious and substantial underestimates for times earlier than the Mesozoic.

Data from a new atlas of facies distribution through time¹⁵ are here used to propose a revised graphical interpretation of North American data. In the absence of up-to-date palaeogeographic maps for individual stages, an attempt was made to determine the approximate maximum extent of sea for each period, using references cited in the atlas and making fairly conservative inferences. This allows a general comparison with Schuchert's data and the world data of the Termiers and Strakhov, plotted in a similar way (Fig. 2).

Schuchert considerably underestimated the former extent of seas from the Cambrian to the Lower Carboniferous. Subsequently, the Schuchert and revised North American curves match quite closely with only a relatively slight but systematic increase in inferred extent of flooding in the revised version. The revised curve matches the similarly plotted Russian curve much more closely than the Schuchert curve. The main differences are that the North American seas attained their maximum in the late Ordovician rather than the middle Ordovician and late Devonian-early Carboniferous, although the values are remarkably similar at around 60%, and the late Jurassic transgression was less extensive than the late Cretaceous. Because the two continents comprise approximately one-third of the Earth's land surface it might reasonably be inferred that the changes in question are primarily expressions of eustatic changes in sea level, with the relatively minor differences being attributable to regional differences in topography and rates of subsidence. This is confirmed by the close match of the two curves with those based on the Termier and Strakhov world palaeogeographic maps. A eustatic interpretation is both simpler and more plausible than the alternative proposed by Sloss and Speed¹⁶, that the continents have moved up and down in concert. An additional point is that the Permo-Triassic minimum must be due to a genuine eustatic fall and not to the silting up of shallow sea during a phase of stillstand. This is because the extent of end Permian and beginning Triassic non-marine sediments is negligible in both the USSR (Fig. 1) and North America.

Discussion

Several different explanations can account for the long-term secular changes under consideration. Earth expansion: besides the fact that the notion of slow earth expansion poses serious physical problems¹⁷ it has already been pointed out that the Phanerozoic overall regression can be explained more conservatively without considering a change in the earth's radius. In addition, the regression ought to have continued back into the Proterozoic, but the end of the Proterozoic was in fact a regressive phase. Thus Lower Cambrian deposits transgress widely on to much older rocks, often Archaean or early Proterozoic in age, a fact that has given rise to the term Lipalian interval. This regressive interval is probably comparable in magnitude with the end-Palaeozoic and late Cainozoic examples.

The alternative expansion model, which involves a much more rapid increase in the earth's radius since the early Mesozoic, leading to dispersal of the fragments of Pangaea^{6,18}, is even more clearly refuted by physical arguments¹⁹⁻²¹ and is incompatible with the dominantly

transgressive character of Mesozoic seas. Holmes⁴ even interpreted the Quaternary secular fall in sea level, superimposed on glacially-controlled oscillations, as a consequence of rapid earth expansion.

Glacial control: the onset of the Cainozoic ice age might be considered to have been responsible for a substantial component of the contemporary drop in sea level. It has been estimated that sea level would rise 46 m if all polar ice was melted⁷. An Antarctic ice sheet first formed in the late Miocene, although ice apparently started to accumulate as early as the late Eocene²². Abstraction of ice from the ocean system can only account for a small proportion of the lowering of sea level since the late Cretaceous high stand, however, if we accept the figure of slightly over 500 m computed from estimated changes through time of oceanic ridge volume²³, and supported independently by an estimate of the overall continental margin sedimentary overlap through the Cainozoic (P. R. Vail, personal communication).

The glacial explanation is even less successful in accounting for the major late Permian-early Triassic regression, which took place after the melting of the extensive late Carboniferous-early Permian Gondwana ice cap and the concomitant establishment of a more equable world climate.

Orogeny: plate tectonics provides a ready mechanism for the thickening and hence uplift of elongate sectors of crust at continental margins overlying subduction zones, by frictional melting of the descending slab giving rise to ascending magmas⁸, and perhaps more locally by the underthrusting of one continental edge beneath another. Thus closure of the Iapetus Ocean at the end of the Lower Palaeozoic in north-west Europe and eastern North America has converted a marginal basin-island arc-trench regime to a tectonically more stable zone of continent which has not been the site of subsequent orogeny but has persisted in most places as a positive area welded to the adjacent Precambrian shields and only locally and occasionally inundated by shallow sea.

With the detailed Russian data of Fig. 1, the clearest indication of orogeny correlating with regression concerns the substantial post-Valanginian eastward withdrawal of the sea in eastern Siberia, which is bound up with the younger Cimmerian Orogeny²⁴. The subsequent Aptian to Campanian transgression never reached as far west as the late Jurassic seas, and the Cimmerian event is the principal reason for the Russian late Cretaceous extent of sea being less than the late Jurassic, hence contradicting the world picture.

Similarly, the late Carboniferous-Permian regression is associated with the multiphase Hercynian Orogeny, commencing in the middle Carboniferous and reaching a climax in the early Permian, and converting the old Uralian and Angara geosynclines into cratonic regimes. More tentatively, the regression commencing in the late Ordovician and reaching a maximum at the end of the Silurian seems to be connected with the Russian equivalent of the Caledonian Orogeny, as manifested around the western and southern margins of the Siberian Shield. In the far east of the country, a notable end-Cretaceous orogeny is reported locally²⁴, and a number of minor Tertiary orogenic phases in the same region might help to account for part of the Cainozoic overall regression.

The North American data of Fig. 2 are, of course, less precise, but it is apparent that a component of the post-middle Ordovician Palaeozoic regression may be attributed to the progressive conversion of the Appalachian and neighbouring regions by the successive Taconic, Acadian and Appalachian orogenies from a geosynclinal-island arc zone into a positive upland area welded to the central North American craton, which lay beyond the reach of subsequent Mesozoic and Cainozoic transgressions. Like-

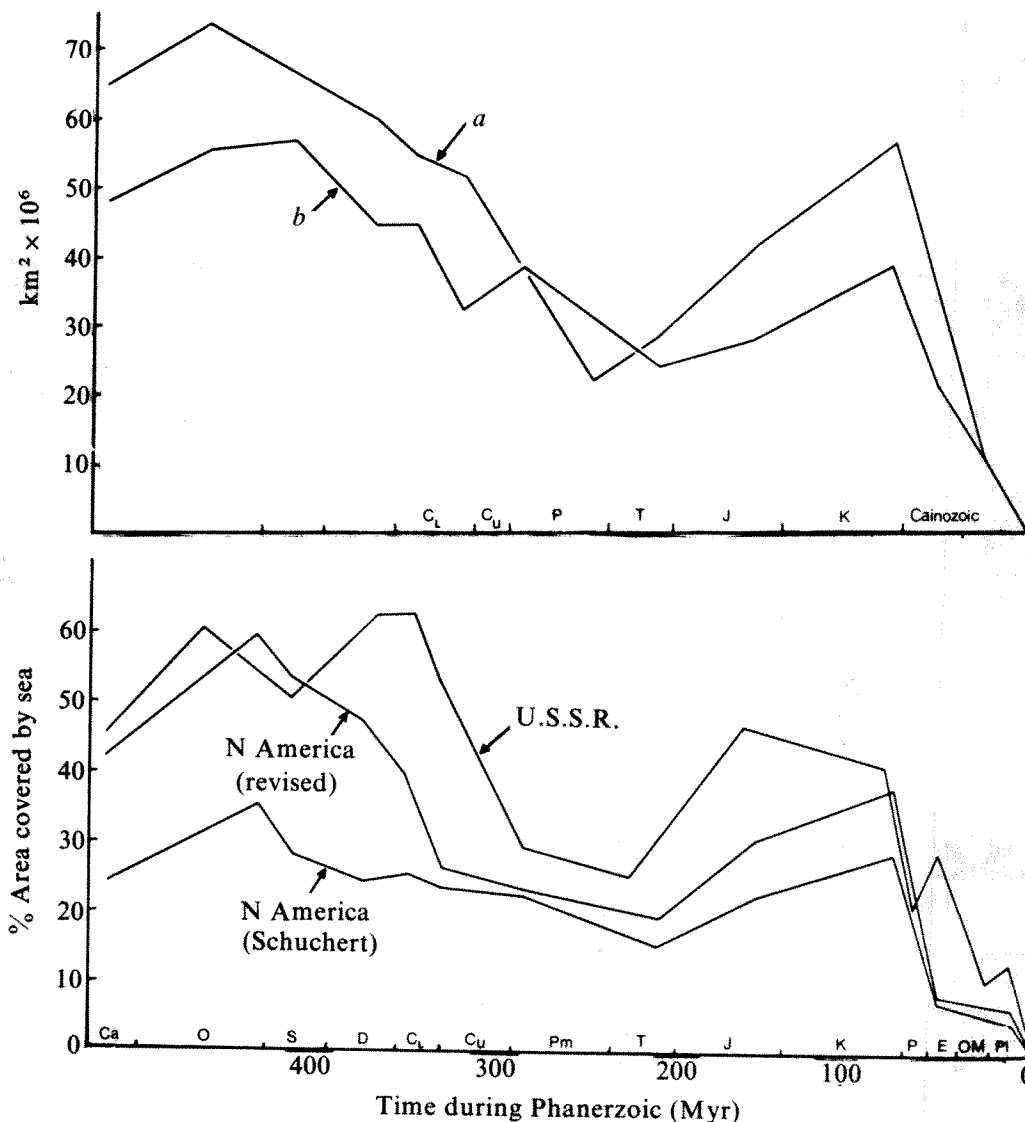


Fig. 2 Maximum degree of marine inundation for each geological period for USSR and North America (according to Schuchert and as revised in this paper, and comparison with the world data of Strakhov (a) and the Termiers (b), derived from ref. 8.

wise, the pronounced post-Cretaceous regression correlates with the Laramide Orogeny in the west of the continent.

That orogeny cannot account for all the regression is shown by examination of the history of extensive shield and platform areas of both continental masses, which were unaffected by Phanerozoic diastrophism. Thus the Russian Platform was extensively covered by shallow sea throughout the great bulk of Phanerozoic time but was completely emergent by the end of the Permian. A post-Cretaceous emergence is also apparent after the extensive late Mesozoic transgressions. Similarly, the Siberian Shield was substantially transgressed in the Cambro-Ordovician but a major withdrawal of the sea commenced in the west towards the close of the Silurian, with a land area emerging between the Ural and east Siberian seas. From middle Jurassic times onwards the sea covered the huge West Siberian depression but withdrew definitively in the Oligocene.

In North America, shallow seas covered a large part of the Canadian Shield from the Ordovician to the Devonian, but withdrew definitively in the Lower Carboniferous, as they did in the American mid-west in the Upper Carboniferous and Permian, and in the USA and Canadian Western Interior after the Cretaceous.

More generally, only a small percentage of the present land surface is occupied by 'Alpine' orogenic zones, yet it has been estimated that continental relief has increased by a factor of about 2.5 in the late Cainozoic²⁵.

Continental underplating: to account for the apparent emergence through the Phanerozoic of such cratonic areas

I formerly argued for a slight thickening by sialic underplating, using a term proposed for the African cratons by Shackleton²⁶. Several lines of evidence suggest, however, that old cratons have remained tectonically inert after the early Proterozoic and have been subjected to relatively little uplift or erosion since their time of formation, an interpretation supported by low heat flow at present²⁷. Persistence of ancient structural patterns and distinctive types of mineral assemblage is also incompatible with significant change at the base of the crust²⁸. Underplating, in fact, invokes a petrological process not required by plate tectonics and not supported by independent evidence. It is probably only relevant, if at all, to the Archaean.

Variations in rate of seafloor spreading: Hays and Pitman²³ attribute the late Cretaceous major transgression to a rapid seafloor spreading in the Pacific and Atlantic from 110 to 85 Myr, with a correspondingly greater extent of 'hot', buoyant oceanic ridge displacing seawater on to the continents. This interpretation relies on the accuracy of correlation of magnetic anomalies and has been questioned by Berggren *et al.*³⁰, who proposed an amended time scale consistent with more-or-less uniform rate of spreading. Even disregarding this criticism, the Hays and Pitman interpretation does not account satisfactorily for the marked secular regression through the Cainozoic. According to the data presented by Larsen and Pitman²⁹, the spreading rate for the Atlantic remained more or less constant from Palaeocene time onwards, and matched that for the late Jurassic and early Cretaceous. The rate was similarly con-

stant for the Phoenix-Pacific plate boundary, while the fuller data for the Farallon-Pacific plate boundary shows a fall at 85 Myr to a lower rate from the Coniacian to the Palaeocene. Thereafter there was a sharp but small reduction at the Palaeocene-Eocene boundary, a rise to a maximum in the Oligocene and subsequently a stepwise decline to the present. The Oligocene, however, is clearly regressive with respect to the Palaeocene and Eocene³¹.

Taking Indian Ocean data into account only complicates the picture further, and in a way not obviously consistent with the Hays and Pitman interpretation³². India moved away from Antarctica at a very rapid rate in the early Tertiary, and was followed by a period when little or no spreading took place west of the 90°E Ridge. Spreading from the Carlsberg Ridge has proceeded at a more or less constant rate of 1.2–1.3 cm yr⁻¹ over the past 30–35 Myr, back into the Oligocene, following a period of very slow spreading back to the late Palaeocene. In the central Indian Ocean the spreading rate has not been constant as in the Atlantic and Pacific, but changed from 5.7 cm yr⁻¹ in the Campanian to ~9 cm yr⁻¹, persisted at that rate until the middle Palaeocene and then decelerated.

Changes in length of ocean ridge system: the most plausible alternative to the spreading rate interpretation to account for the spectacular late Cretaceous transgression, which was obviously a very unusual geological event, is that a large volume of seawater was displaced by creation of new oceanic ridges in the South Atlantic and Indian oceans, since this was the time when effective dispersal of the fragments of Gondwana commenced. Such an interpretation would imply that the Cretaceous regression was due at least partly to the progressive loss of ridges elsewhere by subduction. That this is likely to have happened is evident from the analysis of Larsen and Pitman²⁹. According to their interpretation, in the middle Cretaceous there were four major plates in the Pacific region, the Kula, Farallon, Phoenix and Pacific plates. In the past 110 Myr there has been progressive consumption through subduction beneath Asia and the Americas of ridges separating the Kula from the Farallon, and the Farallon from the Phoenix plate, together with the Pacific-Kula ridge.

On the other hand, the early Tertiary saw the creation of new ridges between Greenland and Scandinavia as the North Atlantic opened, and between Antarctica and Australia. This might account for the worldwide Eocene transgression³¹ shown for the USSR in Fig. 1, which is against the secular Cretaceous trend.

Assessment of the role of ridges in pre-Cretaceous times must be highly speculative because the marine record is lost, but it seems possible that the more or less progressive Jurassic transgression relates in part to the creation of a ridge within the newly-opened central Atlantic, while the striking late Palaeozoic regression could to some extent be due to the consumption of spreading sea floor including ridges that had previously driven continents together to effect the Hercynian Orogeny.

The dominant cause of the longer-term Phanerozoic changes in sea level and overall regression is likely to have been a combination of continental thickening by orogeny consequent on subduction or collision, and variation in the cumulative length of the oceanic ridge system. The best prospect for testing these ideas quantitatively is in the Cretaceous, which has by far the fullest record of geological events per unit of time, both on the continents and ocean floor.

Received 28 June; accepted 2 September 1977.

1. Termier, H. & G. *Histoire Géologique de la Biosphère* (Masson, Paris, 1952).
2. Strakhov, N. M. *Outlines of Historical Geology* (Government Printing Office, Moscow, 1948).
3. Eged, L. *Geofis. Pura Apl.* **33**, 42 (1956).
4. Holmes, A. *Principles of Physical Geology* 2nd edn (Nelson, London, 1965).
5. Dearnley, R. *Physics and Chemistry of the Earth* (eds Ahrens, L. H. et al.) **7**, 1 (1966).
6. Carey, S. W. *Earth Sci. Rev.* **11**, 105 (1975).
7. Armstrong, R. L. *Nature* **221**, 1042 (1969).
8. Hallam, A. *Nature* **232**, 180 (1971).
9. Wise, D. U. *The Geology of Continental Margins* (eds Burk, C. A. & Drake, C. L.) **45** (Springer, New York, 1974).
10. Vinogradov, A. P. (ed.) *Atlas of the Lithological-Palaeogeographic Maps of the USSR* 4 vols (Ministry of Geol., Moscow, 1967–69).
11. Hallam, A. *Earth Sci. Rev.* **5**, 45 (1969).
12. Garrels, R. M. & Mackenzie, F. T. *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
13. Schuchert, C. *Atlas of Paleogeographic Maps of North America* (Wiley, New York, 1955).
14. Johnson, J. G. *Bull. geol. Soc. Am.* **82**, 3263 (1971).
15. Cook, T. D. & Bally, A. W. *Stratigraphic Atlas of North and Central America* (Princeton University Press, Princeton, 1977).
16. Sloss, L. L. & Speed, R. C. *S.E.P.M. Spec. Publ.* **22**, 89 (1974).
17. Birch, F. *Phys. Earth planet. Inter.* **1**, 141 (1968).
18. Owen, H. G. *Phil. Trans. R. Soc. A* **281**, 223 (1976).
19. Hospers, J. & Van Andel, S. I. *Tectonophysics* **5**, 5 (1967).
20. Le Pichon, X. *J. geophys. Res.* **73**, 3661 (1968).
21. Stewart, A. D. *J. geol. Soc.* **133**, 281 (1977).
22. Hayes, D. E. & Frakes, L. A. *Init. Rep. D.S.D.P.* **28**, 919 (1975).
23. Hays, J. D. & Pitman, W. C. *Nature* **246**, 18 (1973).
24. Nalivkin, D. V. *Geology of the U.S.S.R.* (Oliver and Boyd, Edinburgh, 1973).
25. Flint, R. F. *Glacial and Pleistocene geology* (Wiley, New York, 1957).
26. Shackleton, R. M. *Proc. geol. Ass. Lond.* **81**, 549 (1970).
27. Watson, J. V. *Phil. Trans. R. Soc. A* **280**, 629 (1976).
28. Watson, J. V. *Phil. Trans. R. Soc. A* **273**, 443 (1973).
29. Larsen, R. L. & Pitman, W. C. *Bull. geol. Soc. Am.* **83**, 3645 (1972).
30. Berggren, W. A., Mckenzie, D. P., Slater, J. G. & Van Hinte, J. E. *Bull. geol. Soc. Am.* **86**, 267 (1975).
31. Hallam, A. *Am. J. Sci.* **261**, 397 (1963).
32. McKenzie, D. P. & Slater, J. G. *Geophys. J.* **25**, 437 (1971).

Location and bond type of intermolecular contacts in the polymerisation of haemoglobin S

Ruth E. Benesch, Suzanna Kwong, Reinhold Benesch & Rohinton Edalji

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York 10032

The solubility of 14 hybrid haemoglobins composed of α chains with a single substitution and β chains from HbS was compared with that of sickle haemoglobin. A substantial reduction in the insolubility of native deoxyhaemoglobin S results from surface mutations in certain regions of the α chain while changes in other areas have no effect. Also, the chemical nature of the substitution is decisive and points to the type of intermolecular bonding at several loci.

WE described previously the preparation of five haemoglobin double mutants carrying the $\beta 6$ Glu \rightarrow Val mutation as well as an additional one on the α chains^{1,2}. They were used to delineate regions on the surface of the molecule where significant bonding between neighbouring tetramers takes place in the polymerisation of deoxyhaemoglobin S. This work has now been extended to include portions of the α chain which were previously unexplored. In addition, the availability of more than one mutation at the same locus has now made it possible to infer the type of bonding involved in some of these intermolecular contacts.

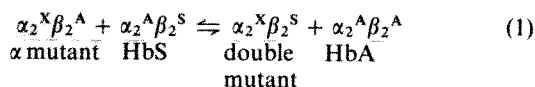
Table 1 Mutant haemoglobins used for preparation of HbS hybrids

No.	Mutant Hb	Locus on α chain	Substitution	Ref.	Method of isolation*	
					α Mutant	Hybrid
1	Sawara	6	Asp→Ala	6	6	1
2	Anantharaj	11	Lys→Glu	7	4	2
3	HbI	16	Lys→Glu	8	3	5
4	Sealy (Hasharon)	47	Asp→His	9,10	2	1
5	Mugino (Kokura)	47	Asp→Gly	11	1	1
6	Montgomery	48	Leu→Arg	12	1	1
7	Shimonoseki	54	Gln→Arg	13	2	1
8	J Mexico	54	Gln→Glu	14	4	2
9	G Phila	68	Asn→Lys	15	—	1
10	Ube II	68	Asn→Asp	16	4	2
11	Mahidol	74	Asp→His	17	6	1
12	Winnipeg	75	Asp→Tyr	18	6	1
13	Q Iran	75	Asp→His	19	6	1
14	O Indonesia	116	Glu→Lys	20	2	1

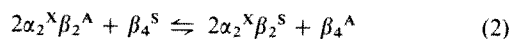
*Conditions for isolation of α mutant and hybrid haemoglobin were as follows: 1, Chromatography on DEAE-Sephadex A-50 and elution with a 24-h linear gradient of 0.05 M Tris-HCl, pH 8.4→7.4. 2, As(1), but pH 8.0→7.4. 3, As(1) but 12-h gradient pH 8.0→7.0. 4, As(1) but 16-h gradient pH 7.7→7.0. 5, As(1) but pH 7.6→6.8. 6, Polyacrylamide gel electrophoresis²¹.

Preparation and characterisation of double mutant haemoglobins

Two different hybridisation reactions were used to prepare double mutant tetramers. The first^{1,2} is based on the exchange reaction



This method is suitable for separating the double mutant except where the mutation on the α chain brings about a change in charge which is equal and opposite to that of the β^S mutation, as for example in HbJ Mexico ($\alpha^{54\text{Gln} \rightarrow \text{Glu}}$). In such a case, the hybrid, $\alpha_2^X\beta_2^S$, will, of course, have the same charge as HbA and can therefore not be separated from it. This difficulty can be overcome by hybridisation of such α mutants with β_4^S since HbA is not a product of this reaction



Method (2) was therefore used in all cases where the effective charge of the double mutant was too near to that of HbA to permit a good separation. This was often encountered in haemoglobins where the formal difference in charge brought about by the mutation was modified by interactions with other residues.

HbS was isolated from the blood of heterozygous donors by chromatography on DEAE-Sephadex³ using a 24-h linear gradient of 0.05 M Tris buffer pH 8.0–7.4.

Hb β_4^S was prepared by treatment of the unfractionated A/S haemolysate with *p*-mercuribenzoate followed by chromatography on CM-cellulose as described by Bucci and Fronticelli⁴. The β^{SHg} fraction was collected, concentrated by ultrafiltration using PM 30 Amicon membranes and the mercury was removed with *N*-acetylpenicillamine as described previously⁵.

The α mutant haemoglobins including the five which were hybridised with HbS before^{1,2}, are listed in Table 1. Only mutants were chosen with substitutions at or near the surface of the molecule. The exact position of each residue was located on a three-dimensional model assembled from Labquip components using the refined coordinates of human deoxyhaemoglobin²². The haemoglobin variants were isolated from the blood of heterozygous donors. The samples were transported in temperature controlled containers (Trans-Temp 313, Fisher Scientific). The purity of the mutant haemoglobins was checked by polyacrylamide gel electrophoresis.

Hybrid tetramers with two β^S chains and α chains from mutants 3, 4, 5, 6, 7, 9 and 14 (Table 1) were prepared by method (1) as described previously^{1,2}. The remainder were made by method (2) using β_4^S as donor of β^S chains. Equal amounts of 1% solutions of α mutant haemoglobin and β_4^S were mixed and incubated in 0.2 M

acetate buffer pH 4.7 for 3–4 h at 0 °C. The $\alpha_2^{\text{Shimo}}\beta_2^S$ hybrid was made by both methods and HbS was also prepared from β_4^S and HbA. The conditions for the isolation of the double mutant haemoglobins are given in Table 1. The identity and purity of the hybrids was established by polyacrylamide gel electrophoresis of the intact haemoglobins and, in some cases, of the mercurocurated subunits.

The oxygen equilibrium curves of all haemoglobins including the hybrids were measured as described before^{23,24} using 10 μ M haemoglobin in 0.05 M bis-Tris buffer pH 7.4 at 20 °C.

The structural and functional integrity of the hybrid tetramers was tested using the criteria applied previously^{1,2}. The oxygen affinities of all the double hybrids were the same as those of the α mutant from which they were derived and, with two exceptions, were within the normal range ($\log p_{50} = 0.43$ – 0.56 at 20 °C and pH 7.4). Hb Mugino and $\alpha_2^{\text{Mugino}}\beta_2^S$ had a somewhat raised oxygen affinity ($\log p_{50} = 0.36$) and Hb Sawara was found to be a really high affinity mutant ($\log p_{50} = 0.02$), contrary to a previous report¹¹. In this case too, the Sawara-S hybrid retained the high affinity.

The oxygenation properties of HbS prepared from HbA and β_4^S by method (2) were identical with those of native HbS ($\log p_{50} = 0.47$).

Solubility in strong phosphate as measure of HbS polymerisation

The solubility in concentrated phosphate buffer, as originally described by Itano²⁵, was chosen for comparing the polymerising tendency of the double mutants with that of HbS, since only small quantities of the double mutants were available (15–30 mg). Several lines of evidence support the assumption that this method measures the same phenomenon as the usual gelling tests on much larger samples: (1) While the precipitation of oxy and deoxyhaemoglobin A as well as that of oxyhaemoglobin S is essentially instantaneous when they are mixed with the phosphate buffer, deoxyhaemoglobin S precipitates slowly, which presumably reflects the time required for the formation of ordered polymers. The suspensions were therefore incubated with gentle shaking for at least 30 min at 25 °C and the filtrates were observed for further precipitation. Incomplete precipitation introduces increasingly large errors as the fraction of haemoglobin in solution decreases with increasing ionic strength. (2) Cottam and Waterman²⁶ concluded from the parallel temperature dependence of the solubility and the gelation that the former constitutes 'a sensitive method of monitoring the aggregation phenomenon of deoxyhaemoglobin S'. (3) Both with some chemically modified HbS molecules²⁷ and in the case of two naturally occurring double mutants, the results obtained by the two methods were similar. Thus HbC Harlem was 2.5 times more soluble than HbS in strong phosphate²⁸ and its minimum gelling concentration was 36 g % instead of 24.5 g % for HbS (ref. 28). Hassan *et al.*²⁹ have shown

Table 2 Solubility of deoxygenated double mutants

Haemoglobin	α -chain locus	Solubility (mol l ⁻¹ × 10 ⁵)
$\alpha_2^A\beta_2^S$	—	0.3
$\alpha_2^{\text{Winnipeg}}\beta_2^S$	75	0.2
$\alpha_2^{\text{Sawara}}\beta_2^S$	6	0.2
$\alpha_2^{\text{Q Iran}}\beta_2^S$	75	0.3
$\alpha_2^{\text{Mahidol}}\beta_2^S$	74	0.3
$\alpha_2^{\text{Ube II}}\beta_2^S$	16	0.9
$\alpha_2^{\text{Mugino}}\beta_2^S$	68	0.9
$\alpha_2^{\text{G Phila}}\beta_2^S$	47	0.9
$\alpha_2^{\text{Anantharaj}}\beta_2^S$	68	1.1
$\alpha_2^{\text{Montgomery}}\beta_2^S$	11	1.5
$\alpha_2^{\text{J Mexico}}\beta_2^S$	48	1.5
$\alpha_2^{\text{Shimo}}\beta_2^S$	54	1.7
$\alpha_2^{\text{O Indonesia}}\beta_2^S$	54	1.8
$\alpha_2^{\text{Sealy}}\beta_2^S$	116	2.0
	47	4.6

The measurements were carried out as described previously³⁰ in 2.04 M phosphate buffer pH 6.8 ($\mu = 4.4$) at 25 °C. The values were obtained by interpolation or extrapolation of solubility plots such as those shown in Fig. 1.

that the double mutant, Hb Stanleyville II/S ($\alpha_2^{78 \text{ Lys}}\beta_2^{6 \text{ Val}}$) is twice as soluble in strong phosphate as native HbS and this is paralleled by an increase in the minimum gelling concentration from 24 to 30 g %.

Effect of α -chain mutations on the solubility of HbS

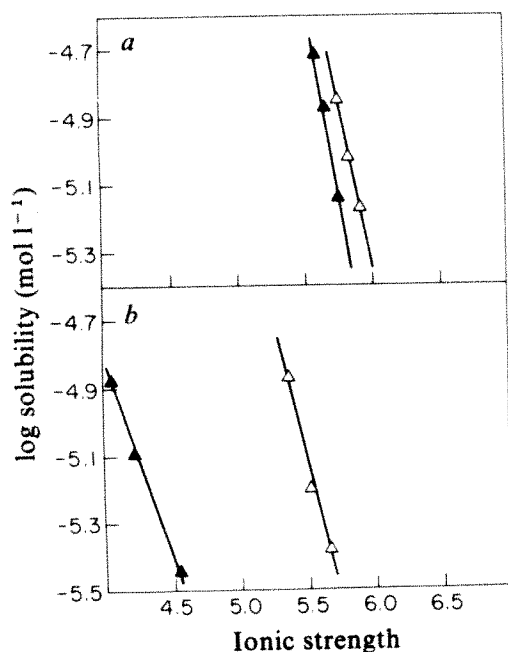
The solubility of the deoxy form of all the double mutants used in 4.4 ionic strength phosphate buffer is given in Table 2. Clearly, the effect of the mutations varies over a wide range from no effect at all at positions 6, 74 and 75 to the 20-fold increase in solubility caused by the replacement of aspartic acid by histidine at position 47.

Another criterion was also used to compare the various double mutants with HbS—the change in ionic strength ($\Delta\mu$) necessary to make the solubility of the oxy and deoxy form equal in each case (Fig. 1). This value was 1.35 for HbS and 0.22 for HbA.

The effect of a mutation in the α chain on the solubility of the tetramer it forms with β^S chains depends not only on the location of the substitution but also on its chemical nature.

The most instructive from this point of view were the three pairs of mutants at positions $\alpha 47$, $\alpha 54$ and $\alpha 68$. The results obtained on the hybrids of these mutants with HbS are shown in Table 3. The

Fig. 1 Solubility of haemoglobins A (a) and S (b) in phosphate buffer pH 6.8 at 25 °C. Open symbols, oxyhaemoglobin; filled symbols, deoxyhaemoglobin.

**Table 3** Effect of α -chain mutations on solubility of HbS

Haemoglobin	Position on α chain	Residue	$\Delta\mu^*$
$\alpha_2^A\beta_2^S$	47 (CD6)	Asp	1.35
$\alpha_2^{\text{Mugino}}\beta_2^S$		Gly	1.07
$\alpha_2^{\text{Sealy}}\beta_2^S$		His	0.58
$\alpha_2^A\beta_2^S$	54 (E3)	Gln	1.35
$\alpha_2^{\text{Shimo}}\beta_2^S$		Arg	0.78
$\alpha_2^{\text{J Mexico}}\beta_2^S$		Glu	0.78
$\alpha_2^A\beta_2^S$	68 (E17)	Asn	1.35
$\alpha_2^{\text{G Phila}}\beta_2^S$		Lys	1.08
$\alpha_2^{\text{Ube II}}\beta_2^S$		Asp	1.10

*Change in ionic strength necessary to make the deoxy and oxy forms equally soluble.

greatest solubilising effect on deoxyhaemoglobin S encountered among 14 different substitutions on the α chain is that due to replacement of aspartic acid by histidine at $\alpha 47$ (Hb Sealy). But, when the substitution at this locus is glycine, as in Hb Mugino, the solubilising influence is greatly reduced (Table 3). We therefore conclude that the aspartate side chain at $\alpha 47$ is engaged in an intermolecular salt bridge which materially contributes to the stability of the helical polymer. Wishner *et al.*³¹ and Edelstein *et al.*³² also concluded that $\alpha 47$ is in an important contact region. If the spacial relations in the fibre can be assumed to be similar to the arrangement of the double strands seen in crystals of deoxyhaemoglobin S the most likely partner for aspartate $\alpha 47$ is lysine $\beta 59$ on the adjacent molecule³¹.

Table 3 shows that the glutamine residue at $\alpha 54$ also provides an important intermolecular contact point. Here, however, the same increase in solubility is observed whether the glutamine is replaced by a positive residue (Hb Shimonoseki) or a negative residue (HbJ Mexico). Although the solubility data show that $\alpha 68$ is a weaker contact point altogether, the nature of the bond must be of the same kind as that at $\alpha 54$, since here again, both a negative and a positive substitution have the same effect. This evidence suggests apolar bonds at these two locations. Hydrophobic bonding is generally assumed to be important in the polymerisation of HbS as, for example, indicated by the temperature dependence of the gel formation³³. In this connection it is pertinent to recall a parallel situation involving the β chains where no gelation occurs when the valine at $\beta 6$ is replaced either by glutamic acid (HbA) or by lysine (HbC).

A second region of the tetramer surface formed by the α chains qualifies as a weaker contact area. It contains residues $\alpha 11$, $\alpha 16$ and $\alpha 116$, brought into proximity by the tertiary structure of the protein. The mutations at these loci (Hb Anantharaj, HbI and HbO Indonesia) all have comparable effects on the solubility of HbS (Table 4).

A particularly significant group of mutants were Hb Sawara ($\alpha 6$), Hb Mahidol ($\alpha 74$) and haemoglobins Winnipeg and Q Iran ($\alpha 75$). In all these cases the hybrid composed of the abnormal α chains and β^S chains had the same solubility as HbS itself (Table 5). The three-dimensional model of the tetramer shows that these three loci ($\alpha 6$, $\alpha 74$ and $\alpha 75$) are situated quite close together with $\alpha 6$ being somewhat recessed from the surface. This part of the surface can therefore be designated as a non-contact region. It should be noted that two of these mutants—haemoglobins Mahidol and Q Iran—carry the same substitution (Asp→His) as does Hb Sealy

Table 4 Effect of α -chain mutations on solubility of HbS

Haemoglobin	Position on α chain	Substitution	$\Delta\mu^*$
S	—	—	1.35
$\alpha_2^{\text{Anantharaj}}\beta_2^S$	11 (A9)	Lys→Glu	0.86
$\alpha_2^{\text{I}}\beta_2^S$	16 (A14)	Lys→Glu	1.15
$\alpha_2^{\text{O Indonesia}}\beta_2^S$	116 (GH4)	Glu→Lys	0.84

*Change in ionic strength necessary to make the deoxy and oxy forms equally soluble.

Table 5 Effect of α -chain mutations on solubility of HbS

Haemoglobin	Position on α chain	Substitution	$\Delta\mu^*$
S	—	—	1.35
α_2 Sawara β_2^S	6 (A4)	Asp→Ala	1.37
α_2 Mahidol β_2^S	74 (EF3)	Asp→His	1.35
α_2 Winnipeg β_2^S	75 (EF4)	Asp→Tyr	1.33
α_2 Q Iran β_2^S	75 (EF4)	Asp→His	1.33

*Change in ionic strength necessary to make the deoxy and oxy forms equally soluble.

($\alpha 47$). As the same change has a maximal effect at $\alpha 47$, but none at $\alpha 74$ and $\alpha 75$, the location on the surface of the molecule is clearly the decisive factor.

The Hb Sawara hybrid also shows that a high oxygen affinity is not incompatible with efficient polymerisation of deoxyhaemoglobin S. The same situation was encountered previously with HbS from which the α C-terminal residue had been removed—desArg HbS (ref. 1).

This method of approach has thus made it possible to define several distinct areas on the surface of the haemoglobin molecule formed by the α chains. One of these is a region of strong intermolecular bonding which includes aspartate $\alpha 47$, leucine $\alpha 48$ and glutamine $\alpha 54$. Of these, $\alpha 47$ aspartate probably forms a salt link with lysine $\beta 59$ on the next molecule while glutamine $\alpha 54$ participates in hydrophobic bonding.

A region where replacement of the normal residues has a smaller effect in solubilising HbS is enclosed by the charged residues at $\alpha 11$, $\alpha 16$ and $\alpha 116$. All three are therefore candidates for electrostatic bonding. Another contact point of comparable significance is the asparagine at $\alpha 68$, engaged in hydrophobic bonding. A weak effect brought about by a single substitution could be the result of multiple contacts in that region so that replacement of only one of them would weaken the contact by only a fractional amount.

Finally, the mutations at $\alpha 6$, $\alpha 74$ and $\alpha 75$ provide an excellent control, since changes at these positions—and in the case of $\alpha 75$ even two different substitutions—have no effect whatever on the solubility of native HbS.

In Fig. 2, the 10 different loci with one or more substitutions, which were included in this study are marked on a schematic representation of the haemoglobin tetramer. But, the distance between the different positions can only be judged from the three-dimensional model and not from this necessarily two-dimensional substitute.

We thank Drs S. Pootrakul, S. Rahbar, D. Labie, J. Rosa, J. Alperin, M. Grey, R. Rieder, T. Hosty, P. Galbraith, T. Imamura and T. Miyaji for blood samples. This work was supported by US PHS grants HL-17552 and HL-05791 from the National Heart and

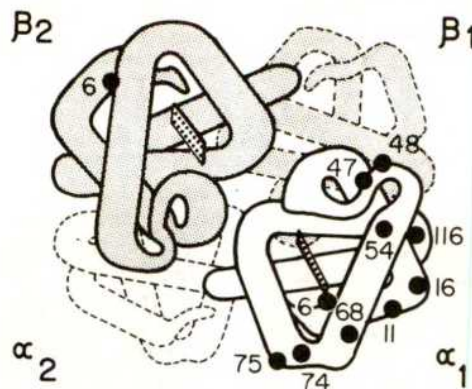


Fig. 2 Schematic model of the haemoglobin molecule showing the locations of the amino acid substitutions.

Lung Institute and grant PCM76 20361 from the NSF. R.B. is a recipient of a US PH research career award from the National Heart and Lung Institute.

Received 24 June; accepted 22 August 1977.

1. Benesch, R. E., Yung, S., Benesch, R., Mack, J. & Schneider, R. G. *Nature* **260**, 219–221 (1976).
2. Benesch, R. E., Yung, S., Benesch, R., Mack, J. & Schneider, R. G. *Proc. Symp. Molecular and Cellular Aspects of Sickle Cell Disease* Dallas, Texas, DHEW Publ. No. 76-1007, 113–131 (1975).
3. Huisman, T. H. J. & Dozy, A. M. *J. Chromat.* **19**, 160–169 (1965).
4. Bucci, E. & Fronticelli, C. *J. Biol. Chem.* **240**, PC551 (1965).
5. Benesch, R., Benesch, R. E. & Enoki, Y. *Proc. natn. Acad. Sci. U.S.A.* **61**, 1102–1106 (1968).
6. Sumida, I., Ohta, Y., Imamura, T. & Yanase, T. *Biochim. biophys. Acta* **322**, 23–26 (1973).
7. Pootrakul, S., Kematorn, B., Na-Nakorn, S. & Suanpan, S. *Biochim. biophys. Acta* **405**, 161–166 (1975).
8. Schneider, R. G., Alperin, J. B., Beale, D. & Lehmann, H. *J. lab. clin. Med.* **68**, 940–946 (1966).
9. Halbrecht, I., Isaacs, W. A., Lehmann, H. & Ben-Porat, F. *Israel J. med. Sci.* **3**, 827–831 (1967).
10. Schneider, R. G., Ueda, S., Alperin, J. B., Brimhall, B. & Jones, R. T. *Am. J. hum. Genet.* **20**, 151–156 (1968).
11. Sumida, I. *Jap. J. hum. Genet.* **19**, 343–363 (1975).
12. Brimhall, B. et al. *Biochim. biophys. Acta* **379**, 28–32 (1975).
13. Miyaji, T., Iuchi, I., Takeda, I. & Shibata, S. *Acta Jap. Jap.* **26**, 531–537 (1963).
14. Jones, R. T., Koler, R. D. & Lisker, R. *Clin. Res.* **11**, 105 (1963).
15. Baglioni, C. & Ingram, V. M. *Biochim. biophys. Acta* **48**, 253–265 (1961).
16. Miyaji, T., Iuchi, I., Yamamoto, K., Ohba, Y. & Shibata, S. *Clin. chim. Acta* **16**, 347–352 (1967).
17. Pootrakul, S. & Dixon, G. H. *Can. J. Biochem.* **48**, 1066–1078 (1970).
18. Vella, F., Wiltshire, B. G., Lehmann, H. & Galbraith, P. *Clin. Biochem.* **6**, 66–70 (1973).
19. Lorkin, P. A. et al. *Br. J. Haemat.* **19**, 117–125 (1970).
20. Baglioni, C. & Lehmann, H. *Nature* **196**, 229–232 (1962).
21. Suzuki, T., Benesch, R. E., Yung, S. & Benesch, R. *Analyt. Biochem.* **55**, 249–254 (1973).
22. Fermi, J. *molec. Biol.* **97**, 237–256 (1975).
23. Benesch, R., Macdull, G. & Benesch, R. E. *Analyt. Biochem.* **11**, 81–87 (1965).
24. Benesch, R. E., Benesch, R. & Yung, S. *Analyt. Biochem.* **55**, 245–248 (1973).
25. Itano, H. A. *Archs Biochem. Biophys.* **47**, 148–159 (1953).
26. Cottam, G. L. & Waterman, M. R. *Biochem. biophys. Res. Commun.* **54**, 1157–1163 (1973).
27. Benesch, R., Benesch, R. E. & Yung, S. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1504–1505 (1974).
28. Bookchin, R. M., Nagel, R. L. & Ranney, H. M. *J. Biol. Chem.* **242**, 248–255 (1967).
29. Hassan, W., Beuzart, Y., North, M. L. & Rosa, J. *Biochem. biophys. Res. Commun.* (in the press).
30. Benesch, R., Benesch, R. E. & Yung, S. *Biochem. biophys. Res. Commun.* **55**, 261–265 (1973).
31. Wishner, B. C., Hanson, J. C., Ringle, W. M. & Love, W. E. *Proc. Symp. Molecular and Cellular Aspects of Sickle Cell Disease*, Dallas, Texas, DHEW Publ. No. 76-1007 1–29 (1975).
32. Edelstein, S. J. et al. *Proc. Symp. Molecular and Cellular Aspects of Sickle Cell Disease*, Dallas, Texas, DHEW Publ. No. 76-1007, 33–59 (1975).
33. Allison, A. C. *Biochem. J.* **65**, 212–219 (1957).

Synchronised transmembrane insertion and glycosylation of a nascent membrane protein

James E. Rothman & Harvey F. Lodish

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Studies of the synthesis and incorporation of the vesicular stomatitis virus glycoprotein into membranes in a synchronised cell-free system demonstrate a tight coupling between polypeptide synthesis and membrane insertion, as a result of which the nascent chain crosses the membrane. The studies reveal a surprisingly precise sequence by which the nascent chain of this membrane glycoprotein is glycosylated in two steps. These findings have important implications for the mechanisms of membrane assembly.

MEMBRANES are continuously formed and remodelled in all living cells, and the problem of how membranes are assembled is central to cell biology. But, the mechanisms by which proteins are incorporated into membranes and the means by which specific proteins are directed only to the appropriate membrane or organelle are obscure. We describe here a novel approach to these problems which has begun to elucidate the nature and temporal sequence of the events responsible for the synthesis and membrane insertion of ectoproteins¹, those integral membrane proteins which have a hydrophilic domain on the extra-cytoplasmic side of the membrane. The external portion(s) of

ectoproteins must somehow cross the permeability barrier of the membrane at some stage. Several considerations¹ suggest that the external portions of the polypeptide chain of an ectoprotein cross the membrane during protein synthesis, but direct evidence has been lacking. The alternative school of thought² holds that ectoproteins can be inserted into membranes after they are completely synthesised.

The glycoprotein (G) of vesicular stomatitis virus (VSV) is such an ectoprotein. Most of the protein of G and all of its carbohydrate are external to the lipid bilayer of the viral envelope, forming the 'spikes' which project radially from the surface of the viral particle³. The G protein is synthesised by ribosomes bound to the endoplasmic reticulum^{4,5} of infected cells and receives at least the core *N*-acetylglucosamine and mannose sugars in this organelle⁶⁻⁸. G is a transmembrane protein of the endoplasmic reticulum membrane, oriented with its sugar-containing portion within the lumen of the organelle, but with about 30 amino acids at its carboxy terminus exposed on the opposite, cytoplasmic side of the membrane^{8,9}. G is ultimately transported from the reticulum to the plasma membrane, during which time the glycosylation is completed by the addition of terminal sialic acid residues^{6,7}, and is subsequently incorporated into the viral envelope following the budding out of the viral nucleocapsid through the cell surface membrane³.

A cell-free system capable of supporting membrane assembly has been described⁹ in which the VSV G protein was translated from viral-specific mRNA in crude extracts of wheat germ. When G was synthesised in the presence of membrane vesicles derived from the rough endoplasmic reticulum of pancreas (but freed of endogenous ribosomes), this membrane protein was glycosylated and was asymmetrically inserted into the vesicle membrane, spanning the lipid bilayer, with the same orientation as the native G protein found in the rough endoplasmic reticulum *in vivo*.

We report here a simple method for synchronising the growth and processing of the G protein in this cell-free system. This technique has revealed a remarkably close degree of coupling between polypeptide synthesis and incorporation into the membrane, and has made it possible to demonstrate a precise sequence by which the nascent chain of the membrane protein is glycosylated.

Synchronisation of protein synthesis

Protein synthesis is started when total VSV mRNA is added to a cell-free extract of wheat germ. After 2 min of protein synthesis, a specific inhibitor of initiation¹⁰, 7-methylguanosine-5'-phosphate (⁷mGp) is added to prevent subsequent initiations. If this interval is sufficiently short in comparison with the time required to complete the G protein, and if all of the nascent glycoprotein chains grow at the same rate, then in principle, all copies of the G protein will be polymerised in synchrony as a cohort population.

Figure 1 shows that an approximation to synchrony can be obtained by this procedure. The synthesis of total VSV protein proceeds linearly for a limited period (Fig. 1a), beyond which no further protein is made, as would be expected if initiation were completely blocked by ⁷mGp. In the absence of ⁷mGp, synthesis is linear for about 60 min (not shown). No completed G protein can be observed until about 45 min, when all chains of G are observed to be completed within a rather brief time span of 10 min (Fig. 1b). Total protein synthesis is apparently complete before the appearance of G protein because G is a minor fraction of the total translation products and greatly exceeds most of the products in chain length.

Coupling of insertion and glycosylation to protein synthesis

To determine the degree to which the processes of insertion and glycosylation of G are coupled to protein synthesis, synthesis of G chains was initiated synchronously in the absence of pancreatic membrane vesicles, and at various times samples were removed and the chains were completed in the presence of vesicles. This

experiment determines the maximum size of nascent glycoprotein which can be made without any association with membranes without affecting proper insertion and glycosylation.

As shown previously⁹, the partially glycosylated form of the glycoprotein (G₁) migrates more slowly (see Fig. 2) through sodium dodecyl sulphate-polyacrylamide gels than does the unglycosylated form (G₀) which results from synthesis in the absence of pancreatic membrane vesicles. The extent of glycosylation was therefore determined as the fraction of G

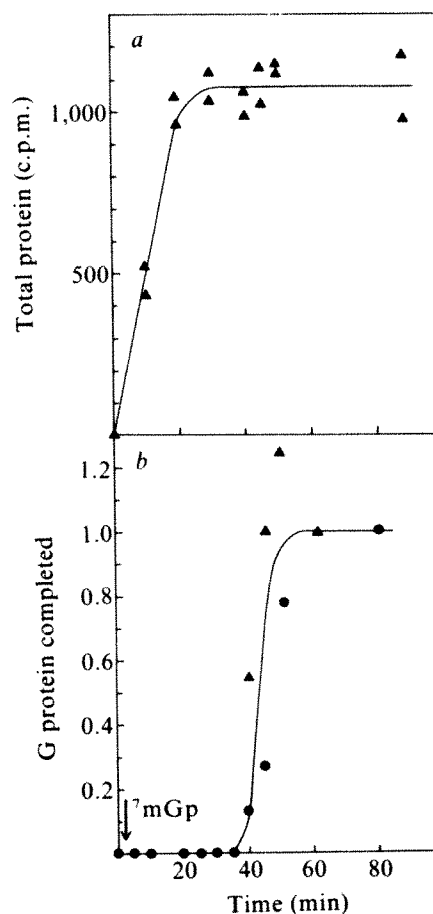


Fig. 1 Synchronisation of protein synthesis. Wheat germ extract and VSV were prepared and proteins were synthesised at 23 °C, essentially as before⁹, in a volume (including all additions) of 0.5 ml containing 0.15 ml wheat germ extract, 20 μ l VSV mRNA, 27 mM HEPES (pH 7.2), 120 mM KCl, 1.5 mM magnesium acetate, 5.6 mM dithiothreitol, 2.6 mM ATP, 0.26 mM GTP, 16 mM creatine phosphate, 0.73 mM spermidine, 42 μ g ml⁻¹ creatine phosphokinase, 0.13 mM each of the 19 amino acids, except methionine, and ³⁵S-methionine (0.5 mCi ml⁻¹) with a specific radioactivity of approximately 600 Ci mmol⁻¹. The mixture was pre-incubated for 1.5 min at 23 °C before adding mRNA at time zero. After 2 min, 25 μ l of a 20 mM solution of ⁷mGp (P. L. Biochemicals) were added, giving a final concentration of 1 mM. *a*, At various times, duplicate samples of 2 μ l were taken, and the total protein synthesised was determined as radioactivity insoluble in boiling 5% trichloroacetic acid (TCA). The amount of acid-insoluble radioactivity at zero time (550 c.p.m.) was subtracted to yield the data in the figure. *b*, At various times, samples of 25 μ l were precipitated with TCA and separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate⁹. The amount of G protein present was determined from scans of autoradiographs of the dried gels. The triangles and circles represent data from two experiments, run in identical conditions. The amounts of G protein shown are values obtained by normalisation to the amount of G protein made at the longest time point shown for each experiment, in order to permit the two experiments to be compared directly. Note that the concentration of ⁷mGp required to achieve satisfactory inhibition of initiation (> 99%) is very sensitive to the KCl concentration and that the time for chain completion can vary markedly ($\pm 50\%$) between independently prepared extracts of wheat germ.

protein in the G_1 form. Trypsinisation of vesicles after protein synthesis removes peptides from the extreme C-terminal region of G_1 , yielding a large fragment which migrates slightly faster than G_1 (but more slowly than G_0) on acrylamide gels (Fig. 2) and is protected from further tryptic degradation by the permeability barrier of the membrane vesicle^{8,9} wall through which G_1 is inserted. G_0 yields no analogous protected portion⁹. The extent to which the G protein was properly inserted into the membrane was, therefore, assayed as the fraction of radioactivity in the total G product ($G_0 + G_1$) preserved in the membrane-protected tryptic fragment which runs between G_0 and G_1 on gels. Even if all copies of G were inserted correctly, some loss of radioactivity would be expected since three C-terminal peptides containing ³⁵S-methionine radioactivity are removed by trypsin in the process of conversion of G_1 to the protected fragment⁹.

Figure 2 shows that when chains are completed in the presence of membranes after 6 min of synchronous synthesis in the absence of membranes, the resulting G protein is all in the G_1 , or glycosylated form, and yields a membrane-protected fragment on trypsinisation. A dramatic change takes place thereafter. Few of the G chains made for 9 min in the absence of membranes, and then completed in the presence of membranes, are glycosylated, and little if any membrane-protected fragment is observed (Figs 2, 3). Both glycosylation and proper insertion are lost coordinately and remarkably rapidly after 6 min. Indeed, both processes are lost within a span of 3 min, when the window in which initiation was permitted was 2 min. Consequently, prolonging synthesis of a G chain (which was initiated at 0 min) in the absence of membranes beyond the critical time of about 6 min by as little as 1 min will result in a marked reduction of proper insertion and of glycosylation. A fourfold increase in the concentration of membranes does not cause a significant increase in the critical time of about 6 min (not shown), indicating that this time is not related to the rate at which ribosomes can

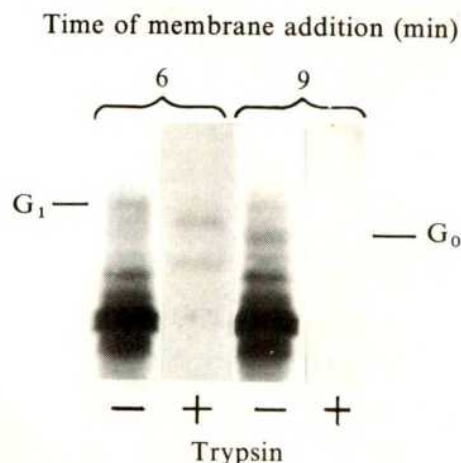


Fig. 2 Membrane addition experiment. At the indicated times, 50 μ l samples of the same protein synthesis incubation as Fig. 1 (Δ) were removed, 0.5 μ l of pancreatic microsomal membranes (prepared as before⁹; stock suspension had an A_{280} of 50, as measured in 1% sodium dodecyl sulphate) were added, and incubation was continued at 23 °C until 53 min had elapsed from the time mRNA was added. One half of each sample was precipitated with TCA⁹, while the other half was treated with trypsin-TPCK (0.5 mg ml⁻¹) at 23 °C for 15 min before TCA precipitation. Shown here is the autoradiogram of the gel of the translation products for the 6 and 9 min time points. Only the region of the gel containing G is presented; no time-dependent changes were observed for the other viral proteins. The gel was impregnated with scintillant³³ before drying, and the film was exposed for 40 d at -70 °C. The band below G_1 in the trypsin-treated lanes has methionine containing tryptic peptides of G (F. Katz, personal communication), and varies greatly in amount between experiments. Its significance is unclear.

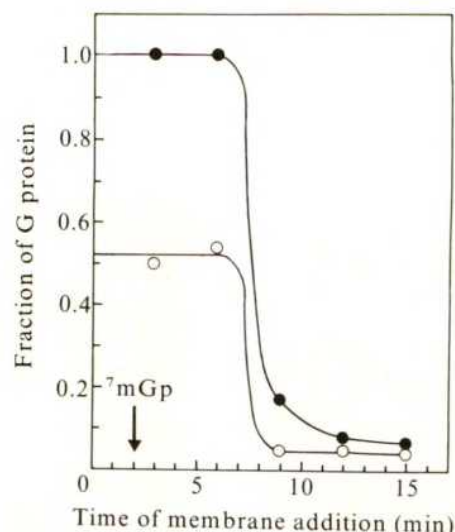


Fig. 3 Effect of the time of membrane addition on glycosylation and proper insertion. Same experiment as Fig. 2. The autoradiogram of the gel was scanned, and the amounts of G_0 and G_1 (lanes without trypsin treatment) and of the large membrane-protected tryptic fragment of G_1 (lanes with trypsin treatment) were determined from the peak heights. The extent of glycosylation (●) is $G_1/(G_0 + G_1)$. The extent of protection of G by membranes (○) is the amount of fragment (running between G_0 and G_1) divided by $G_0 + G_1$ determined from the lane without trypsin of the same time point.

encounter membranes when the two are first mixed. This suggests that the critical time is dependent on some intrinsic property of the nascent G protein. This view is strongly supported by experiments in which the time for completion of G was about 23 min (using a different wheat germ preparation from Fig. 3), and the critical time for proper insertion was only 3 min. The critical time is therefore proportional to the time required for completion of the polypeptide.

If it is assumed that the growth of the G polypeptide is proportional to time, then the approximate length of the chain at the critical time can be determined. G has a molecular weight of ~63,000 (ref. 11) and therefore contains approximately 550 amino acid residues. In the experiment of Fig. 3, the chain was completed in 40 min, so about 80 residues would have been completed at the critical time. Of these, about 40 are present within the large subunit of the ribosome^{12,13}, so that only 40 residues would be available for any interaction with membrane vesicles. Synthesis of no more than 10–15 additional residues in the absence of membranes at this critical chain length results in the loss of proper insertion into the membrane and of glycosylation.

It can be concluded that both insertion into the membrane and glycosylation are closely coordinated with polypeptide chain synthesis, for membranes are required almost from the outset.

Sequence of glycosylation

The finding (Fig. 4) that protein synthesis in wheat germ extracts is unaffected by high concentrations of the detergent Triton X-100 has permitted the times of the glycosylation events to be determined in a simple experiment, and has led to the discovery of a novel intermediate in glycosylation. The lack of effect of Triton at concentrations as high as 1% (or possibly higher) is in marked contrast to the complete inhibition by the bile salt deoxycholate at much lower concentrations, as found for the reticulocyte system¹⁸. Concentrations of Triton as high as 1% would be expected to completely solubilise the pancreatic vesicle membranes, and might be expected to cause an abrupt inhibition of glycosylation by producing a random physical segregation of the nascent G chain (with attached ribosome), the glycosyltransferases, and the presumed lipid-linked sugar

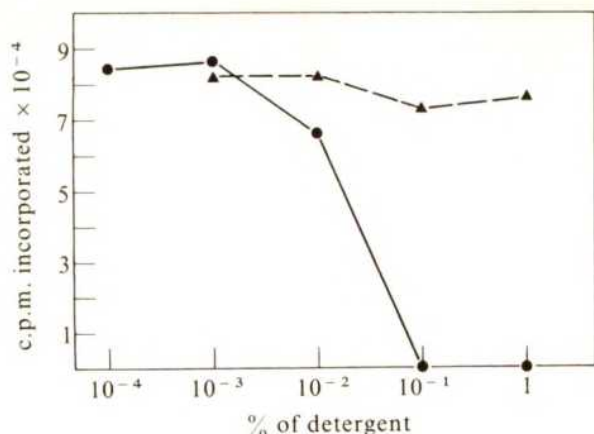


Fig. 4 Effect of detergents on protein synthesis. Conditions of protein synthesis were essentially the same as Fig. 1, except no ³H-mGp was added. Shown is the amount of TCA-insoluble protein made in 60 min per 2 µl of reaction as a function of the concentration (w/v %) of either Triton X-100 (▲) or sodium deoxycholate (●).

substrates into different detergent micelles. Indeed, all of the G protein which results from translation of VSV mRNA in the presence of pancreatic membranes and 1% Triton X-100 is in the G₀ (unglycosylated) form (Fig. 5).

These results imply that the timing of glycosylation can be explored by synchronising protein synthesis with ³H-mGp as before but with the exception that membrane vesicles are present from the outset. At various times, samples are taken and Triton X-100 is added to inhibit further glycosylation, and the polypeptide chains are completed in the presence of the detergent. From the resulting distribution of G chains between glycosylated and unglycosylated forms as a function of the time of detergent addition, the times of glycosylation events can be inferred. Thus, when detergent is added before any glycosylation has taken place, no subsequent glycosylation can occur, so all of the resulting G will migrate in the G₀ position. When Triton is added after all glycosylation is completed, only the G₁ form will result.

Figure 5 shows that up to 9 min of synchronous synthesis, no glycosylation has taken place since only G₀ is observed. After 29 min, mainly G₁ is observed, so glycosylation is mainly complete well before the chain is finished. In between, and particularly around 20 min, a previously unknown form of G predominates, migrating on the gel in between G₀ and G₁. The amounts of these three forms as a function of the time of addition of detergent are shown in Fig. 6. The novel form of G, called G_{1/2}, is clearly a kinetic intermediate in the formation of G₁. This form binds specifically to columns of concanavalin A-Sepharose 4B, as judged by the methods used previously⁹ (data not shown), and is therefore (as expected from its intermediate mobility in gels) an intermediate in glycosylation.

The VSV virion glycoprotein contains two essentially identical oligosaccharide side chains¹⁴. As it is unlikely that both of the sites are glycosylated at the same time, and as the Asn-linked oligosaccharide would be derived by *en bloc* transfer from a lipid-linked oligosaccharide^{15,16}, an intermediate form in which one of the two sites is glycosylated would be expected. It was confirmed recently by analysis of the kinetics of degradation of G₁ by endoglycosidase H that G_{1/2} contains only one of the two oligosaccharides (J. Rothman, in preparation). This enzyme attacks each oligosaccharide at only one site, generating partial degradation products in proportion to the number of glycosylated Asn residues. It was found that G₁ was converted to G_{1/2}, which was then converted to G₀.

Since glycosylation is completed before the completion of the polypeptide backbone (Fig. 6), it is clear that the transfer of oligosaccharides is to the nascent chain. The rather precise kinetics of glycosylation probably indicate that each of the two

successive transfers of oligosaccharides to the nascent chain can only occur only after specific lengths of the nascent chain have been made because the timing of these events is determined only by the time required to complete G. When 23 min were required for completion of G chains (using a different wheat germ preparation from that used in Fig. 6), G_{1/2} was maximal at 12 min, and G₁ was half-maximal at 17 min, as compared with 22 min for G_{1/2} and about 30 min for G₁ when about 43 min were required for completion of G (Fig. 6).

Implications for the mechanism of insertion

Figure 7 presents a diagram of what is currently known of the sequence of events which occur during the synthesis of the VSV glycoprotein.

Our finding (Fig. 3) of a very early requirement for membranes implies that the insertion of G protein into the membrane has already begun when 80 or fewer residues are made, of which only 40 or fewer can be available for any interaction with membranes. The experiment only indicates the longest chain length which still permits subsequent insertion, so these figures place an upper limit on when insertion actually occurs. Glycosylation events are evidently not the basis for the early membrane requirement as both glycosylation steps occur well after the requirement for membranes is expressed (Fig. 7). Glycosylation is therefore not important for insertion of the membrane protein, at least in the initial stages. Presumably, insertion into the membrane proceeds continuously after it has begun.

In addition, our results strongly suggest that the nascent chain spans the lipid bilayer such that the protein is extruded across the membrane as it is being polymerised, as predicted from considerations of membrane asymmetry¹. This conclusion follows immediately from the fact that the chain is glycosylated while it is still attached (through tRNA) to the ribosome on the 'cytoplasmic' side of the membrane, providing it is assumed that the glycosylation steps take place on the opposite side of the membrane, within the vesicle.

Implications for the mechanisms of glycosylation

The belief that glycosylation is restricted to the extracytoplasmic surface of membranes is widespread and is based on the fact

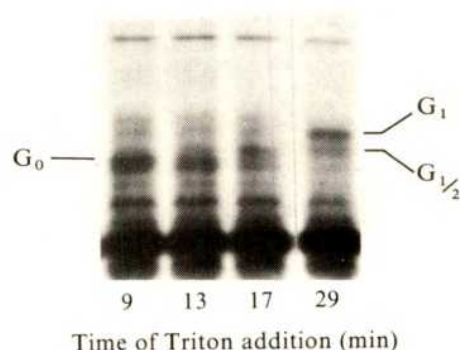


Fig. 5 Triton X-100 addition experiment. Protein synthesis was performed in the same conditions as Fig. 1, except the final volume was 0.95 ml and pancreatic microsomal membranes (20 µl) were present before adding mRNA at time zero. At 2 min, 1 mM ³H-mGp was added. At each indicated time, a sample (25 µl) was removed, 2.5 µl of 10% (w/v) Triton X-100 was added, and incubation at 23 °C was continued until 53 min had elapsed from the time of addition of mRNA. Samples were then precipitated with TCA and electrophoresed as before⁹. Only the regions of the autoradiogram of the gel containing G are shown since no time-dependent changes in other viral proteins were observed. The gel was impregnated with scintillant³³ before drying, and was autoradiographed for 40 d at -70 °C. The band above G₁ was observed in the presence or absence of membranes, varied between experiments, and was not time-dependent. The G₁ chains required between 40 and 45 min for completion in the presence of membranes in this experiment, as determined by the methods of Fig. 1 (data not shown). The rate of elongation of proteins is therefore unaffected by the presence of membranes.

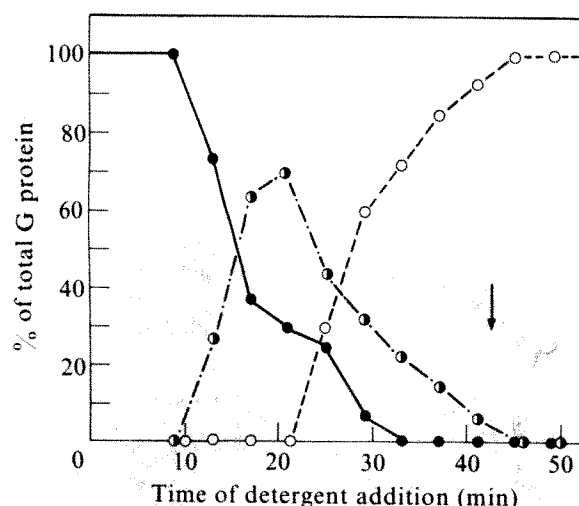
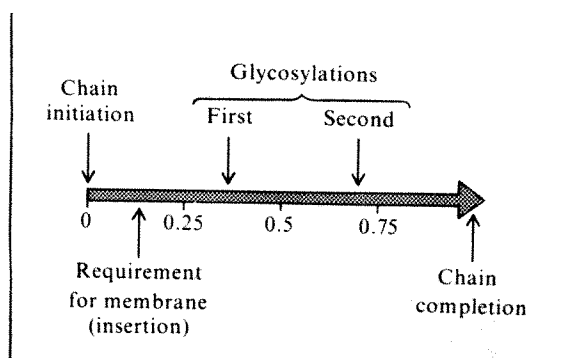


Fig. 6 Sequence of glycosylation. The amounts of G-specific translation products, G_0 , $G_{1/2}$ (the band between G_0 and G_1 in Fig. 5), and G_1 were determined for each time point from the peak heights of the corresponding bands in the acrylamide gel for the experiment which is partially presented in Fig. 5. The amount of each form is expressed as a % of the total of the three forms. G_0 (●), $G_{1/2}$ (●), G_1 (○). Arrow indicates polypeptide chain completion.

that membrane carbohydrate is found only on that side^{1,2}. Consistent with this, we have found (Fig. 3) that both glycosylation and proper insertion of the VSV glycoprotein are lost coordinately when membrane vesicles are added too late, in spite of the fact that the glycosylation steps themselves do not take place until well after this requirement for membranes is expressed (Fig. 7). This indicates that the late glycosylation depends on the early insertion of the nascent chain into the membrane on the 'cytoplasmic' side. This implies that the late glycosylation steps occur when the nascent chain of the G protein is present on the opposite, luminal side of the vesicle membrane.

We have presented here the first direct evidence that the nascent chain of a membrane glycoprotein is glycosylated. Kiely *et al.*¹⁷ showed that the majority of the carbohydrate is attached only to those peptide chains of ovalbumin (a secreted protein) that have been essentially completed. The timing of the two glycosylation steps of the VSV glycoprotein (Fig. 6) is remarkably precise. Each glycosylation takes place only after a specific length of the polypeptide chain is made. One explanation for this would be that the order and timing of the glycosylations is determined only by the order of the two acceptor residues in the amino acid sequence, that is, when the acceptor Asn residues first appear on the luminal side of the membrane.

Fig. 7 Sequence of events during the synthesis of G protein. The numbers below the arrow indicate the fraction of the chain completed, assuming the chain grows at a constant rate. The time of the first glycosylation is when G_0 is half of its original value, and the time for the second glycosylation is when G_1 is half maximal (Fig. 6).



Another plausible explanation would be that only when specific lengths of chain are made (and perhaps only for small intervals of chain length) is the nascent membrane protein in the appropriate conformation to act as an acceptor. In this case, an Asn would not generally be glycosylated at a time which corresponds to its position in the amino acid sequence. Such a conformation would presumably require that the Asn acceptor site of the nascent chain be in intimate proximity with the luminal surface of the lipid bilayer, in order to permit enzymatic transfer from the dolichol-oligosaccharide donor, located at the same interface.

Role of signal sequences

The considerations discussed above and elsewhere^{4,19} imply that the ribosomes which manufacture G protein are bound to the membrane by the nascent chain, if by no other interaction. Our results show for the first time that membrane-bound ribosomes engaged in the secretory process can be derived from free ribosomes after initiation of protein synthesis, a central aspect of the 'signal hypothesis'^{20,21}. Thus, when G chains are started in the absence of membranes on free ribosomes, and then subsequent initiation is blocked, these same ribosomes must become bound to the membrane in a functional configuration in order to permit the insertion and glycosylation of the resulting G protein which is observed when membranes are added at sufficiently early times (Fig. 3). Earlier work²¹ did not distinguish whether initiation occurred on added free ribosomes before or after they became bound to the membrane.

It then follows that these free ribosomes must possess a 'signal' which directs them to the correct membrane to bind in a functional way, permitting the nascent chain to be extruded through the membrane, as proposed in the 'signal hypothesis'^{20,21}. This signal could, in principle, be found in sequences of mRNA or of the nascent polypeptide itself. Support for the localisation of the 'signal sequence'²¹ to the N terminus of the nascent chain has come from the original finding of a precursor to the immunoglobulin light chain containing an additional sequence at the N terminus²⁰ and the subsequent finding that a wide spectrum of proteins secreted by pancreas possess essentially identical N-terminal extensions which are removed from the proteins in the endoplasmic reticulum²². Much additional work has shown that, quite generally, secreted proteins²³⁻³¹ (as well as a membrane ectoprotein³²) have similar N-terminal extensions some 15-30 residues in length. Unfortunately, direct evidence for the function of these extensions as the proposed signal sequences has been lacking.

Our findings now provide direct evidence for an N-terminal signal sequence which functions in the predicted manner²¹. Thus, the requirement for membranes during synthesis of G protein is not expressed until some 40 residues are found outside the ribosome, a length of chain just sufficient to contain any of the N-terminal extensions cited above. On the basis of the signal hypothesis, membranes could not be required until all of the signal sequence is translated and outside the large ribosomal subunit. The rapid loss of proper insertion of G when no more than 10-15 additional residues are made would then indicate that the signal sequence contained within the N-terminal 40 residues can only function during a short interval in the synthesis of the glycoprotein. There is as yet no evidence for a significant cleavage at the N terminus of G, but there is no obvious reason why an N-terminal signal sequence need be cleaved off.

The mechanisms used by the VSV glycoprotein to enter membranes can be expected to be relevant to most or all cellular ectoproteins since the limited coding capacity of the viral genome (five proteins, all structural components of the virion) requires that it utilises cellular factors for glycoprotein glycosylation and insertion. Indeed, the diverse sources of the components (plant ribosomes and animal membranes) which function together in the cell-free system indicate a high degree of evolutionary conservation of the mechanisms involved. The results obtained do not reflect unique properties of the particular

components used since the glycoprotein produced in the cell-free system is indistinguishable from the glycoprotein found in the endoplasmic reticulum of infected cells⁹. Further investigations using the cell-free system may provide more detailed insights into the mechanism by which the nascent chain crosses the membrane, and the relationship of signal sequences to this mechanism.

This research was supported by grants from the USNIH and the American Cancer Society. J. E. R. is a postdoctoral fellow of the Damon Runyon Foundation. We thank Dr. Richard Palmiter for useful discussions, Susan Froshauer and Martin Brock for technical assistance and Marianne Robotham for assistance with the manuscript.

Received 29 June; accepted 22 August 1977.

1. Rothman, J. E. & Lenard, J. *Science* **195**, 743-753 (1977).
2. Bretscher, M. S. *Science* **181**, 622-629 (1973).
3. Lenard, J. & Compans, R. W. *Biochim. biophys. Acta* **344**, 51-94 (1974).
4. Morrison, T. & Lodish, H. F. *J. biol. Chem.* **250**, 6955-6962 (1975).
5. Grubman, M. J., Moyer, S. A., Banerjee, A. K. & Ehrenfeld, E. *Biochem. biophys. Res. Commun.* **62**, 531-538 (1975).
6. Knipe, D. M., Baltimore, D. & Lodish, H. F. *J. Virol.* **21**, 1128-1139 (1977).
7. Hunt, L. A. & Summers, D. F. *J. Virol.* **20**, 637-645 (1976); **20**, 646-657 (1976).
8. Katz, F. N. & Lodish, H. F. (submitted).
9. Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G. & Lodish, H. F. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3278-3282 (1977).

10. Hickey, E. D., Weber, L. A. & Baglioni, C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 19-23 (1976).
11. Both, G. W., Moyer, S. A. & Banerjee, A. K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 274-278 (1975).
12. Blobel, G. & Sabatini, D. D. *J. Cell Biol.* **45**, 130-145 (1970).
13. Malkin, L. I. & Rich, A. *J. molec. Biol.* **26**, 329-346 (1967).
14. Robertson, J. S., Etchison, J. R. & Summers, D. F. *J. Virol.* **19**, 871-878 (1976).
15. Waechter, C. J. & Lennarz, W. J. *Rev. Biochem.* **45**, 95-112 (1976).
16. Pless, D. D. & Lennarz, W. J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 134-138 (1977).
17. Kiely, M. L., McKnight, G. S. & Schimke, R. T. *J. biol. Chem.* **251**, 5490-5495 (1976).
18. Olsnes, S., Spaeren, U., Heiberg, R. & Pihl, A. *Biochem. J.* **130**, 297-299 (1972).
19. Lodish, H. F. & Froshauer, S. *J. Cell Biol.* **74**, 358-364 (1977).
20. Milstein, C., Brownlee, G. G., Harrison, T. M., & Mathews, M. B. *Nature new Biol.* **239**, 117-120 (1972).
21. Blobel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 835-851 (1975); **67**, 852-862 (1975).
22. Devillers-Thierry, A., Kindt, T., Scheele, G. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5016-5020 (1975).
23. Burstein, Y. & Schechter, I. *Proc. natn. Acad. Sci. U.S.A.* **74**, 716-720 (1977).
24. Shields, D. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2059-2063 (1977).
25. Kemper, B., Habener, J. F., Ernst, M. D., Potts, J. T. & Rich, A. *Biochemistry* **15**, 15-19 (1976).
26. Chan, S. J., Keim, P. & Steiner, D. F. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1964-1968 (1976).
27. Strauss, A. W., Donohue, A. M., Bennett, C. D., Rodkey, J. A. & Alberts, A. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1358-1362 (1977).
28. Szczesna, E. & Boime, I. *Proc. Natn. Acad. Sci. U.S.A.* **73**, 1179-1183 (1976).
29. Sussman, P. M., Tushinski, R. J. & Bancroft, F. C. *Proc. natn. Acad. Sci. U.S.A.* **73**, 29-33 (1976).
30. Dannies, P. S. & Tashjian, A. H., Jr *Biochem. biophys. Res. Commun.* **70**, 1180-1189 (1976).
31. Inouye, H. & Beckwith, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1440-1444 (1977).
32. Inouye, S., Wang, S. S., Sekizawa, J., Halegoua, S. & Inouye, M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1004-1008 (1977).
33. Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335-341 (1975).

letters to nature

Radio haloes around BL Lacertae objects AO0235+164 and 4C03.59

THE rapid variations in intensity and linear polarisation of several BL Lacertae sources at centimetre and optical wavelengths have indicated the presence of multiple non-thermal components, often appreciably smaller than 1 pc (refs 1, 2). But there is no clear evidence for radio structures extended over kiloparsecs around any of the known variable BL Lac sources which have been scanned with narrow beams of a few arcs³⁻⁷. The absence of extended radio structure in brighter and more variable BL Lac sources has also been inferred from their flat or inverted radio spectra and has led to the suggestion that these are extremely 'young' QSOs which have yet to develop a steady, non-thermal background⁸⁻¹⁰. We report here the lunar occultation and interplanetary scintillation (IPS) observations of two BL Lac sources, AO0235+164 and 4C03.59 at 327 MHz. One of the two occultations of the former source, which is known to be an exceptionally violent variable at centimetre, infrared and optical wavelengths, took place in November 1975 near the epoch of its major outburst¹¹⁻¹³. These observations indicate that at 327 MHz, part of the emission of both these sources originates in components larger than 1 arc s.

The Arecibo occultation source AO0235+164 was identified with a 19-mag stellar object and was shown to be a radio and optical variable having an inverted radio spectrum and a featureless, steep optical spectrum^{3,16,17}. During late 1975, when this BL Lac source flared up dramatically, spectrometry revealed two optical absorption-line systems^{14,15} at redshifts of $z=0.524$ and 0.852 and also HI absorption¹⁵ at $z=0.524$. The strikingly rapid flux variations of this source impose extreme constraints on the incoherent synchrotron interpretation for rapidly varying extragalactic sources, if their redshifts are cosmological^{12,13,18}. The other BL Lac source 4C03.59 also has a steep optical spectrum^{3,19,20} but only a normal, straight radio spectrum²¹ with a slope of -0.6 and has been classified as a possible radio variable²². Visual images of both these BL Lac objects exhibit a jet-like nebulousity extending up to ~ 3 arc s from the stellar nucleus^{13,17,20}.

The lunar occultation and IPS observations at 327 MHz were made using the Ooty radio telescope. The methods of reducing such observations are described elsewhere²³⁻²⁵. The results are given in Table 1, Figs 1 and 2. Occultation scans were observed for three position angles across AO0235+164 on 28 August and 18 November, 1975 and for four position angles across 4C03.59 between December 1973 and October 1974. The IPS observations were reduced using PKS1148-00 as calibrator, for which observations at Ooty have shown that its scintillation index rises to a maximum value of $85 \pm 5\%$ (S. Ananthakrishnan, personal communication).

After applying corrections for beam-broadening to the restored strip scans of Fig. 1, we estimate the half-power width of an equivalent gaussian source to AO0235+164 to be 3.0 ± 1.4 , 6.3 ± 1.8 and 3.7 ± 1.5 arc s in position angles of 21° , 66° and 140° , respectively. Thus AO0235+164 is resolved significantly at 327 MHz. We have also carried out IPS observations of this source on 10 days during April and May 1977, which indicate a scintillating component of size < 0.1 arc s containing a flux of 0.75 ± 0.15 Jy out of the total 1.07 ± 0.11 Jy at 327 MHz (Table 1). A simple interpretation of these measurements would be that at 327 MHz

Table 1 The observed parameters at 327 MHz

	AO0235+164	4C03.59
RA (1950)	02 ^h 35 ^m 52.61 \pm 0.04s (02 35 52.63 \pm 0.04) [†]	23 ^h 35 ^m 34.28 \pm 0.03s (23 35 34.25 \pm 0.03) [†]
Dec. (1950)	+16° 24' 04.5 \pm 0.5" (+16 24 04.3 \pm 0.4) [†]	+03° 10' 11.6 \pm 0.4" (+03 10 11.8 \pm 0.5) [†]
Flux density*	1.07 \pm 0.11 Jy	4.3 \pm 0.3 Jy
Flux of the IPS component	0.75 \pm 0.15 Jy	0.4 \pm 0.2 Jy
Size of the IPS component	<0.1 arc s	0.25 \pm 0.05 arc s

* The flux scale is as defined by Veron, Veron and Witzel²⁶.

[†] The values inside brackets are the centroid positions measured at 2.7 GHz using the RRE interferometer^{16,22} and are in excellent agreement with the positions of the identified optical objects, measured on the Sky Survey prints to an accuracy of ± 0.4 arc s in each coordinate^{16,27}.

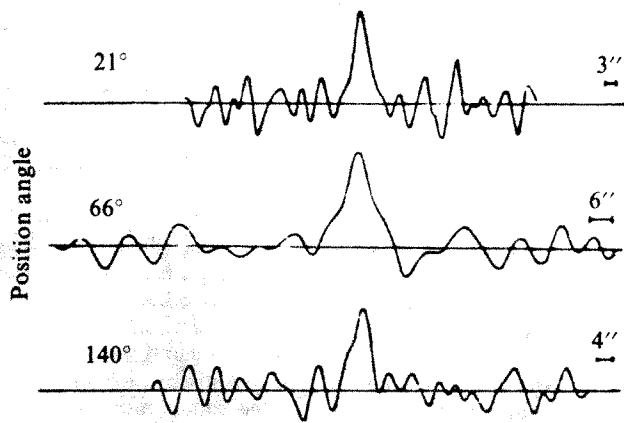
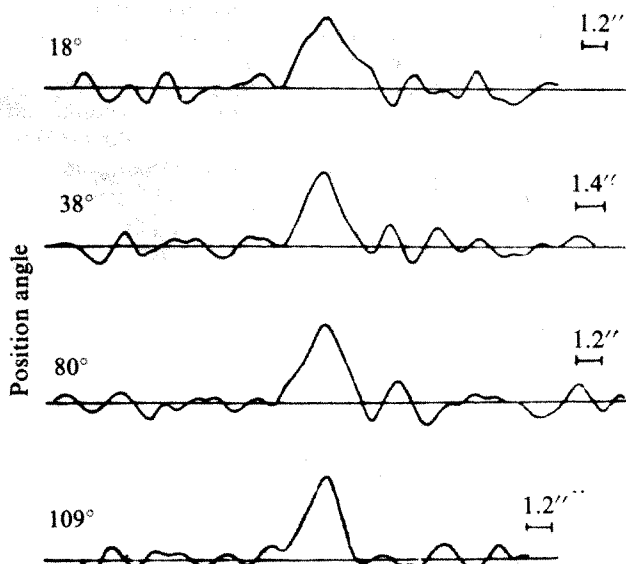


Fig. 1 The strip-brightness distributions across AO0235+164 are shown for the three indicated position angles. The resolutions are indicated by bars. Right ascension increases to the left. The scan in position angle 66° was observed on 18 November 1975 near the peak of the major radio outburst of this source^{11,12}.

the source consists of a core of size <0.1 arc s which accounts for nearly two-thirds of the total emission and an underlying extended component having a size of a few arc s. During VLBI observations²⁸, carried out in 1970 with a fringe spacing of 0.05 arc s, the source showed a maximum amplitude of 0.97 ± 0.27 Jy at 430 MHz, as compared with the total flux of 1.50 ± 0.16 Jy measured by Sutton *et al.* at 408 MHz²⁹. These two measurements indicate that either the entire flux is contained in a gaussian source of size ~ 0.02 arc s which was only partially resolved by the VLBI or, alternatively, that the measured visibility was due to an unresolved core of size <0.01 arc s and the remainder flux comes from a broad component. The latter interpretation would be consistent with the simple model suggested above.

After correcting the strip scans shown in Fig. 2 for beam-broadening, the half-power width of the source, 4C03.59(2335+031), is estimated to be 1.5 ± 0.5 arc s. The source profile in position angle 109° shows a faint but probably real extension towards east. This could be identified with the optical nebulosity of a few arc s which is seen extending from the stellar object identified with this source²⁰. In addition to the above structure, there is also

Fig. 2 Strip-brightness distributions across 4C03.59 are shown for the four indicated position angles. The resolutions are indicated by bars. Right ascension increases to the left.



evidence for a more compact, scintillating core contributing $10 \pm 5\%$ of the total flux at 327 MHz (Table 1).

The extended radio features of size ≥ 1 arc s detected in these two BL Lac sources have brightness temperatures exceeding $\sim 10^9$ K at 327 MHz. Hence their emission at metre wavelengths is likely to be predominantly non-thermal. Assuming that the absorption-line redshift $z=0.852$ for AO0235+164 represents its cosmological redshift and for Einstein-de Sitter model with $H=50$ km s⁻¹ Mpc⁻¹, the extended component of this source has a size of ~ 50 kpc and a minimum energy density of $\sim 10^{-10}$ erg cm⁻³ in the form of relativistic electrons and magnetic field.

Like many QSOs, the BL Lac source AO0235+164 exhibits multiple absorption systems¹⁴. The absorption could arise either in the intervening galaxies along the line of sight or within gas clouds located near its nucleus and being driven away due to its large radiation pressure^{30,31}. An important requirement in the latter model is that the gas clouds do not expand freely during their outflow, though the mechanism of their confinement is not clear³²⁻³⁴. The confinement may occur due to external pressure caused by the extended non-thermal component, provided the simple core-halo model of the source is valid. But, the present observations are also consistent with a more complex structure without a halo. It may also be noted that the mildly relativistic speeds of the outflowing absorbing clouds, as are inferred³⁵ from the multiple-redshift systems in QSOs and AO0235+164, are attainable only in a period of $\geq 10^5$ yr after the ejection³². It is interesting that a similar age of $\geq 10^5$ yr is also implied for AO0235+164 by the measured size of its extended component, taking this non-thermal component to be an expanded remnant of the past activity in its nucleus.

I thank Professor G. Swarup and Dr M. N. Joshi for their valuable comments.

GOPAL-KRISHNA

Radio Astronomy Centre,
Tata Institute of Fundamental Research,
P.O. Box 8, Ootacamund 643 001, India

Received 15 June; accepted 31 August 1977.

- Altschuler, D. R. & Wardle, J. F. C. *Mem. R. astr. Soc.* **82**, 1-67 (1976).
- Kinman, T. D. *Astrophys. J.* **205**, 1-5 (1976).
- Stein, W. A., O'Dell, S. L. & Strittmatter, P. A. *Rev. Astr. Astrophys.* **14**, 173-195 (1976).
- Kapahi, V. K. *Nature phys. Sci.* **234**, 49-50 (1971).
- Conway, R. G. & Stannard, D. *Mon. Not. R. astr. Soc.* **160**, 31-33 (1972).
- Colla, G. *et al. Astr. Astrophys.* **38**, 209-223 (1975).
- Argue, A. N., Kenworthy, C. M., Ryle, M. & Shakeshaft, J. R. *Nature Phys. Sci.* **241**, 139-139 (1973).
- Altschuler, D. R. & Wardle, J. F. C. *Nature* **255**, 306-310 (1975).
- Pollock, J. T. *Astrophys. J. Lett.* **198**, L53-L56 (1975).
- Usher, P. D. *Astrophys. J. Lett.* **198**, L57-L60 (1975).
- MacLeod, J. M., Andrew, B. H. & Harvey, G. A. *Nature* **260**, 751-752 (1976).
- Ledden, J. E., Aller, H. G. & Dent, W. A. *Nature* **260**, 752-754 (1976).
- Rieke, G. H., Grasdalen, G. L., Kinman, T. D., Hintzen, P., Wills, B. J. & Wills, D. *Nature* **260**, 754-759 (1976).
- Burbidge, E. M., Caldwell, R. D., Smith, H. E., Liebert, J. & Spinrad, H. *Astrophys. J. Lett.* **205**, L117-L120 (1976).
- Roberts, M. S. *et al. Astr. J.* **81**, 293-297 (1976).
- Argue, A. N., Kenworthy, C. M. & Stewart, P. M. *Astrophys. Lett.* **14**, 99-104 (1973).
- Spinrad, H. & Smith, H. E. *Astrophys. J.* **201**, 275-276 (1975).
- Terrell, J. *Astrophys. J. Lett.* **213**, L93-L97 (1977).
- Strittmatter, P. A., Carswell, R. F., Gilbert, G. & Burbidge, E. M. *Astrophys. J.* **190**, 509-514 (1974).
- Craine, E. R., Tapia, S. & Tarengi, M. *Nature* **258**, 56-57 (1975).
- Wall, J. V. *Aust. J. Phys. Astrophys. Suppl.* no. 24 1-63 (1972).
- McEwan, N. J., Browne, I. W. A. & Crowther, J. H. *Mem. R. astr. Soc.* **80**, 1-59 (1975).
- Kapahi, V. K., Joshi, M. N., Subrahmanya, C. R. & Gopal-Krishna *Astr. J.* **78**, 673-680 (1973).
- Venkatakrishnan, K. L. & Swarup, G. (in preparation).
- Bhandari, S. M., Ananthakrishnan, S. & Rao, A. P. *Aust. J. Phys.* **27**, 121-128 (1974).
- Veron, M. P., Veron, P. & Witzel, A. *Astr. Astrophys. Suppl.* **13**, 1-53 (1974).
- Singal, A. K., Gopal-Krishna & Venugopal, V. R. (in preparation (1977)).
- Galt, J. A., Broten, N. W., Legg, T. H., Leparskas, H. J. A. & Yen, J. L. *Mon. Not. R. astr. Soc.* **178**, 301-305 (1977).
- Sutton, J. M., Davies, I. M., Little, A. G. & Murdoch, H. S. *Aust. J. Phys. Astrophys. Suppl.* no. 33, 1-44 (1974).
- Mushotzky, R. F., Solomon, P. M. & Strittmatter, P. A. *Astrophys. J.* **174**, 7 (1972).
- Scargle, J. D. *Astrophys. J.* **179**, 705-719 (1973).
- Kippenhahn, R., Perry, J. J. & Röser, H.-J. *Astr. Astrophys.* **34**, 211-224 (1974).
- Mathews, W. G. *Astrophys. J.* **189**, 23-31 (1974).
- Weymann, R. *Astrophys. J.* **208**, 286-291 (1976).
- Rees, M. J. *Astrophys. J. Lett.* **160**, L29-L32 (1970).

A constraint on the universal baryon density from the abundance of ${}^7\text{Li}$

THE observed interstellar abundance of ${}^2\text{H}$ has been used^{1,2} to estimate the mean baryon density (ρ_b) of the Universe. This follows, because (1) there is no plausible source for ${}^2\text{H}$ other than the primordial big bang and (2) the production of ${}^2\text{H}$ in a standard big bang decreases rapidly with increasing ρ_b . If one then assumes that all ${}^2\text{H}$ was formed in a big bang, the observed abundance² of this nuclide requires a value of ρ_b sufficiently low¹ that, for a cosmological constant $\Lambda=0$, the present expansion of the Universe will continue forever and the Universe is open. A major weakness in this argument is that another source of ${}^2\text{H}$ may be found. It has been suggested, for example, that ${}^2\text{H}$ could be made in shock waves accompanying a supernova explosion; this now seems unlikely⁴, but other mechanisms will certainly be suggested, so that it is important to obtain confirmation of the above conclusion. The predicted production of ${}^7\text{Li}$ in a big bang² varies rapidly with ρ_b and could be used to estimate ρ_b if the fraction of the observed ${}^7\text{Li}$ made in the big bang were known. Unfortunately there are many possible sources⁴ of ${}^7\text{Li}$ and such estimates must be regarded with scepticism. In this note we point out that ${}^7\text{Li}$ can be used to place an upper limit on ρ_b , even if other production mechanisms are important, and that this limit also strongly favours an open universe. This possibility arises because the big bang production of ${}^7\text{Li}$ increases with increasing ρ_b (for

$\rho_b > 10^{-31}$) so that an upper limit is obtained by attributing all of the observed ${}^7\text{Li}$ to the big bang.

We have adopted here Boesgaard's⁵ value of the Li abundance which yields⁶ a fractional abundance by mass of ${}^7\text{Li}$, $X_7 = 5 \times 10^{-9}$. Assuming the big bang must not synthesise more than this amount leads to $\rho_b \leq 1.1 \times 10^{-30} \text{ g cm}^{-3}$. As is shown in Fig. 1, this is substantially less than the critical value ρ_c necessary to close a $\Lambda=0$ Friedman universe.

The uncertainty in X_7 is perhaps a factor of two; the meteoritic value⁶, for example, is $X_7 = 8 \times 10^{-9}$. Substantially larger values have been seen⁵ in a small number of red giant stars, but these values presumably reflect a local production mechanism. Allowing for a factor of two uncertainty gives an upper limit closer to ρ_c , but still favouring an open and forever expanding universe.

The existence of mechanisms which destroy ${}^7\text{Li}$ weakens the limit on ρ_b since the big bang may then have made more ${}^7\text{Li}$ than is now observed; conversely, discovery of additional sources of ${}^7\text{Li}$ strengthens the limit. Astration of primordial material is presumably the most important destruction process. Estimates of the fraction of matter which has passed through stars are rather uncertain but are typically about 0.5. It has been pointed out^{4,7}, however, that infall of primordial material from the galactic halo may be significant and would tend to compensate for the effects of astration for those nuclei produced in the big bang. Other sources of ${}^7\text{Li}$ are generally rather speculative⁴, except for production in the cosmic rays which yields roughly 10% of the observed ${}^7\text{Li}$. Since these various effects tend to offset each other, the observed value of X_7 seems reasonable but subject to uncertainty.

If it is a good approximation to ignore both astration and sources of ${}^2\text{H}$ and ${}^7\text{Li}$ other than the big bang, their observed abundances each separately determine the density. An estimate based on the ${}^2\text{H}$ abundance X_D is shown in Fig. 1, and is in good agreement with the density obtained from ${}^7\text{Li}$. Effects of astration would tend to worsen this agreement. Thus when other possible contributions to ${}^7\text{Li}$ are better understood, the requirement that the big-bang contribution to X_7 and X_D yield the same value of ρ_b may be a strong constraint on allowable astration.

We assumed above that the cosmological constant $\Lambda=0$. While this is consistent with the available data, a non-zero value cannot be excluded, except on aesthetic grounds, and its effects must be considered. It has been found¹¹ that for reasonable values of Λ , the limits on ρ_b from the ${}^2\text{H}$ and ${}^7\text{Li}$ abundances are essentially unchanged. But, the simplest relationship between ρ_b and the curvature and evolution of the Universe is no longer valid¹¹.

In summary, the simplest and most straightforward assumptions concerning the origin of ${}^7\text{Li}$ and the nature of the big bang expansion require an upper limit for the present universal density of $\rho_b = (1.1 [+1.1 \text{ or } -0.4]) \times 10^{-30} \text{ g cm}^{-3}$. Given that the Universe is indeed a Friedman universe with zero cosmological constant, the agreement between the present limit and that based on ${}^2\text{H}$ strongly supports the conclusion of Gott *et al.*¹ that the Universe is open and will continue to expand forever.

This research was supported by the USNSF.

Note added in proof: It has come to our attention that conclusions similar to those reached here have been discussed by G. Steigman at the Harvard Neighborhood Meeting on Cosmology, October 1975.

SAM M. AUSTIN
C. H. KING

Cyclotron Laboratory and Physics Department,
Michigan State University,
East Lansing, Michigan 48824

Received 27 June; accepted 15 August 1977.

- Gott, J. R., Gunn, J. E., Schramm, D. N. & Tinsley, B. M. *Astrophys. J.* **194**, 543–553 (1974).
- Schramm, D. N. & Wagoner, R. V. *A. Rev. nucl. Sci.* (in the press).
- Epstein, R., Arnett, W. D. & Schramm, D. N. *Astrophys. J. Suppl.* **31**, 111–141 (1976).
- Reeves, H. *A. Rev. Astr. Astrophys.* **12**, 437–469 (1974).
- Boesgaard, A. M. *Pub. astr. Soc. Pac.* **88**, 353–66 (1976).
- Cameron, A. G. W. *Space Sci. Rev.* **15**, 121–46 (1973).
- Audouze, J. & Tinsley, B. M. *Astrophys. J.* **192**, 487–500 (1974).
- Wagoner, R. V. *Astrophys. J.* **179**, 343–360 (1973).
- Woody, D. P., Mather, J. C., Nishioka, N. S. & Richards, P. L. *Phys. Rev. Lett.* **34**, 1036–1039 (1975).
- York, D. G. & Rogerson, J. B. *Jr Astrophys. J.* **203**, 378–385 (1976).
- Tinsley, B. M. *Phys. Today* **30**, 32–38 (1977).

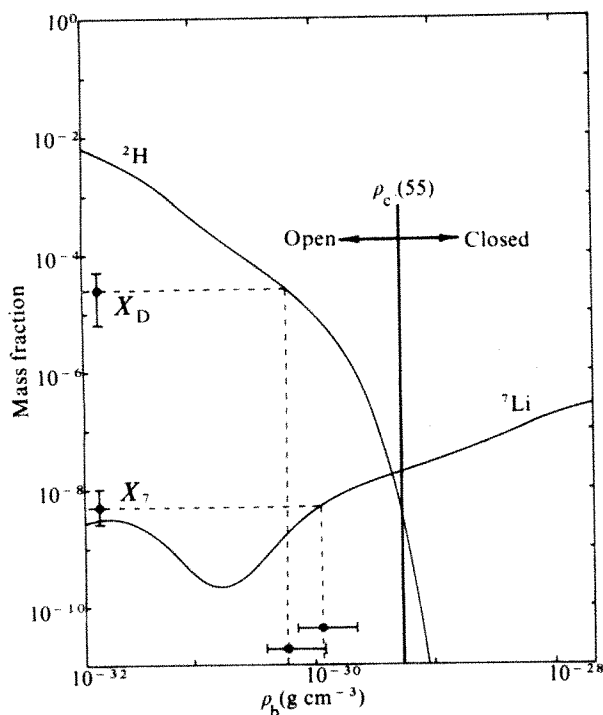


Fig. 1 Abundances of ${}^2\text{H}$ and ${}^7\text{Li}$ produced in a standard big bang (adapted from ref. 8). The present black body temperature is taken to be 2.90 K, see ref. 9.) The vertical line labelled ρ_c (55) is the density necessary to close a Friedman universe with $\Lambda=0$, if $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$ (in general $\rho_c = 5.7 \times 10^{-30} (H_0/55)^2$). The point labelled X_7 is the mass fraction of ${}^7\text{Li}$ corresponding to the abundance given by Boesgaard⁵, while that labelled X_D is the mass fraction of ${}^2\text{H}$ from the summary of ref. 2. (This latter value is smaller than that used by Gott *et al.*¹, mostly because they include an estimate of the effects of astration). The uncertainty indicated for X_7 is a factor of two in either direction while that for X_D covers the range from a factor of four smaller to a factor of two larger¹⁰. Corresponding values of ρ_b and their uncertainties are also shown. The value of ρ_b determined from the ${}^7\text{Li}$ abundance is only an upper limit if there are significant sources of ${}^7\text{Li}$ other than the big bang.

Quantitative analysis of the Dermott-Gold theory for Uranus's rings

DERMOTT and Gold¹ have attempted to explain the locations of Uranus's rings in terms of resonances between ring particles and pairs of satellites, such that each particle librates about consecutive conjunctions of the satellites. The libration argument is given by

$$K = \lambda_1 - (p+1)\lambda_2 + p\lambda_3$$

where $\lambda_i = n_i t + \text{constant}$, $i = 1, 2, 3$, are the mean longitudes of the ring particle and the satellites, and p is an integer. Despite the apparent success of the theory in predicting the main observed features of the rings, it is necessary to investigate the strength of the resonances in more detail before any conclusions can be drawn. This letter summarises the outcome of such an investigation which supplements the previous largely qualitative analysis¹.

Aided by a study by Wilkens² of possible three-body resonances involving one minor and two major planets, I derived an equation of motion analogous to that of a pendulum

$$\frac{d^2 K}{dt^2} = c \cdot \sin K$$

where c is found to be roughly proportional to $m_2 m_3 (a_2/a_3)^p$, that is the product of the masses of the two satellites times the ratio of their semi-major axes to the p -th power. In Table 1, the first column gives the mass products adopted by Dermott and Gold for various pairs of satellites. The second column contains the range of values for $p = (n_1 - n_2)/(n_2 - n_3)$ required to span the observed width of the ring system—roughly 45,000–52,000 km. The corresponding range of c -values and of libration periods ($P = 2\pi c^{-1/2}$) are given in the final two columns (the quoted periods are for zero-amplitude librations; the period increases slowly with the amplitude, being twice as large for 164° as for 0°). Since $c > 0$, librations can occur only about $K = 180^\circ$.

On the basis of the magnitudes of the product $m_2 m_3$ and the resulting ring pattern, Dermott and Gold concluded that the observed pattern is probably due primarily to the Ariel-Titania and Ariel-Oberon pairs. From the values of c , however, we now see that the correction factor $(a_2/a_3)^p$ makes Miranda play the key role rather than Ariel, in spite of the small mass of the former. Furthermore, the single combination Miranda-Ariel dominates over all the others in terms of efficiency and predicts ring radii for $p = 7, 8$ and 9 which are very close to the radii obtained by Marsden³ for the ϵ_2 , γ , and α rings, respectively. Therefore, when applied to this and other satellite pairs, the Dermott-Gold mechanism, as modified here, seems to have the capability of explaining the observed ring locations. It is not clear, though, how much statistical weight this argument should be given, one problem being that the model may predict too many rings—which, incidentally, provides hope for a future observational test. The relatively low p values of the pairs involving Miranda may also add some new credibility to the theory. It is interesting to speculate that even lower values for p —and higher ones for c —would be attainable for a hypothetical pair of satellites involving Miranda and an as yet undiscovered satellite inside it. After all, the 80,000-km wide gap between the rings and Miranda is not likely to be entirely empty.

On the other hand, the very long libration periods derived in Table 1, when compared with revolution periods of only about a third of a day for the ring particles, put very tight constraints on the semi-major axes of librating particles. A radial displacement of the order of $\Delta a_1 = a_1 \sqrt{c/n_1} \leq 50$ m is enough to disrupt the extremely weak particle-satellite-satellite coupling

—and there are several competing forces that might set up a disturbance of that magnitude. Elliot *et al.*⁴ have estimated ring widths of from 12 to 85 km and particle sizes < 6 km. The theory cannot account for these observations unless the particles are assumed to have slightly eccentric orbits. One important question not yet answered is whether the restoring force of the particle 'pendulum' is sufficiently strong to overcome the Poynting-Robertson effect for reasonable particle sizes.

Table 1 Ranges of p , c , and $P = 2\pi c^{-1/2}$

Satellites	$m_2 m_3 \times 10^{11}$	p	$c(1,000 \text{ yr})^{-2}$	$P(1,000 \text{ yr})$
Miranda, Ariel	19	7–9	53–18	0.87–1.5
Miranda, Umbriel	5	4–6	5.0–1.1	2.8–5.9
Miranda, Titania	32	3–5	6.9–0.69	2.4–7.5
Miranda, Oberon	25	3–4	1.8–0.32	4.7–11
Ariel, Umbriel	99	15–20	4.6–0.73	2.9–7.4
Ariel, Titania	580	8–11	2.1–0.15	4.4–16
Ariel, Oberon	447	7–10	0.32–0.01	11–62
Umbriel, Titania	170	20–26	$(88–3.7)10^{-4}$	67–320
Umbriel, Oberon	131	15–19	$(63–2.1)10^{-5}$	$(2.5–14)10^3$
Titania, Oberon	770	66–83	$(56–0.32)10^{-8}$	$(0.84–11)10^4$

In conclusion, although sceptical of the Dermott-Gold theory, despite its ingenuity and elegance, I believe that a decisive test of the theory has to await further observational details of Uranus's rings. In the meantime, it would seem advisable to look for alternative ring theories. For example, Colombo (personal communication) points out that it may be of significance that the period associated with the ϵ ring is in the approximate ratios 5:4, 6:5, 9:8, and 11:10 to those associated with the α , β , γ , and δ rings. One should not discount the possibility, either, that the rings may be transient features not locked into any kind of resonance at all.

This work was supported by NASA grant NGR 09-015-213.

KAARE AKSNES

Center for Astrophysics,
Harvard and Smithsonian Observatories,
Cambridge, Massachusetts 02138

Received 5 August; accepted 31 August 1977.

1. Dermott, S. F. & Gold, T. *Nature* **267**, 590–593 (1977).
2. Wilkens, A., & Sitzungsber., D., *Math-Natn. Abt. Bayer, D., Akad. Wiss., D., Zu Munchen*, **1**, 71–101 (1933).
3. Marsden, B. G. *IAU Circ. No. 3061* (1977).
4. Elliot, J. L., Dunham, E. & Mink, D. *Nature* **267**, 328–330 (1977).

Revenge of tiny Miranda

DERMOTT and Gold¹ have proposed a resonance model for the rings of Uranus. They assume the rings are, in fact, arcs composed of small particles librating about stable resonances determined by pairs of satellites, either Ariel and Titania or Ariel and Oberon. Dermott and Gold dismiss as insignificant resonances involving tiny Miranda. We report here that, by a wide margin, the strongest resonances are all associated with Miranda. Furthermore, we show that the hypothesis that the rings are made up of librating particles, while original and ingenious, is incorrect.

Before considering the quantitative analysis of the resonance model, we make two minor points. First, when allowance is made for the orbital motion (assumed prograde) of the ring material between occultations, it is found that the two occulting regions of each ring were physically only 35° (α ring) to 46° (ϵ ring) apart. Thus, only short arcs are required to fit the observations. Second, while large systematic errors may remain in the calculated absolute ring radii, the spacings between the rings are well determined and must be accurately predicted by a resonance theory. In Table 1, the spacings predicted by Dermott and Gold are compared with those deduced by Elliot *et al.*² and Marsden³. The discrepancies seem to be larger than observational error and exhibit no systematic trend.

We have calculated the strengths of the resonances between a ring particle in circular orbit and either a single satellite or a pair of

satellites. The resonance strength is expressed as the magnitude of the resonant term in the disturbing potential, R , felt by the ring particle. Figure 1 shows the strengths of the most important resonances in the radial range $42 \times 10^3 \text{ km} < a < 54 \times 10^3 \text{ km}$, which spans the ring radii. The only two-body resonances included in Fig. 1 are the 4:1 and 5:1 resonances with Miranda. Two-body resonances involving other satellites also lie in the range of the rings. The strengths, however, are too small for them to show in Fig. 1.

The strongest resonances are the two-body resonances with Miranda which occur where

$$4n_M - n - 3 \frac{d\tilde{\omega}_M}{dt} = 0$$

or

$$4n_M - n - \frac{d\tilde{\omega}_M}{dt} - \frac{d\Omega}{dt} - \frac{d\Omega_M}{dt} = 0$$

Here, n_M and n are the mean motions of Miranda and the ring particle, Ω_M and Ω are the longitudes of their ascending nodes, and $\tilde{\omega}_M$ is the longitude of Miranda's periape. The resonance strengths are given by Brouwer and Clemence⁵.

$$R_1^4 = \frac{GM m_M}{48a M} \frac{a}{a_M} e_M^3 \left[-256\alpha + \left(142 + 114\alpha \frac{d}{d\alpha} + 21\alpha^2 \frac{d^2}{d\alpha^2} + \alpha^3 \frac{d^3}{d\alpha^3} \right) b_{1/2}^{(1)}(\alpha) \right]$$

and

$$R_2^4 = \frac{GM m_M}{4a M} \frac{a}{a_M} e_M (\sin i/2)^2 \left[\left(8 + \alpha \frac{d}{d\alpha} \right) b_{3/2}^{(2)}(\alpha) \right]$$

where M is the mass of Uranus, m_M and e_M the mass and eccentricity of Miranda, i the mutual inclination of the ring and Miranda's orbit, and $\alpha \equiv a/a_M$.

The functions $b_s^{(i)}$ are Laplace coefficients.

There are three 5:1 resonances, corresponding to

$$5n_M - n - 4 \frac{d\tilde{\omega}_M}{dt} = 0$$

$$5n_M - n - 2 \frac{d\tilde{\omega}_M}{dt} - \frac{d\Omega}{dt} - \frac{d\Omega_M}{dt} = 0$$

and

$$5n_M - n - 2 \frac{d\Omega}{dt} - 2 \frac{d\Omega_M}{dt} = 0$$

Using formulae given by Peirce⁶, we obtain the resonance strengths:

$$R_1^5 = \frac{GM m_M}{384a M} \frac{a}{a_M} e_M^4 \left[-3125\alpha + \left(1569 + 1556\alpha \frac{d}{d\alpha} + 402\alpha^2 \frac{d^2}{d\alpha^2} + 36\alpha^3 \frac{d^3}{d\alpha^3} + \alpha^4 \frac{d^4}{d\alpha^4} \right) b_{1/2}^{(1)}(\alpha) \right]$$

$$R_2^5 = \frac{GM m_M}{16a M} \frac{a}{a_M} e_M^2 (\sin i/2)^2 \left[\left(85\alpha + 20\alpha^2 \frac{d}{d\alpha} + \alpha^3 \frac{d^2}{d\alpha^2} \right) b_{3/2}^{(2)}(\alpha) \right]$$

$$R_3^5 = \frac{3GM m_M}{8a M} \frac{a}{a_M} (\sin i/2)^4 \left[\alpha^2 b_{5/2}^{(3)}(\alpha) \right]$$

where a and α now refer to the position of the 5:1 resonance. All of these strengths depend sensitively on the uncertain values of e_M and i . Greenberg⁴ has determined that $e_M \approx 0.012$ and $i \approx 4^\circ$, though

he regards the latter as "extremely model dependent". Using these values, and noting, therefore, that only R_1^4 and R_1^5 are likely to be reasonably accurate, we obtain

$$R_1^4 \approx 6.6 \times 10^{-13} \frac{GM}{a}$$

$$R_2^4 \approx 1.1 \times 10^{-11} \frac{GM}{a}$$

$$R_1^5 \approx 9.1 \times 10^{-15} \frac{GM}{a} \quad (7)$$

$$R_2^5 \approx 3.0 \times 10^{-13} \frac{GM}{a}$$

and

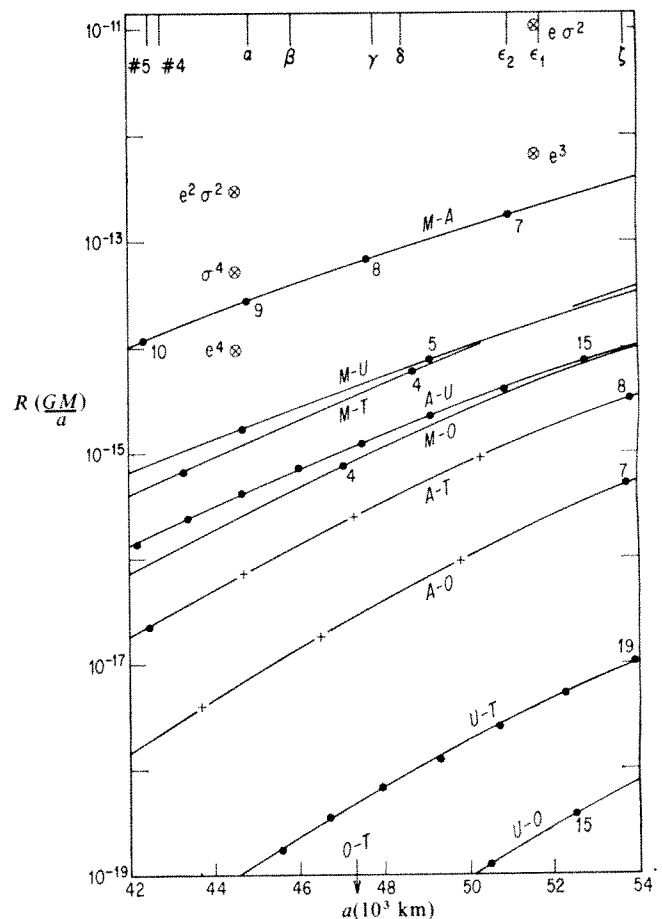
$$R_3^5 \approx 5.2 \times 10^{-14} \frac{GM}{a}$$

Next, we consider three-body resonances of the form

$$qn - (p+q)n_B + pn_A = 0 \quad (8)$$

where p and q are integers and n the mean motions with the subscripts A and B denoting the outer and inner satellites respectively. Both satellite orbits are assumed to be circles and to be outside the rings. The dominant resonant term in the disturbing potential arises as follows. Outer satellite A perturbs the orbit of inner satellite B, producing oscillatory variations in its radius and longitude with frequencies $p(n_A - n_B)$. The potential at the ring due

Fig. 1 The strengths of two- and three-body resonances in the neighbourhood of Uranus's rings. \otimes , The 4:1 and 5:1 resonances with Miranda discussed in the text and identified by $e = e_M$ and $\sigma = \sin(i/2)$; \bullet , the three-body resonances for $q=1$ and values of p as indicated. The resonances previously¹ associated with the rings are shown as +.



to B moving on its perturbed orbit contains terms with frequencies $qn - (p + q)n_B + pn_A$. These include the resonant term and many short period terms. Additional, but smaller (by a factor $\lesssim 0.1$), resonant terms arise from the attraction of A moving on an orbit perturbed by B. Finally, there are much smaller resonant terms due to the interaction between the direct perturbations of the ring by each satellite moving on its unperturbed circular orbit. The dominant resonant term is easily shown to be

$$\begin{aligned} R_{pq} = & \frac{GM}{2a} \frac{m_A m_B}{M^2} \frac{a_A^2}{a_B^3} \frac{n_A^2}{[p^2(n_B - n_A)^2 - n_B^2]} \times \\ & \times \left\{ h_q(\alpha) \left[f_p(\beta) - \frac{2n_B}{p(n_B - n_A)} g_p(\beta) \right] + g_q(\alpha) \times \right. \\ & \times \left. \left[\left(1 + \frac{3n_B^2}{p^2(n_B - n_A)^2} \right) g_p(\beta) - \frac{2n_B}{p(n_B - n_A)} f_p(\beta) \right] \right\} \quad (9) \end{aligned}$$

where a is orbital radius, m the satellite mass and $\alpha \equiv a/a_B$, $\beta \equiv a_B/a_A$. The functions f_k , g_k and h_k may be expressed in terms of Laplace coefficients (ref. 5) $b_k^{(j)}$ by

$$f_k(x) = x b_{3/2}^{(k)}(x) - \frac{1}{2} [b_{3/2}^{(k+1)}(x) + b_{3/2}^{(k-1)}(x)] + \delta_{k1} \quad (10)$$

$$g_k(x) = \frac{1}{2} [b_{3/2}^{(k-1)}(x) - b_{3/2}^{(k+1)}(x)] - \delta_{k1} \quad (11)$$

$$h_k(x) = \frac{1}{2} [b_{3/2}^{(k-1)}(x) + b_{3/2}^{(k+1)}(x)] - b_{3/2}^{(k)}(x)/x + 2\delta_{k1} \quad (12)$$

We have calculated the strengths of all $q = 1$ resonances due to each pair of satellites using power series approximations for the Laplace coefficients and satellite parameters from Greenberg⁴. The results are displayed in Fig. 1, with a smooth curve connecting the resonances due to each satellite pair. It is apparent that resonances involving tiny Miranda, the innermost satellite, dominate the field. The Ariel-Titania and Ariel-Oberon resonances advocated by Dermott and Gold¹ are relatively weak and presumably incapable of determining the ring locations.

Figure 1 shows that the strong Miranda-Ariel resonances lie close to four of the rings: Millis *et al.*'s ring 5 (ref. 7) and Elliot *et al.*'s rings α , γ and ϵ_2 , (ref. 2). Differences between Marsden's³

Table 1 Observed compared with resonant ring spacings

Ring pair	Spacing (km)*		
	Marsden ³	Elliot <i>et al.</i> ²	Resonance model ¹
α, β	949	926	1,026
γ, δ	680	672	800
ϵ_1, ϵ_2	632	~ 600	483
			Difference (km)
			+ 89
			+ 124
			~ - 130

*Calculated values are averages of occultation entry and exit results.

calculated positions for these rings and the resonances corresponding to $p = 10, 9, 8$ and 7 are 155, 96, 157 and 111 km, respectively, for an assumed $J_2 = 0.013$ for Uranus. As a consequence of the approximate Laplace relation satisfied by the mean motions of Miranda, Ariel and Umbriel⁸, several weaker Miranda-Umbriel and Ariel-Umbriel resonances also approximately coincide with the above rings. Furthermore, the 4:1 resonance with Miranda is located about 114 km inside Marsden's³ position for the ϵ_1 ring.

Despite the near coincidences between the locations of the strongest resonances and some of the rings, the rings cannot be made up of librating material. The maximum radial width of an arc of librating particles is

$$W = 8 \left(\frac{aR}{3GM} \right)^{1/2} a \quad (13)$$

From the resonance strengths, we obtain $W \approx 0.7$ km for the 4:1 resonance and $W \lesssim 0.07$ km for the strongest Miranda-Ariel resonances. By comparison, the widths of the observed rings range from 1–10 km for the inner rings and 30–100 km for the ϵ ring or

rings. Furthermore, libration occurs about a relative maximum of the potential energy in the frame rotating with the resonant mean motion. Thus, inelastic collisions among the particles would be destabilising and lead to the dissolution of a compact arc of librating material. We conclude that if the ring positions are determined by resonances, the control is more subtle than previously suggested. One possibility is that the rings are the crests of nonlinear density waves in an optically thin disk of particles. Calculations of the resonant excitation of density waves in Saturn's rings have shown that even weak resonances produce nonlinear waves⁹.

We thank G. Colombo for helpful discussions and acknowledge the support of NASA Grant NGL 05-002-003.

PETER GOLDREICH
PHILIP NICHOLSON

Division of Geological and Planetary Sciences,
California Institute of Technology,
Pasadena, California 91125

Received 1 August; accepted 31 August 1977.

1. Dermott, S. F. & Gold, T. *Nature* **267**, 590–593 (1977).
2. Elliot, J. L., Dunham, E. & Mink, D. *Nature* **267**, 328–330 (1977).
3. Marsden, B. G. *IAU Circ.* No. 3061 (1977).
4. Greenberg, R. *Icarus* **24**, 325–332 (1975).
5. Brouwer, D. & Clemence, C. M. *Methods of Celestial Mechanics* 490–494 (Academic, New York, 1961).
6. Peirce, B. *Astr. J.* **1**, 1–8, (1849).
7. Millis, R. L., Wasserman, L. H. & Birch, P. V. *Nature* **267**, 330–331 (1977).
8. Sinclair, A. T. *Mon. Not. R. astr. Soc.* **171**, 59–72 (1975).
9. Goldreich, P. & Tremaine, S. *Icarus* (submitted).

Identification of water frost on Callisto

THE 3.1- μ m absorption band of water frost has been tentatively identified in spectra of the Galilean satellites Ganymede and Callisto. This is the first positive evidence for water frost on the surface of Callisto. The depth of this absorption band on both satellites is about twice as deep as is inferred from the depths of the water frost bands at 1.5 and 2.0 μ m. Previous high resolution spectral studies ($\Delta\lambda/\lambda \sim 0.02$) of the Galilean satellites in the 1.0–2.5 μ m region have identified water frost on the surfaces of Europa (J II) and Ganymede (J III) from the presence of characteristic water frost absorption bands at 1.5 and 2.0 μ m^{1–3}. All studies failed to positively identify water frost on either Io (J I) or Callisto (J IV) but were used to set an upper limit of 5–25% (surficial coverage) for the amount of water frost on Callisto¹. The spectrum of water frost also has a characteristic band at about 3.1 μ m which is stronger than the ones at shorter wavelengths^{3–5}. Previous studies have shown a decreased reflectance between 3 and 4 μ m for Callisto, but water frost could not be positively identified because of low spectral resolution^{6,7} and poor signal-to-noise and the problem of using the Moon as a standard because of the onset of lunar thermal emission beyond 2.5 μ m (refs 1,2).

Broadband (J, K, and L) and narrowband (3.0–3.8 μ m) observations of Ganymede and Callisto were made on the nights of 7 November 1976 and 29 November 1976 (UT) with the Mount Lemmon 28-inch infrared telescope which

Table 1 Flux calibration

Wavelength (μ m)	$\Delta\lambda$ (μ m)	Zero magnitude flux density	
		(W cm ⁻² μ m ⁻¹)	(W m ⁻² Hz ⁻¹)
1.25 (J)	0.3	3.40×10^{-13}	1.77×10^{-23}
2.22 (K)	0.5	4.14×10^{-14}	6.80×10^{-24}
3.03	0.07	1.28×10^{-14}	3.92×10^{-24}
3.12	0.1	1.10×10^{-14}	3.57×10^{-24}
3.43	0.2	7.62×10^{-15}	2.99×10^{-24}
3.45 (L)	0.9	7.43×10^{-15}	2.58×10^{-24}
3.73	0.5	5.56×10^{-15}	2.46×10^{-24}

J and K values from Low and Rieke⁸. All others are intermediate values from the K and L values of Low and Rieke⁸.

employs a rocking secondary mirror to chop the beam 25 arc s on the sky. The infrared observational techniques and equipment are described elsewhere⁸. The filters used are listed in Table 1 along with the zero magnitude flux calibration. α Per and β Gem were the standard stars for these observations. The standard star calibrations derived from Johnson *et al.*⁹ are given in Table 2. The standard star observations were internally consistent to better than 0.05 mag and all satellite measurements were made within 0.05 air mass of a standard star measurement. 'Intermediate' air mass corrections were used⁸. Relative infrared reflectances were normalised to $R_\lambda(1.25 \mu\text{m}) = 1.0$ after subtracting the appropriate solar colour. The solar magnitudes used are given in Table 2 and are derived from Low and Rieke⁸ and Labs and Neckle¹⁰. The log of satellite observations is listed in Table 3.

The results of the observations are given in Table 4 and illustrated in Fig. 1. The spectra clearly show an absorption feature centred between 3.1 and 3.4 μm . The band depth in the Ganymede spectrum is consistent with at least as much surficial coverage of water frost as estimated by Pilcher, *et al.*¹ and Kieffer and Smythe³. The absorption band in the spectrum of Callisto is shallower than that of Ganymede, but is of the same general shape. This indicates the presence of an optically significant amount of frost on Callisto and is consistent with a surface exposure of water frost which is somewhat less than on Ganymede.

Table 2 Stellar calibration

Wavelength (μm)	Sun*	Magnitude α Per†	β Gem†
1.25	-27.81	0.87	-0.49
2.22	-28.23	0.56	-1.09
3.03	-28.28	0.51	-1.14
3.12	-28.29	0.51	-1.14
3.43	-28.30	0.49	-1.16
3.45	-28.30	0.49	-1.16
3.73	-28.32	0.47	-1.18

*1.25- μm (J) magnitude calculated from the filter bandpass and the solar spectrum of Labs and Neckel¹⁰. 2.22 μm (K) from Low and Rieke⁸. All others are interpolated values from K and L magnitudes of Low and Rieke⁸.

†1.25- μm (J) and 2.2- μm (K) magnitudes from Johnson, *et al.*⁹. All others are interpolated values from K and L magnitudes of Johnson, *et al.*⁹.

This absorption feature is deeper than might be expected on both satellites. Assuming that the rest of the surface is covered by a 'grey' component (for example, silicate), Pilcher *et al.*¹ used the observed visual albedo and the observed depths of the 1.5- and 2.0- μm water frost features (upper limits for the case of Callisto) to determine the most probable surface coverage of water frost for Ganymede and Callisto. The depth of the 3.1- μm band can be predicted from the relative depths of the 1.5-, 2.0- and 3.1- μm water frost features, the estimated surface coverage of water frost, and albedo of the frost and 'grey' material. The predicted depth of the band is only about half as deep as observed on both Ganymede and Callisto (see Fig. 2a). One possibility is that the non-frost component has a flat relative reflectance from the visual through 2.5 μm and a lower reflectance in the 3-4- μm region. This may be indicative of the presence of dark hydrated minerals (for example, carbonaceous chondritic material) whose spectra show water absorption in the 3-4- μm region, but whose water absorption features shorter than 3 μm are suppressed by the presence of dark material (W. Salisbury, personal communication and ref. 11). Alternatively, the relative inconsistency between the band depths at 1.5 and 2.0 μm compared with 3.1 μm may be due to the intimate mixing of a significant amount of the 'observed' water frost with the 'grey' material, thus suppressing the short wavelength

Table 3 Log of Data

Date (UT)* (November 1976)	Wavelength (μm)	Magnitude J III J IV
7.34	1.25	4.36
7.35	3.0	5.17
7.35	3.1	5.53
7.36	3.4	5.49
7.36	3.45	5.04
7.36	3.7	4.96
7.41	3.7	4.80
7.42	1.2	3.42
7.43	3.0	5.41
7.43	3.1	5.90
7.43	3.4	6.34
7.44	3.45	5.68
29.28	3.45	5.05
29.28	3.7	4.99
29.29	2.2	4.16
29.30	1.2	4.43
29.32	3.4	5.44
29.32	3.0	5.04
29.33	3.1	5.64

*Each point consists of 2-3 separate measurements at the same wavelength taken less than 10 min apart.

bands relative to the much stronger 3.1- μm band. Either of these would be consistent with the models for the formation and evolution of the Galilean satellites that predict relatively unaltered surface crust of water frost and silicates for Callisto¹².

Recent airborne telescopic observations of the Galilean satellites and laboratory studies of hydrated minerals bear out both of these possibilities. The telescopic observations covering the spectral region 1-5 μm ($\Delta\lambda/\lambda \sim 0.03$) show a very strong absorption feature centred at about 2.8 to 3.0

Fig. 1 Relative reflectance of the satellites J III (Ganymede) (●) and J IV (Callisto) (○) normalised to $R_\lambda(1.25 \mu\text{m}) = 1.0$. The broadband L point (3.45 μm) has been deleted for clarity.

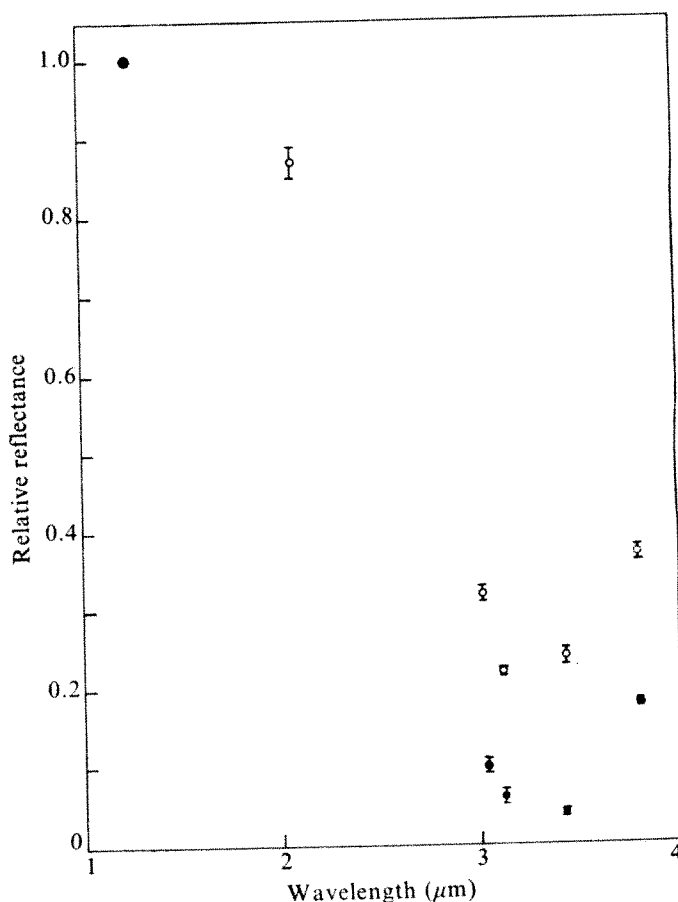


Table 4 Normalised reflectance

Wavelength (μm)	J III (6-7 November 1976)	J IV (6-7 November 1976)	J IV (28-29 November 1976)	J IV (Mean)
1.25	1.00	1.00	1.00	1.00
2.22			0.87 ± 0.02	0.87 ± 0.02
3.03	0.10 ± 0.01	0.31 ± 0.01	0.37 ± 0.02	0.32 ± 0.01
3.12	0.06 ± 0.01	0.22 ± 0.005	0.21 ± 0.01	0.22 ± 0.005
3.43	0.04 ± 0.005	0.22 ± 0.01	0.26 ± 0.01	0.24 ± 0.01
3.45	0.08 ± 0.005	0.34 ± 0.005	0.36 ± 0.01	0.34 ± 0.005
3.73	0.18 ± 0.005	0.37 ± 0.01	0.38 ± 0.01	0.37 ± 0.01

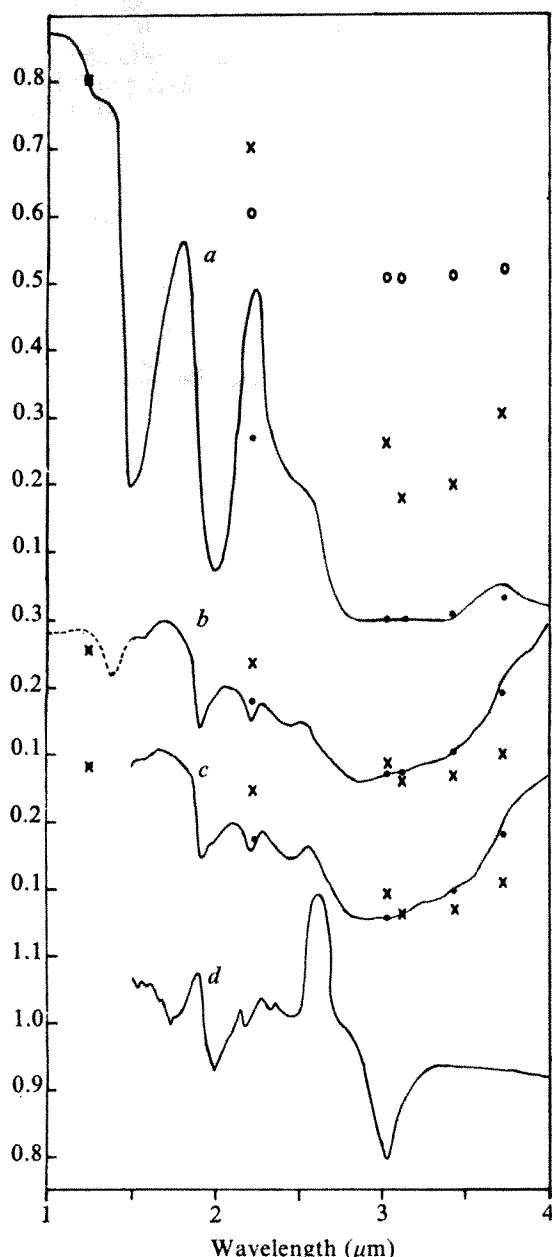


Fig. 2 Comparison of the relative spectral reflectance of Callisto to possible surface materials. *a*, Water frosts (absolute reflectance); *b*, montmorillonite, $(\text{Mg, Fe, Al})_4(\text{OH})_2(\text{Si, Al, Fe})_8\text{O}_{20} \cdot n \cdot 6\text{H}_2\text{O}$; *c*, Montmorillonite, cooled to about 150 K (reflectance, arbitrary units); *d*, Ratio *c/b*. For each curve, \times and \bullet represent the relative reflectances (for the filter band passes used) of Callisto and the materials in question, respectively. \circ represent the relative reflectances in the filters of a mixture of water ice and a 'grey' material as estimated by Pilcher, *et al*¹ for the surface composition of Callisto (10% water frost and 90% 'grey' material). *a* is from Smythe⁴. All other curves are from Lebofsky¹⁵. The extension of curve *b* down to 1.0 μm was taken from the results of Nash and Fanale¹⁶.

μm which has been tentatively identified as being due to water of hydration¹³. These data are consistent with the results presented here, but are not of sufficient signal-to-noise to make an absolute match.

Using X-ray diffraction techniques¹⁴ it has been shown that when hydrated minerals (bentonite-water pastes in these studies) are frozen, all but two or three monomolecular layers of the interlamellar water migrates into the pore space to form ice. Reflectance studies (1.5–4.0 μm , $\Delta\lambda/\lambda \sim 0.002$) of room temperature and frozen ($\sim 150\text{ K}$) montmorillonite and other minerals are being conducted¹⁵. Preliminary results are presented in Fig. 2, which shows that the water of hydration bands at 1.9 and 2.8 μm are decreased in strength and shifted to longer wavelengths due to the formation of water ice in the pore space. The absorption bands due to the water ice can be seen at 2.0 and 3.0–3.1 μm (Fig. 2*d*). It is clear from Fig. 2 that the hydrated minerals alone do not match the spectrum of Callisto because of the presence of bands at 1.4 and 1.9 μm and the differences in the 3–4- μm band shape, but it seems that this may be overcome by the addition of dark carbonaceous material (to suppress the 1.4- and 1.9- μm feature) and some water frost (to increase the band depth in the 3–4- μm region).

Based on the previous identification of water frost on the surface of Ganymede from shorter wavelength data, we infer the presence of water frost on the surface of Callisto from similar bands at 3–4 μm on both satellites. The depths of these bands may also indicate the presence of water of hydration, though how the frost and hydrated mineral may be mixed are as yet unknown.

I thank the University of Arizona, Lunar and Planetary Laboratory for telescope time and equipment, also Drs G. Rieke, F. Fanale, T. Johnson, D. Matson, and G. Veeder for helpful discussions. I was supported by a National Research Council Resident Research associateship during this research. This paper is JPL Planetology Publication 78-9 and was carried out under contract NAS 7-100, sponsored by the Planetary Geology Program Office, Office of Space Science, NASA.

LARRY A. LEBOFSKY*

Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena, California 91103

Received 13 June; accepted 30 August 1977.

*Present address: Lunar and Planetary Laboratory, University of Arizona, Tucson, Arizona 85721.

- Pilcher, C. B., Ridgeway, S. T. & McCord, T. B. *Science* **178**, 1087–1089 (1972).
- Fink, U., Dekkers, N. H. & Larson, H. P. *Astrophys. J.* **179**, L 155–159 (1973).
- Kieffer, H. H. & Smythe, W. D. *Icarus* **21**, 506–512 (1974).
- Smythe, W. D. *Icarus* **24**, 421–427 (1975).
- Irvine, W. M. & Pollack, J. B. *Icarus* **8**, 324–360 (1968).
- Gillett, F. C., Merrill, K. M. & Stein, W. A. *Astrophys. Lett.* **6**, 247–249 (1970).
- Hansen, O. L. *Icarus* **26**, 24–29 (1975).
- Low, F. J. & Rieke, G. H. *Meth. Exp. Phys.* **12A**, 415–462 (1974).
- Johnson, H. L., Mitchell, P. I., Iriarte, B. & Wisniewski, W. Z. *Commun. Lunar Planet. Lab.* **63**, 99–110 (1966).
- Labs, D. & Neckel, H. *Solar Phys.* **15**, 79–87 (1970).
- Johnson, T. V. & Fanale, F. P. *J. geophys. Res.* **78**, 8507–8518 (1973).
- Consolmagno, G. J. & Lewis, J. S. *Jupiter Studies of the Interior, Atmosphere, Magnetosphere and Satellites*, 1035–1051 (1976).
- Pollack, J. B., Witteborn, P., Erickson, E., Strecker, D., & Baldwin, H. presented at 8th Annual Planetary Geology Principal Investigator's Symposium, St. Louis, Missouri (1977).
- Anderson, D. M. & Hoekstra, P. *Am. Proc. Soil Soc.* **31**, 498–504 (1965).
- Lebofsky, L. A. presented at 8th A. Planet. Geol. Principal Investigator's Symp., St. Louis, Missouri (1977).
- Nash, D. B. & Fanale, F. P. *Icarus* **31**, 40–80 (1977).

Isotopic composition of uranium in chondritic meteorites

THE isotopic composition of uranium has been extensively measured for terrestrial U ores by precise gas mass spectrometry^{1,2}; measurements on other rock-types and minerals are limited³⁻⁵. Analyses of lunar material⁶⁻⁸ have shown the $^{238}\text{U}/^{235}\text{U}$ ratio to be the same, within experimental error, as the accepted value for terrestrial U (from pitchblende), namely 137.88 ± 0.14 (ref. 8). Measurements of the isotopic composition of U in five meteorites are reported here. The data show that variations in the U isotopic composition exist in these chondrites, and indicate the presence of components of low $^{238}\text{U}/^{235}\text{U}$ ratio. For meteorites there does not seem to have been any published attempt at a direct measurement of the $^{238}\text{U}/^{235}\text{U}$ ratio. In meteorite chronology it is generally assumed⁹⁻¹¹ that the U isotopic composition in meteorites is the same as that on Earth.

The samples used consisted of whole meteorite fragments free from fusion crust and broken from the interior of a given meteorite. A modified form of the method given by Arden and Gale¹² was used for the separation of U, which was carried out in clean-room conditions. The separated uranium was loaded, in water, on to an outgassed zone-refined V-shaped Re filament, previously completely covered on its top surface with a thin film of colloidal graphite and carefully evaporated

to dryness. Rigid control was maintained throughout on filament preparation, loading and running conditions. The mass spectrometer and ion-counting system used have been described¹².

The isotopic abundance of U, using NBS 950a standard, was measured in the range 0.2 to 20×10^{-9} g. The measured mean $^{238}\text{U}/^{235}\text{U}$ ratios ranged from 136.82 to 138.33 with a single-run internal precision of about $\pm 0.2\%$ ($1\sigma_m$). To check for any possible interference effects or suppression of emission, some runs were made after passing the U through the separation procedure and with an excess of tri-*n*-butyl phosphate. The variable mass fractionation between runs as measured by the standard deviation of replicate measurements was $\pm 0.37\%$.

The total range of U standard measurements about the mean value of 137.59 was $\pm 0.5\%$, which is similar to that obtained with the use of $^{233}\text{U}/^{235}\text{U}$ spike¹³ at the $0.1\text{-}\mu\text{g}$ U level. No measurable in-run fractionation, over the temperature range $1,600\text{--}1,700^\circ\text{C}$, was observed. The U loaded on to the filament was in a highly purified state and no residue was visible. On the standard and sample runs a 'clean' spectrum was obtained. The metal-oxide ion ratio was of the order of 1,000:1 and the ^{238}U count rates were in the range 10^3 to 0.7×10^6 p.p.s. Sample data, measured U isotopic compositions, whole meteorite concentrations and computed overall whole meteorite isotopic compositions are given in Table 1.

The results show that U in the meteorites analysed is a mixture of components of widely different $^{238}\text{U}/^{235}\text{U}$ ratios. One

Table 1 Measured uranium isotopic compositions and concentrations

Meteorite	Mass (g)	Decomposition	Acid*	Measured isotopic composition†		U‡ (10^{-9} g per g)	$^{238}\text{U}/^{235}\text{U}$ §
				$^{238}\text{U}/^{235}\text{U}$	<i>m</i>		
Parnallee LL3 (BM 34792)	1.62417	HF/HNO ₃	(a) HNO ₃	137.07 ± 0.26	46	10.60 ± 0.04	134.3
			(b) HCl	93.98 ± 0.34	27	0.468 ± 0.004	
			(c) HNO ₃	72.46 ± 0.85	17	0.020 ± 0.002	
Barwell L5 (BM 1966, 59)	1.58409	HF/HNO ₃	(a) HNO ₃	137.99 ± 0.36	27	12.38 ± 0.05	137.5
			(b) HCl	133.53 ± 1.04	17	0.702 ± 0.005	
			(c) HNO ₃	40.23 ± 0.73	6	0.010 ± 0.002	
Allende C3V (SI)	1	HF/HNO ₃	(a) HNO ₃	137.38 ± 0.30	32	15.01 ± 0.06	136.9
			(b) HCl	131.11 ± 0.37	20	0.410 ± 0.005	
			(c) HNO ₃	45.65 ± 1.13	22	0.020 ± 0.002	
	2	HF	(a) HCl	132.32 ± 0.34	32	5.86 ± 0.03	136.1
			(b) HNO ₃	139.11 ± 0.46	20	7.80 ± 0.03	
			(c) HCl	63.19 ± 0.19	20		
Bruderheim L6 (B.93 UA)	1.49691	HF/HNO ₃	(a) HNO ₃	137.14 ± 0.18	30	7.47 ± 0.03	136.6
			(b) HCl	122.26 ± 0.79	23	0.249 ± 0.003	
Richardton H5 (100 h ASU)	1	HF/HNO ₃	(a) HNO ₃	123.51 ± 0.21	31	9.37 ± 0.04	124.4
			(b) HCl	135.77 ± 0.36	22	0.779 ± 0.005	
	2	HF	(a) HCl	134.66 ± 0.38	16	4.27 ± 0.02	106.8
			(b) HNO ₃	92.07 ± 0.12	40	5.58 ± 0.02	
	3	HF/HNO ₃	(a) HNO ₃	136.71 ± 0.14	15	8.76 ± 0.04	133.1
			(b) HCl	103.72 ± 0.14	39	0.808 ± 0.006	
	4	HF/HNO ₃	(a) HNO ₃	136.50 ± 0.28	21	9.72 ± 0.05	136.5
			(b) HCl	136.58 ± 0.21	38	0.96 ± 0.01	
	5	HF/HNO ₃	(a) HNO ₃	134.98 ± 0.36	19	10.22 ± 0.06	134.6
			(b) HCl	132.51 ± 0.43	29	1.54 ± 0.02	
(BM 1920, 511)	6	HF/HNO ₃	(a) HCl	135.68 ± 0.22	34	10.08 ± 0.04	135.6
			(b) HNO ₃	132.10 ± 0.55	23	0.228 ± 0.004	

Sources of Meteorites: ASU, Arizona State University (Centre for Meteorite Studies); UA, University of Alberta; SI, Smithsonian Institution; BM, British Museum (Natural History).

* (a), Dried residue heated, in autoclave for 1–2 d at $\sim 180^\circ\text{C}$ with 7 ml of either 7M HNO₃ or 6M HCl. The solution was removed, a further similar volume of the acid added and the heating cycle repeated. The two extracts were combined. (b), As for (a), with next acid. (c), A very small residue often remained after (a) and (b). For some of the samples this residue was heated with 16M HNO₃ or 6M HCl at $\sim 180^\circ\text{C}$ for 4 d.

† Measured ratios were corrected for dead-time but not for blank. (Complete blank ~ 0.01 ng, separation blank ~ 0.004 ng.). *m*, No. of data cycles (each cycle consists of the mean of 5 measured ratios). σ_m , Standard deviation of means.

‡ Calculated using measured $^{238}\text{U}/^{235}\text{U}$ ratio, but not corrected for blank.

§ Overall $^{238}\text{U}/^{235}\text{U}$ ratio for the whole meteorite sample—computed from the total no. of atoms of the respective isotopes in each extraction.

component of terrestrial, or near terrestrial, isotopic composition is usually present at the normal abundance level (~ 10 parts in 10^9) and is readily dissolved (after HF attack) by HCl or HNO_3 . The others with lower measured $^{238}\text{U}/^{235}\text{U}$ ratios are present in low abundance in the whole meteorite, and, as indicated by the prolonged acid attack at elevated temperature and pressure required to release the components (see Table 1), seem to be contained in very resistant phases. The distribution of the latter components within the whole meteorite samples is probably very heterogeneous. Fission track studies in chondrites show that changes in U concentration by factors of 10^5 may be observed over distances of $\sim 10^{-2}$ cm (ref. 14).

The dissolution process, however, can influence the contribution made by each component to the U isotopic composition in each fraction. After decomposition, the dried complex fluoride residue is leached with a high concentration of acid at elevated temperature and pressure. This dynamic equilibrium process releases species occluded in the precipitate so that they remain in the solution phase, and has been shown to give quantitative solution of U ('major component') in HCl (ref. 12) and HNO_3 . If, however, one or more component phases are present which are attacked by HNO_3 or HCl but possibly only to a very limited extent (in these experimental conditions) by HF or HF/ HNO_3 during sample decomposition, then the rate and extent of dissolution of this phase depends on several factors. Among these are its physical state (dispersed surface coating or discrete mineral grains) actual time and length of exposure to the acid during the leaching process, as well as the activity of the acid in solutions of varying ionic strength. (The data obtained for the U standards, as well as the detailed analytical procedure used may be obtained from the author.)

Some possible mechanisms by which heterogeneities could arise are: (1) In the two-spike model, Wasserburg *et al.*¹⁵ predicted that the r -process nuclei occurred in three stages; a large amount of initial production early in the history of the galaxy, followed by a relatively quiescent period ($\sim 3 \times 10^9$ yr) terminated by a nucleosynthetic event that possibly initiated the separation of the solar system. Incomplete mixing of matter from the two events in the relatively short time interval between the predicted terminal spike at 4.8 to 4.6 Gyr and the formation of the solar system could occur: matter from the last nucleosynthetic event would have a low $^{238}\text{U}/^{235}\text{U}$ ratio. (2) The presence of grains in the solar nebula that had previous existence in the interstellar medium could, if incorporated into the meteorite parent body, result in a variable but low $^{238}\text{U}/^{235}\text{U}$ ratio¹⁶. (3) Grains with a low $^{238}\text{U}/^{235}\text{U}$ ratio could result from the explosion of a Type II supernova which could have triggered the collapse of a nearby interstellar cloud and led to the formation of the solar system¹⁷. (4) Chemical fractionation of Cm and U in some region of the early solar system. The nuclide ^{247}Cm decays to ^{235}U ; thus if a large $^{247}\text{Cm}/\text{U}$ fractionation occurred it would have produced enhancement in the abundance of ^{235}U , resulting in a low $^{238}\text{U}/^{235}\text{U}$ ratio¹⁸.

I thank M. C. B. Abranches for evaluation of the mass spectrometry of U. This study was supported by a grant from the NERC.

JOHN W. ARDEN

Department of Geology and Mineralogy,
University of Oxford,
Parks Road, Oxford, UK

Received 4 August; accepted 1 September 1977.

1. Cowan, G. A. & Adler, H. H. *Geochim. cosmochim. Acta* **40**, 1487 (1976).
2. Hamer, A. N. & Robbins, E. J. *Geochim. cosmochim. Acta* **19**, 143 (1960).
3. Doe, B. R. & Newell, M. F. *Am. Mineral* **50**, 613 (1965).
4. Rosholt, J. N., Harshman, E. N., Shields, W. R. & Garner, E. L. *Econ. Geol.* **59**, 570 (1964).
5. Rosholt, J. N., Garner, E. L. & Shields, W. R. *U.S. Geol. Survey Prof. Paper* 501-B, B84-B87 (1964).
6. Rosholt, J. N. & Tatsumoto, M. *Geochim. cosmochim. Acta Suppl.* **1**, 1499 (1970).
7. Fields, P. R., Diamond, H., Metta, D. N., Stevens, C. M. & Rokop, D. J. *Geochim. cosmochim. Acta Suppl.* **2**, 1571 (1971).
8. Barnes, I. L., *et al.* *Geochim. cosmochim. Acta Suppl.* **3**, 1465 (1972).
9. Tatsumoto, M., Unruh, D. M. & Desborough, G. A. *Geochim. cosmochim. Acta* **40**, 617 (1976).
10. Chen, J. H. & Tilton, G. R. *Geochim. cosmochim. Acta* **40**, 635 (1976).

11. Hutchison, R., Gale, N. H. & Arden, J. W. *Nature* **254**, 678 (1975).
12. Arden, J. W. & Gale, N. H. *Anal. Chem.* **46**, 687 (1974).
13. Ridley, R. G., Daly, N. R. & Dean, M. H. *Nucl. Instr. Meth.* **34**, 163 (1965).
14. Fleischer, R. L. *Geochim. cosmochim. Acta* **32**, 989 (1968).
15. Wasserburg, G. J., Schramm, D. N. & Hüneke, J. C. *Astrophys. J.* **157**, L91 (1969).
16. Clayton, D. D. *Earth planet. Sci. Lett.* **35**, 398 (1977).
17. Cameron, A. G. W. & Truran, J. W. *Icarus* **30**, 447 (1977).
18. Blake, J. B. & Schramm, D. N. *Nature phys., Sci.* **243**, 138 (1973).

Stacking faults in dolomite

DOLOMITE, an important rock-forming mineral, is a mixed carbonate possessing trigonal symmetry ($R\bar{3}$), with alternate layers of Mg and Ca atoms separated by planes containing the carbonate groups. Its ordered structure offers the possibility of extended planar faults of low energy and Barber¹ has shown evidence for stacking disorder in dolomite of high metamorphic grade. There is no previous evidence, however, for the creation of extended faults as a direct result of deformation processes. We have prepared single crystals, approximately 25 mm² in cross section and 10 mm long, and tested them in compression at temperatures up to 800 °C while subjected to a confining pressure of either 3 or 7 kbar. Specimens were mostly oriented to give a large resolved shear stress on the basal planes while minimising the stress for mechanical twinning on the $f = \{01\bar{1}2\}$ planes. (We use four-digit indices for planes and directions referred to the structural hexagonal unit cell, with $c = 16.01$ Å and $a = 4.81$ Å.) It has been shown (optically)² that slip on (0001) and twinning on $\{01\bar{1}2\}$ are common modes of deformation on dolomite. Using TEM methods, Barber¹ has shown that slip on $\{01\bar{1}2\}$ can also occur in dolomite. The current observations were also made by high voltage transmission electron microscopy, using sections which were thinned first mechanically and then by ion bombardment.

The extended planar faults are generated by slip on the basal planes and when viewed with the electron beam aligned parallel to a $\langle 2\bar{1}\bar{1}0 \rangle$ direction, they are visible edge-on as very fine lines of low contrast. They are thus easily distinguishable from long screw-type basal dislocations which are profuse in most samples. By tilting a foil of known orientation, ($2\bar{1}\bar{1}0$), through a measured angle about the $[01\bar{1}0]$ zone axis (the line of intersection of a fault with the foil plane) and knowing the foil thickness, the fault plane has been shown to be unequivocally (0001). Local specimen thickness was determined easily because of the presence of small cleavage cracks on $\{10\bar{1}4\}$.

Figure 1a shows the faults in minimum contrast, using a 03 $\bar{3}0$ reflection; the dislocations terminating the faults are clearly visible. The faults also have minimum contrast for reciprocal lattice vector $g = 11\bar{2}0$. In Fig. 1b, the beam is still nearly parallel to $[2\bar{1}\bar{1}0]$, but both $01\bar{1}5$ and $02\bar{2}10$ reflections are operating to give strong fault contrast. In Fig. 1c a tilt of approximately 20° is applied to show fringed fault contrast, using $+g = 1\bar{1}08$ and $-g$, while in Fig. 1d the fault is invisible ($g = 211\bar{1}8$) and the partial dislocations are in contrast. Although these observations were taken at 1,000 kV and dolomite is elastically anisotropic, the clarity of these contrast changes strongly suggests that we can calculate the displacement vector R in the usual manner, taking $g \cdot R = n$, where n must be zero or an integer for zero contrast with a given reciprocal lattice vector g . Using the above data, this gives as a likely value for R the vector $\frac{1}{3}[1\bar{1}00]$, which is not a proper translation vector of the dolomite structure. As might be anticipated, the vector lies in the basal plane and, for example, when applied to a magnesium atom, translates it at 30° to the normal $[1\bar{2}\bar{1}0]$ slip vector to a position directly above a calcium atom. This creates a stacking fault in the structure (not an antiphase boundary): the stacking fault is removed with a further displacement of $\frac{1}{3}[01\bar{1}0]$, giving

$$\frac{1}{3}[1\bar{1}00] + \frac{1}{3}[01\bar{1}0] = \frac{1}{3}[1\bar{2}\bar{1}0]$$

which is the normal slip vector of length 4.81 Å. This reaction, of course, identifies the observed partial dislocations. We have

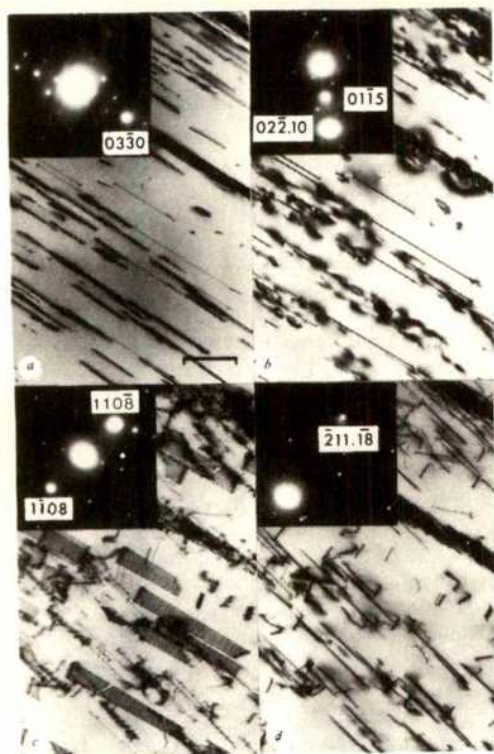


Fig. 1 Basal stacking faults in dolomite single crystal 4071 XD, compressed at 500 °C under a confining pressure of 3 kbar to a strain of 2.5% at a strain rate of 10^{-5} s^{-1} . The foil plane is (2110) and the faults are imaged at 1 MeV in various diffraction conditions: *a*, 0330 reflection; scale bar, 1 μm ; *b*, 0115 and 022.10 reflections; *c*, 1108 and 1108 reflections; *d*, 211.18 reflection.

found that such stacking faults occur extensively in dolomite crystals compressed at temperatures of 300–500 °C.

We have also observed that faint fringe contrast is sometimes created by the passage of dislocations on the {0112} slip planes. We have failed, however, to find a diffraction vector which brings the fringes into strong contrast and the fringes do not always terminate at dislocations but sometimes extend throughout the active part of the slip plane. It thus seems that the faint fringes, which indicate residual elastic strain, may be associated with a small fractional displacement vector, as occurs in orthopyroxene³.

Evidence for stacking faults generated by slip in carbonates is new and it further emphasises the differences in mechanical behaviour between dolomite and calcite⁴. Until the work of Turner and Orozco⁵ on metamorphic calcite there was no firm evidence for basal slip in calcite, although it is the favoured low-temperature mechanism in dolomite. Braillon and Serughetti⁶ have reported small (0001) planar faults in calcite but have attributed them to growth twinning. Our results, which demonstrate the existence of extensive stacking faults in dolomite, together with partial dislocations, are therefore novel and important.

The work was supported by SRC grant GR/A 14599. We thank E. Amundarein, Magnesitas-Navarra, for dolomite crystals.

Physics Department,
University of Essex,
Wivenhoe Park,
Colchester, Essex, UK

D. J. BARBER

Lawrence Livermore Laboratory,
University of California,
P.O. Box 808, Livermore,
California 94550

H. C. HEARD

M. S. PATERSON

Research School of Earth Sciences,
Australian National University,
Canberra, A.C.T.,
Australia

H. R. WENK

Department of Geology and Geophysics,
University of California,
Berkeley,
California 94720

Received 8 August; accepted 2 September 1977.

1. Barber, D. J. *Tectonophysics* 39, 193 (1977).
2. Higgs, D. V. & Handin, J. *Bull. Geol. Soc. Am.* 70, 245 (1959).
3. McLaren, A. C. & Etheridge, M. A. *Contrib. Mineral. Petrol.* 57, 163 (1976).
4. Barber, D. J. & Wenk, H. R. *Tectonophysics* (in the press).
5. Turner, F. J. & Orozco, M. *Contrib. Mineral. Petrol.* 57, 83 (1976).
6. Braillon, P. & Serughetti, J. *Phys. Stat. Sol.* 33, 405 (1976).

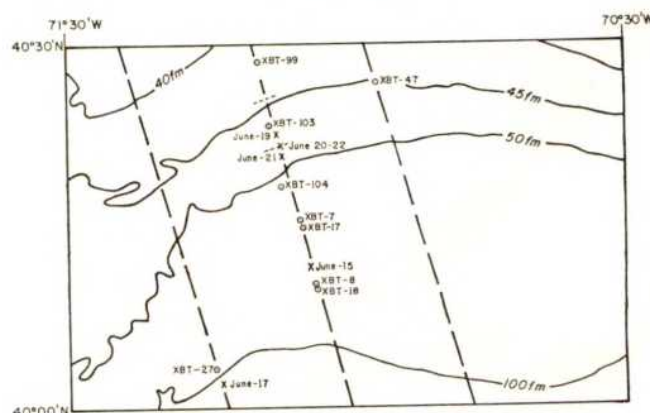
Acoustic imaging of the New England shelf-slope water mass interfaces

PULSED high frequency acoustics has been shown to have unique applications in the detection and measurement of oceanic phenomena such as internal waves¹⁻³ and dispersion of ocean-dumped sewage sludge^{4,5} or dredge material⁶. With moderate to high pulse repetition rates, a virtually continuous record of water column scattering strength is available to depths of the order of 500 m from a ship travelling at speeds of 4 or 5 knots. We used this feature to detect acoustically the interfaces associated with an intrusion of cold New England shelf water into the warmer slope water during a 9-d period in June 1976.

From 15 to 23 June 1976 measurements of effects of internal waves were made from the National Oceanic and Atmospheric Administration (NOAA) ship George B. Kelez in an area on the New England continental shelf partly shown in Fig. 1. Although measurements were made on three tracklines shown, most of the work concentrated on the centre trackline. A 1-kW 20-kHz pulsed acoustic system, specially modified for detection of weak signals scattered from the water column, was operated continuously throughout the cruise. Field operation of this system has been described previously^{4,7}. Profiles using expendable bathythermographs (XBT) and a conductivity-temperature-depth probe (CTD) were obtained at regular intervals.

Throughout the cruise, the acoustic record revealed a reflecting layer (Fig. 2) of limited horizontal extent and

Fig. 1 Region of New England continental shelf where water mass interfaces were observed. Contours are in fathoms, dashed lines are tracklines along which most work was performed. X with a date denotes an observation of the intersection of the scattering layer offshore boundary and the ship's trackline. The light dashed lines represent the width of the intrusion observed on 22 June.



rather variable location. Some observations of the offshore boundary of this layer are indicated in Fig. 1. The layer was observed in all cases (18 observations) to have a slope opposite to that of the bottom. As shown along the ship's trackline, the layer displayed apparent widths of 2.8–7.4 km. The last few records (for example, Fig. 2) of the scattering layer also show a second layer higher in the water column and parallel to the surface, but apparently descending to join the ascending lower layer at their offshore boundary. This made a sufficiently dramatic feature to finally get our attention. We then obtained XBT and CTD profiles through the feature when the cruise plan permitted.

A series of three XBT profiles (Fig. 3) across the layers revealed that they occur in a region of transition between two distinct water masses. XBT 99 shows the bottom shelf water to have a temperature of about 7 °C while XBT 104 shows the minimum temperature of the deeper slope water to be about 10 °C. XBT 103, taken in the region where the layers were present shows interleaving of 7 °C water with 10 °C water. The depths of the upper and lower steep

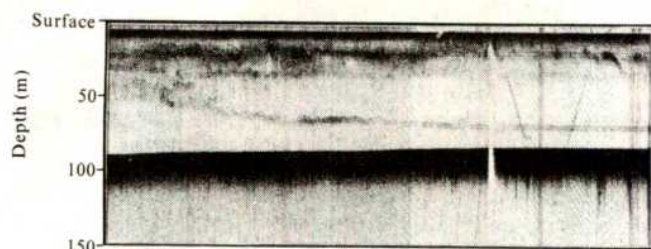


Fig. 2 A portion of the acoustic image showing the scattering layers associated with the intrusion interfaces (frequency 20 kHz). The left portion of the image, with darker background, represents a distance of 5.6 km. The portion to the right of the interrupted bottom signal was made with the ship on station for a CTD profile. The CTD echo signal is visible on the image.

temperature gradients in XBT 103 coincide with the depths of the acoustically reflecting layers measured at the moment the XBT 103 profile was taken. The gradient depths of XBT 103 (taken at 0729 GMT, 22 June) may be compared with the depths of the reflecting layers in Fig. 2, observed at 1325 GMT, 22 June. The slightly later acoustic image was chosen for its higher quality and clearer manifestation of the layers joining at the offshore boundary. In the right hand portion of Fig. 3 (the region of lighter background), the Kelez took station and a CTD profile was made and can be seen falling and then rising through the layers. The CTD temperature profile was similar to XBT 103 but showed very much steeper temperature gradients at the top and bottom of the 7 °C intrusion.

The horizontal transition from 7 to 10 °C water is a familiar feature. It is well known⁸ that by June the surface shelf water has warmed due to increased net solar radiation while the bottom shelf water remains cold, typically about 7 °C. The slope water is not cooled as much during winter mixing and has a minimum temperature of about 10 °C. During winter the shelf-slope water front is a well formed feature⁸, but spring surface warming over the shelf and slope removes the horizontal temperature contrast except in the lower water column. The reflecting layers manifested in the acoustic image are associated with nearly horizontal interfaces formed by interleaving of the shelf and slope water masses during spring and summer.

The location of the shelf-slope water interface on the centre trackline migrated about 18 km northwards during the nine-day span of observations. Reflecting layers observed on 15 and 16 June were confirmed to be shelf-slope water interfaces by XBT pairs (7, 8, and 17, 18 respectively) taken north and south of the observed layers. On 17 June a layer observation on the western trackline, confirmed by XBT 26 (not shown on map) and XBT 27, showed the shelf-slope

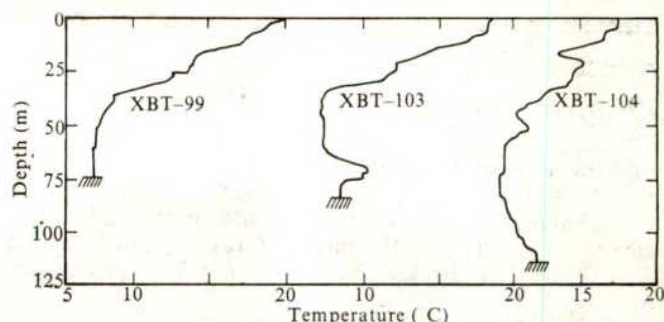


Fig. 3 Three XBT profiles (locations shown in Fig. 1) showing the changing make-up of the water column across the region in which acoustic scattering layers were identified as shelf-slope water mass interfaces. The temperature scale is actually three scales joined at the 20 °C points.

water interface to be further south. We observed no reflecting layer associated with the water-mass interface on the eastern trackline but found the transition took place between XBT 46 42° 32'N and XBT 47 on 18 June. It would seem, then, that the shelf-slope water transition zone was in a north east-south west direction in the vicinity of this experiment. Curiously, from 19 through 22 June the deep water boundary of the transition zone was roughly stationary as shown in Fig. 1. Thus the boundary moved 18 km along the centre trackline from 16 to 19 June and moved very little in the next four days.

The shelf-slope water transition zone apparently fluctuates significantly in location on a time scale of a few days. Also, Cresswell¹⁰ has observed distinct parcels of shelf water in the deeper slope water indicative of a mixing process. If the conditions for scattering at these water mass interfaces are often suitable for acoustic scattering, it may be possible to observe acoustically the shelf-slope water exchange process occurring in the summer on the New England continental shelf. XBT and CTD profiles would be needed only occasionally to confirm that the scattering layers are the interfaces of interest. Most of the ship time would then be spent covering the area of interest in a regular fashion with sufficient frequency to resolve water mass movements as they occur.

FRED C. NEWMAN
JOHN R. PRONI
DONALD J. WALTER

Sea-Air Interaction Laboratory,
Atlantic Oceanographic and Meteorological Laboratories,
Environmental Research Laboratories,
National Oceanic and Atmospheric Administration,
Miami, Florida, 33149

H. MICHAEL BYRNE

Pacific Marine Environmental Laboratory,
Environmental Research Laboratories,
National Oceanic and Atmospheric Administration,
Seattle, Washington, 98105

Received 26 July; accepted 14 September 1977.

1. Weston, D. E., Review, J., Jones, F. R. & Ranster, J. W. *Rep. ARL/L/R88* (Admiralty Res. Lab., Teddington, 1970).
2. Proni, J. R. & Apel, J. R. *J. geophys. Res.* **80**, 1147–1151 (1975).
3. Fischer, F. H. & Squier, E. D. *J. acoust. Soc. Am.* **58**, 1315–1317 (1975).
4. Proni, J. R., Newman, F. C., Sellers, R. L. & Parker, C. *Science* **193**, 1005–1007 (1976).
5. Proni, J. R. *et al. J. geophys. Res.* (submitted).
6. Proni, J. R., Rona, D. C., Lauter, C. A. Jr & Sellers, R. L. *Nature* **254**, 413–415 (1975).
7. Proni, J. R. *et al. Deep Sea Res.* **23**, 831–837 (1976).
8. Beardsley, R. C. & Flagg, C. N. *Mem. Soc. R. Sci. Liège* **6**, 209–225 (1976).
9. Wright, W. R. *J. mar. Res.* **34**, 1–14 (1976).
10. Cresswell, G. M. *Rep.* 67–35 (Woods Hole Oceanogr. Inst., Massachusetts, 1967).

Effect of climate on chemical composition of fossil bones

THE suggestion that microanalysis of nitrogen, fluorine and uranium in fossil bones should be used for age determination¹⁻⁴ assumes that the concentration of these elements varies uniformly in time. Vonach⁵ has plotted calibration curves for the N and F content of fossil bones from the past 10⁴ yr. The nitrogen content, N , can be approximated by

$$\log N = -0.135 \log t + 0.681$$

where N is weight per cent and t is age in years. The chemical changes that occur in buried bones depend on the composition of the surrounding minerals and the environmental conditions⁶. The concentration of a given element in bone is also influenced by biochemical differences between samples. Consequently, large fluctuations in N can be expected around the gross trend described by Vonach. We report here a fluctuation of the N content of bones that seems to correlate with climatic change.

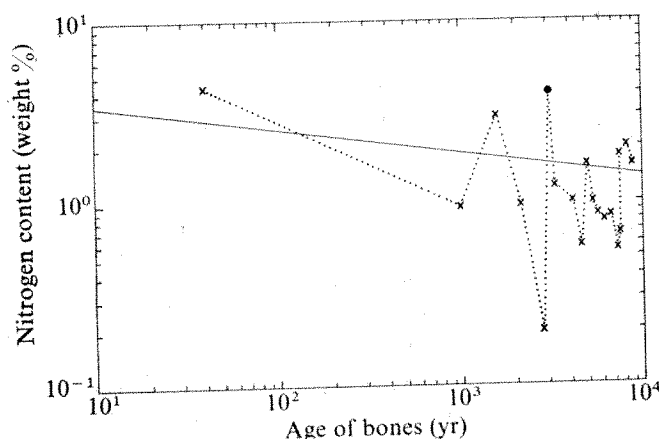


Fig. 1 N content of bones as a function of absolute age. . . . x . . . , Our results; ●, at 3,100 yr and —, from ref. 5.

We investigated the concentration of N, Fe, Al and Ca in bones from the past 9,000 yr. We chose this time interval because of the relatively large number of dated samples found and the significant climatic change during the interglacial phase. We used human vertebrae of known ages buried in the Great Hungarian Plain.

The N, Fe and Al content was determined by fast and thermal neutron activation analyses using the $^{14}\text{N}(n,2n)^{13}\text{N}$, $^{56}\text{Fe}(n,p)^{56}\text{Mn}$ and $^{27}\text{Al}(n,\gamma)^{28}\text{Al}$ reactions, respectively. The

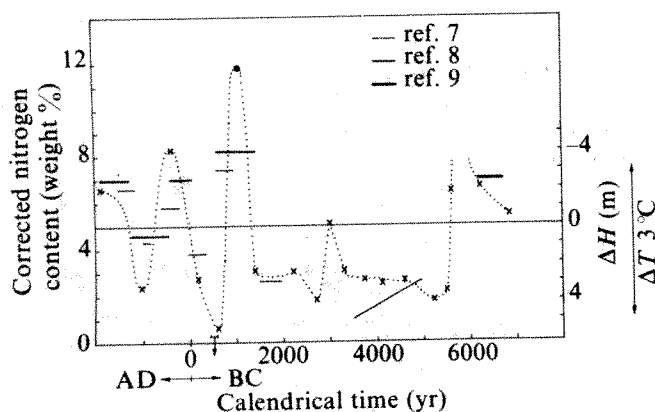


Fig. 2 Corrected N content of bones (. . . x . . .) and the sea level difference (straight lines) as a function of calendrical time.

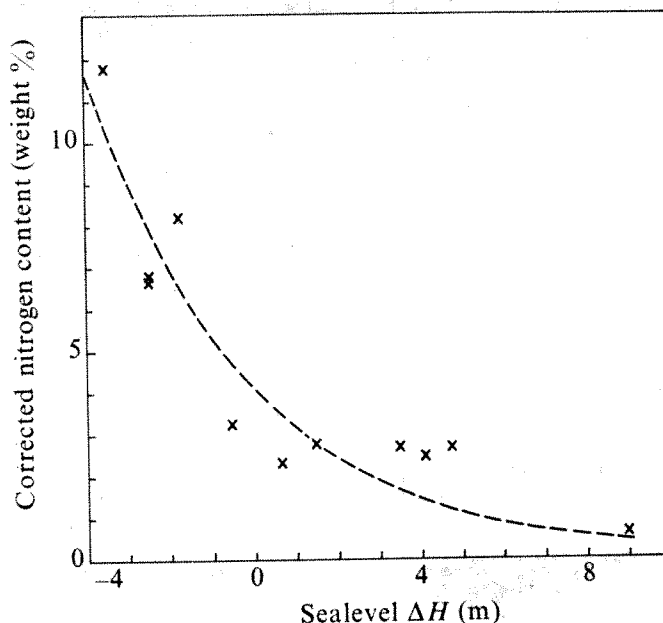


Fig. 3 Corrected N content of bones as a function of sea-level difference. $N = 4.1405 \exp(-0.26\Delta H)$.

concentration of Ca and Fe was measured by X-ray fluorescence using an Si(Li) detector and ^{55}Fe and ^{125}I exciting sources. Figure 1 shows N content as a function of the age of the bone. There is a fine structure (dotted curve) superimposed on the Vonach's gross trend (continuous curve).

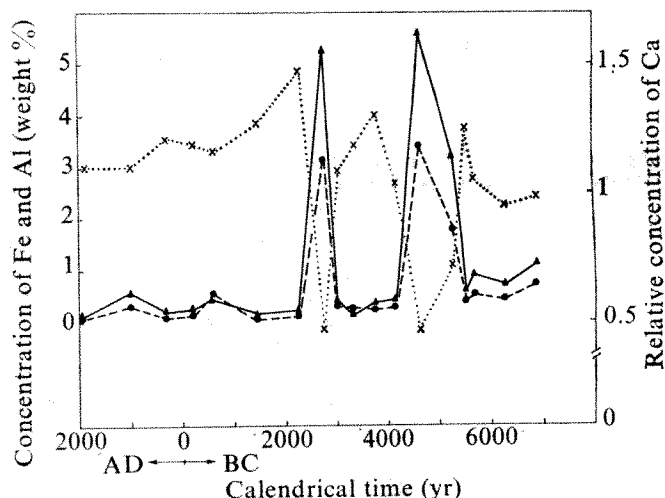


Fig. 4 Concentration of Al(▲), Fe(●) and Ca(×) in bones as a function of calendrical time.

The fluctuations in the data cannot be explained by biochemical differences between individuals and the uncertainty in the determination of N, for which the error does not exceed $\pm 10\%$. Results of *in vivo* neutron activation analysis of patients have shown that deviations in N content are less than $\pm 30\%$ even for different diseases^{12,13}.

There seems to be a periodicity of about 2,000 yr between the minima of the fluctuations. Accepting the gross trend for N content, we normalised the measured data to the date of burial. It should be noted, however, that the corrected N contents in some cases are abnormally larger than those obtained for modern bones. These normalised values and the sea-level differences, ΔH , from the present-day value⁷⁻⁹ are given in Fig. 2 as a function of calendrical time. An extremely high value of sea level (9 m) was given by Brooks¹¹ for 500 BC. Figure 2 shows a correlation between N content and sea level. The correlation can be approximated

by the following relationship: $N = 4.1405 \exp(-0.26\Delta H)$ (Fig. 3). Brooks has pointed out a linear correlation between climate and sea level. On the basis of the data for sea level⁷ and temperature⁹ for the interval from 1,000 BC to 5,000 BC, a value of $\Delta H/\Delta T \sim 2.6$ m per °C can be estimated. It can be concluded that the minima in N content indicated in Fig. 1 coincide fairly well with the climatic maxima. It seems to be a correlation between the 'neo-glacial cycle'¹⁰ and the N content of bones. The deviations from the predicted solid line in Fig. 1 can be attributed to the change in the climate of the age from which the bones originate.

The measured values for Al, Ca and Fe content of human bones as a function of calendrical time are given in Fig. 4. Both the trends and the absolute values for iron measured by X-ray fluorescence and neutron activation are in good agreement within $\pm 5\%$, proving the reliability of the methods applied. Figure 4 shows changes in the concentrations of all the elements investigated.

An opposite trend is shown for Fe and Al on the one hand, and Ca and N on the other hand. Chemical separation showed that the Fe and Al contents belong to the organic part of the bone.

We thank Professor J. Nemeskéry for discussions and for bone samples.

CS. M. BUCZKO

L. VAS

Isotope Laboratory

Institute of Experimental Physics,
Kossuth University,
Debrecen, Hungary

Received 28 March; accepted 11 August 1977.

1. Middleton, J. *Proc. Geol. Soc. Lond.* **4**, 431–433 (1844).
2. Oakley, K. P. in *Anthropology Today* (Kroeber, Chicago, 1953).
3. Oakley, K. P. in *The Scientist and Archaeology*, (ed. Pyddoke, E.) (Oxford University Press, London, 1963).
4. Oakley, K. P. in *Science in Archaeology* (eds Brothwell, D. R. & Higgs, E. S.) (Praeger, London, 1963).
5. Vonach, V. *Atomki Közlemények* **18**, 247–258 (1976).
6. Protsch, R., *Ninth Congress Union Internationale des Sciences Préhistoriques et Protohistoriques*, Nice, 180–211 (1976).
7. Fairbridge, R. W. *Physics and Chemistry of the Earth* **4**, 99–185 (1961).
8. Komlódi, J. M. *Természet Világa* **12**, 535–540 (1971).
9. Ambrózy, P., Czelnai, R. & Götz, G. *Fizikai Szemle* **27**, 54–62 (1977).
10. *IAEA Bulletin* **19**, 32–35 (1977).
11. Brooks, C. E. P. *Climate Through the Ages* (London, 1950).
12. Cohn, S. H., Dombrowski, C. S., Hauser, W., Klopfer, I. & Atkins, H. L. J. *clin. Endocrinology Metabolism* **33**, 719–728 (1971).
13. Cohn, S. H., Cinque, T. J., Dombrowski, C. S. & Letteri, J. M. J. *Lab. clin. Med.* **79**, 978–994 (1972).

Physiological exclusion of magnesium from *Mytilus edulis* calcite

CALCITE produced by many marine organisms (including molluscs¹, brachiopods², coccolithophorids³, and planktonic foraminifera^{4,5}) contains only about 5% as much magnesium as is predicted by laboratory studies of distribution coefficients^{6,7} (ratio of the Mg : Ca ratio in the solid against that in the solution). Understanding the cause of this low magnesium content is important to our comprehension of the calcification process. It is also of considerable interest to the field of low temperature geochemistry for several reasons. First, calcite is the major authigenic component of marine sediments, and its magnesium content is an important feature of its bulk composition. Second, the controls on the magnesium content of invertebrate skeletal must be understood if Mg : Ca ratios of calcitic fossils are to be used as indicators of sea salt palaeochemistry⁸ or sea surface palaeotemperature⁹. Third, the mechanism of magnesium inclusion may determine the role of magnesium in regulating selective dissolution of the tests from planktonic foraminifera⁴. Fourth, since calcite solubility depends on the magnesium content of the calcite, the mode of magnesium inclusion is a factor to be considered in calculating the degree of carbonate saturation in the

oceans. Finally, factors governing the mode of magnesium incorporation into skeletal calcite are important to sedimentary petrologists using magnesium as a diagenetic indicator. We report here that the mussel *Mytilus edulis*, which normally secretes low magnesium calcite, incorporates anomalously high amounts of magnesium into its calcitic shell layer when it is grown in solutions of higher than normal magnesium content. Thus in normal conditions *Mytilus* physiologically excludes magnesium from its shell-forming fluid, and at higher magnesium concentrations ion regulatory systems breakdown, causing a substantial increase in the amount of magnesium coprecipitated into *Mytilus* shell calcite.

Young *M. edulis* (7–15 mm long) were collected from Narragansett Bay, Rhode Island and cultured for 8 weeks in aerated 4.5-l glass aquaria. The water was changed at weekly intervals and the mussels were fed a suspension of *Isochrysis*. Twenty mussels were placed in each tank. The culture solutions were prepared by mixing one part natural seawater with one part synthetic 'seawater'. A range of Mg : Ca ratios in the culture solutions was obtained by changing the proportions of sodium and magnesium in the synthetic 'seawater' solution. A constant total ionic strength (0.64 ± 0.04 M) could then be maintained between different composition solutions while also maintaining a constant calcium concentration similar to that of seawater (9.3 mM) in all the solutions except the lowest Mg : Ca ratio solution. To make the lowest Mg : Ca ratio solution (Mg : Ca = 0.75) a calcium concentration of 33 mM was used in the culture solution.

M. edulis forms both calcitic and aragonitic shell layers. Calcite samples of shell grown during this experiment were collected from the newly grown calcitic-shell edges outside the pallial line. These samples were analysed by atomic absorption spectrophotometry for magnesium and calcium^{5,9}. Ratios of the molar concentrations in both the solution and shell are reported since the ratios more clearly show the relative fractionation effects between the two ions. The growth of each mussel was recorded by measuring its length at the beginning and end of the experiment.

The Mg : Ca atom ratios of the newly grown shell are compared with those of the culture solutions in Fig. 1. Normal seawater has a Mg : Ca ratio of 5.2. Mussels grown in artificial media with the same Mg : Ca ratio as natural seawater produce the same shell Mg : Ca ratio as a control set of mussels grown in 100% natural seawater. Mussels grown in solutions with a Mg : Ca ratio less than 7 show a gradual increase in the shell Mg : Ca ratio with increases in the solution Mg : Ca ratio, except for the lowest solution ratio sample which has a slightly elevated shell ratio. As the Mg : Ca ratio of the solution increases above 7 there is a dramatic increase in the amount of magnesium incorporated into the shell.

Inorganic distribution coefficient data cannot account for the Mg : Ca ratios of the shells. Distribution coefficient data determined by Katz⁷ for low Mg : Ca ratio solutions predict that calcite precipitated from a solution with a Mg : Ca = 0.75 (the lowest ratio tested) will have a calcite Mg : Ca ratio of 40×10^{-3} ; this is 20 times more magnesium than we find in *Mytilus* shells grown in these conditions. Studies of the inorganic precipitation of calcite from seawater reveal Mg calcites containing >10 mol per cent MgCO_3 (ref. 6), although Berner⁶ suggests that 2–7 mol per cent MgCO_3 is the most stable composition in the presence of seawater. Distribution coefficient data are not available for solution Mg : Ca ratios of >5. But, surface-solution equilibrium exchange data show that near a Mg : Ca solution ratio of five surface distribution coefficients decrease¹⁰; if this is any guide, we would also expect bulk distribution coefficients to decrease. The increase in mag-

nesium observed in our experiments is directly opposite to this expectation and suggests that inorganic processes are not responsible for regulating the magnesium content of *Mytilus* calcite.

Growth rate has been suggested as a factor controlling the inorganic composition of calcified tissues¹¹. A range of growth rates was observed for the mussels cultured in each Mg:Ca ratio solution. Mussels in the two lowest Mg:Ca ratio solutions grew slowly and only a few grew sufficiently to allow sampling (≥ 1 mm new growth). Mussels in the other solutions examined grew at about the same rate (from 1 to 4 mm in length in 8 weeks). Mussels placed in a solution with a Mg:Ca ratio of 13.5 did not grow. There was no correlation between growth rate and the shell Mg:Ca ratio of mussels grown in the same solution. High Mg:Ca ratios did not significantly influence growth rate except in the one case noted above. In this experiment growth rate was not a factor influencing the magnesium composition of *Mytilus* shell calcite.

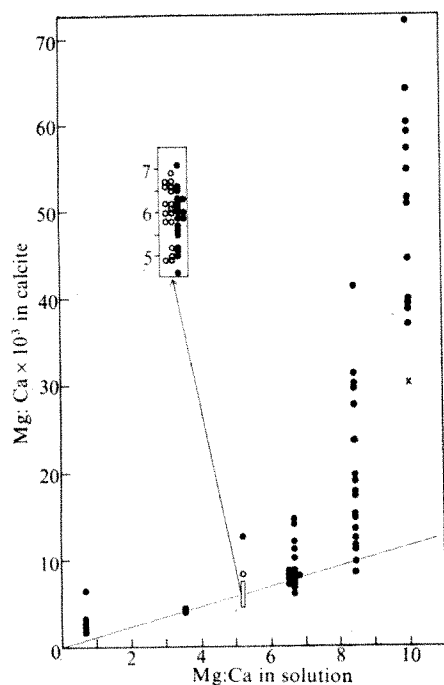


Fig. 1 The calcite shell Mg:Ca atom ratio for each mussel plotted against the Mg:Ca atom ratio of the solution in which it was grown. ○, Samples of mussels grown in natural seawater; ●, mussels grown in synthetic medium. × Represents the metal ratio determined from a composite sample by X-ray diffraction.

Anomalous increases in the magnesium content of these calcite shells could occur if another mineral phase such as brucite ($\text{Mg}(\text{OH})_2$) or hydromagnesite ($3\text{MgCO}_3 \cdot \text{Mg}(\text{OH})_2 \cdot 3\text{H}_2\text{O}$) were precipitated; we carried out X-ray diffraction studies to check this possibility. Mussels grown in 100% natural seawater were almost pure calcite ($d_{211(104)} = 3.035 \text{ \AA}$), whereas a sample taken from mussels grown in the highest Mg:Ca ratio solution had a d spacing of 3.027 \AA . This change in d spacing corresponds to a 2.5 mol per cent increase in MgCO_3 , as calculated from data showing the dependence of d spacing on calcite magnesium content¹². From this increase a Mg:Ca ratio of 31×10^{-3} is calculated for the high magnesium calcite sample. While this ratio falls a little short of the Mg:Ca ratio determined by atomic absorption, it is still several times greater than the ratio that would be obtained by a linear extrapolation from the origin through the normal

seawater ratio (see Fig. 1). The only other peak observed in the high magnesium sample had a d spacing of 17.67 \AA . This d spacing does not correspond to any magnesium containing mineral listed in the tables of the American Society for Testing Materials. High d spacings are often associated with organic material; whether or not this peak represents an elevated shell organic matrix content and whether or not magnesium is associated with it, is conjecture at this point. The X-ray data are in agreement with the atomic absorption data and indicate that an anomalous increase in the coprecipitation of magnesium into *Mytilus* calcite occurs when mussels are grown in solutions containing a higher than normal magnesium content.

Magnesium in high concentrations inhibits the nucleation¹³ and growth⁶ of calcite. Simkiss¹⁴ proposes that the mantle of some molluscs may regulate the flow of material to the crystallisation site so as to exclude crystal growth inhibitors such as magnesium and inorganic phosphate ions. We suggest that *Mytilus* does in fact have the capacity to extensively regulate the magnesium ion activity of the solution from which its shell calcite is grown. *Mytilus* may attempt to maintain an optimum magnesium and calcium composition in this fluid. In normal seawater conditions *Mytilus* produces a low magnesium calcite as a result of this ion fractionation process. At very low Mg:Ca solution ratios the ion fractionation process may discriminate less against magnesium so that the normal, or close to the normal, solution composition is maintained. This is suggested by the slightly elevated shell Mg:Ca ratio at the lowest solution ratio. When *Mytilus* is exposed to Mg:Ca solution ratios much above those of normal seawater, its ion regulatory capability apparently becomes overloaded and breaks down, thus allowing an influx of magnesium ions into the fluid from which new shell is precipitated. The result, as shown in Fig. 1, is a dramatic increase in the amount of magnesium found in *Mytilus* shell calcite.

While data are not available on species other than *Mytilus*, it is probable that other marine organisms which produce low-Mg calcite, such as planktonic foraminifera and coccolithophorids, do so by a physiological fractionation process rather than as a result of any inorganic processes.

We thank Pierre E. Biscaye for performing X-ray diffraction work. We also thank the staff of the Rhode Island Nuclear Science Center for their assistance, A. N. Sastry for animal culture facilities, and M. E. Q. Pilson for reviewing the manuscript. N.S.F. provided financial support (DES37125).

ROBERT B. LORENS
MICHAEL L. BENDER

Graduate School of Oceanography,
University of Rhode Island
Kingston, Rhode Island 02881

Received 18 November 1976; accepted 1 September 1977.

1. Milliman, J. D. *Marine Carbonates* (Springer, New York, 1974).
2. Lowenstam, H. A. *J. Geol.* **69**, 241–260 (1961).
3. Thompson, G. & Bowen, V. T. *J. mar. Res.* **27**, 32–38 (1968).
4. Savin, S. & Douglass, R. G. *Geol. Soc. Am. Bull.* **84**, 2327–2342 (1973).
5. Bender, M. L., Lorens, R. B. & Williams, D. F. *Micropaleontology* **21**, 448–459 (1975).
6. Berner, R. A. *Geochim. cosmochim. Acta* **39**, 489–504 (1975).
7. Katz, A. *Geochim. cosmochim. Acta* **37**, 1563–1586 (1973).
8. Kilbourne, R. T. & Sen Gupta, B. K. *S. East. Reg. G. S. A. abstr. programs* 408–409 (1973).
9. Lorens, R. B., Bender, M. L. & Williams, D. F., *J. Sediment Pet.* (in the press).
10. Moller, P. & Parekh, P. P. *Mar. Chem.* **3**, 63–77 (1975).
11. Zolotarev, V. N. *Geochem. Int.* **11**, 347–353 (1975).
12. Goldsmith, J. R. & Graf, D. L. *Am. Mineral.* **43**, 84–101 (1958).
13. Pytkowicz, R. M. *J. Geol.* **73**, 196–199 (1965).
14. Simkiss, K. *Comp. Biochem. Physiol.* **16**, 427–435 (1965).

Polonium and plutonium in an intertidal food chain

LARGE quantities of seaweeds (kelp) are washed ashore along the coasts of California. Estimates range from 10^4 to 10^6 tonnes (10^7 – 10^9 kg) wet weight per yr. These algal masses provide food for insects and other invertebrates on the beaches. The plants carry with them a variety of di-, tri-, and polyvalent cations of heavy metals, largely adsorbed or chelated to superficial acidic polysaccharides and surface slimes of bacterial origin. Among the heavy metals one can detect small amounts of radioactive elements, including some that emit high-energy α particles such as the natural polonium (^{210}Po , half life 138 d) and the artificial plutonium nuclides ($^{239,240}\text{Pu}$, half lives 24,360 yr and 6,580 yr respectively)¹. In surface waters of the oceans, polonium is born from the decay of ^{210}Pb which is continuously replenished by decay of radon released from the Earth's crust. The plutonium isotopes occur in ocean waters as a consequence of fallout from atomic test explosions some 15–20 yr ago. We have examined the extent to which these elements may be transferred from the ocean to the terrestrial biosphere by means of kelp, kelp flies and their predators. Fears have been expressed that radioactivity from the algae could be reaching man through the terrestrial food chain. Our investigations suggest that, on the basis of current radiation thresholds for human health and safety, such fears are groundless.

Samples of cast weed, largely comprising blades and stipes of *Macrocystis pyrifera* (L.) C.A. Ag. but containing also a few per cent of *Egria laevigata* Setch. and *Pelagophycus porra* (Leman) Setch. and marine grasses (*Phyllospadix* and *Zostera*), were collected from the beach at La Jolla, California, in June and August 1975, at a time when kelp flies were much in evidence. Adult flies (mostly *Fucilia rufitibia* Stein and *F. separata* Stein) were caught over clumps of decaying weed by the use of a 1-mm mesh nylon net. About 30,000 flies, weighing 100 g wet weight (27 g dry weight) were collected in about 30 min. A few of the associated predatory tiger beetles (*Cicindela* sp.) were also netted, as individuals, on nearby sand. For comparative purposes we also obtained some 50 g (dry weight) of common houseflies (*Musca domestica* L.) which had been raised in a laboratory (Department of Entomology, University of California, Riverside) on a commercial fly-larva medium (CSMA, Ralston-Purina Co.).

All samples were dried in an oven at 80 °C overnight. Polonium and plutonium were determined in subsamples of 1.0 g and 20.0 g respectively. The flies and beetles were 'wet-ashed' by heating in a mixture of nitric and perchloric acids. As markers, ^{208}Po and ^{242}Pu were added to monitor the chemical recoveries of ^{210}Po and $^{239,240}\text{Pu}$ from the samples. The α activities of these elements were measured with silicon surface-barrier detectors in combination with pulse-height analysers.

As shown in Table 1, the contents of polonium in laboratory-raised houseflies, and in the diet on which they were raised as larvae, were negligible (10 and 30 pCi per kg dry weight) compared with the kelp flies (250 pCi per kg dry weight). The polonium content of the kelp flies from the beach was found to be about half of that of the cast weed, on the basis of comparable dry weights. (That of laboratory-reared *Musca* was about one third that of their larval food.) This proportional reduction may be attributable to mechanical selection by the fly larvae of the softer, interior parts of the seaweeds, which have been shown to contain considerably less of the adsorbed heavy metals (including polonium) than the surface layers². Alternatively, there may have been discrimination against uptake of polyvalent metallic cations in the insect gut, selective metabolic processes in the tissues, or active excretion of such cations.

The polonium content in the predatory tiger beetles was found to be about one-half that of kelp flies, a fact which may be attributable to similar mechanical or metabolic discrimination. (It would be interesting to determine the polonium content of other predators of kelp flies, for example birds such as spotted sandpipers (*Petrochelidon pyrrhonota*) or swallows (*Hirundo rustica*), but we did not attempt this.) In neither of the two successive steps

Table 1 Polonium and plutonium contents of algae and flies from a California shore

		^{210}Po	$^{239,240}\text{Pu}$	Po/Pu	^{210}Pb
Beach					
Kelp:	<i>Macrocystis</i>	480 \pm 40	2.0 \pm 0.3	250	380 \pm 40
	and others	510 \pm 25			
Flies:	<i>Fucilia</i> : whole adults	290 \pm 15	1.1 \pm 0.2	260	220 \pm 30
	hard parts	247 \pm 18			
	expressed 'juice'	277 \pm 13			
		< 5			
Beetles:	<i>Cicindela</i>	164 \pm 12			
Laboratory					
Fly-larva food (CSMA)		30 \pm 3			
Flies:	<i>Musca</i> : whole adults	10 \pm 4			

Values are pCi per kg dry weight; shown as mean \pm s.e.m.

of the food chain studied here did we observe a relative enrichment of the radionuclides. On the contrary, at each step the relative polonium concentration was halved, while the plutonium (and ^{210}Pb) content of the kelp flies was also only half that of the content of the decayed kelp on which they fed.

A crude extract of the blood, gut contents and other soft parts of the kelp flies was prepared by homogenising a sample of the fresh flies in a Waring blender with distilled water for 45 s and centrifuging at 13,000 r.p.m. (20,000g) for 10 min. The liquid fraction was then clarified by filtration. The residues retained by the filter and at the bottom of the centrifuge tube consisted of cuticles, heads, wings, legs and bristles. This fraction contained essentially all of the polonium of the intact flies (see Table 1), indicating that most of the polonium ingested by the insect larvae with their food (the decaying kelp) had been incorporated in the cuticles of the adult flies.

In ascending the food chain from the kelp to the flies, the Po/Pu ratio remained unchanged. In contrast, corresponding values for *Macrocystis* plants and for bryozoa removed from their surfaces were respectively 230 and 1,000¹, while even larger ratios, as high as 10^5 or 10^6 , were found in the internal organs of several species of marine fishes (V.F.H., unpublished), suggesting that in these latter systems there is a more effective discrimination against Pu, or in favour of uptake of Po, or both, than in the kelp flies.

Our observations indicated that on a warm summer day there may be as many as 10^7 flies per km of beach near the Scripps Institution of Oceanography, in an area where the estimated annual tonnage of cast weed is about 10^5 kg km⁻¹ (ref. 3). The kelp brings with it some 10^7 pCi of polonium and 4×10^4 pCi of plutonium per km of beach annually. If we assume that there are 10 generations of kelp flies per yr (in summer⁴ a generation time of kelp flies may be as short as 10 d), it can be calculated that about 0.2% of the polonium and 0.2% of the plutonium adsorbed or in some other way fixed to the major seaweeds may find its way into a terrestrial food chain.

It should be emphasised that although the kelp flies may occur in large numbers along beaches on warm days, such as those observed during the great seaweed-fly plague in southern England in 1953–54⁵, the radiation values with which we are concerned here present no obvious hazards to human health. The accidental inhalation of a few kelp flies would involve the ingestion of merely 10^{-4} pCi of ^{210}Po and 10^{-6} pCi of $^{239,240}\text{Pu}$, while the deliberate ingestion of tens of thousands of such flies would be too involved and unappetising an exercise to consider seriously.

We thank Dr Willis W. Wirth (Smithsonian Institution, Washington) for identifying the kelp flies, and Mr Ian Moore (University of California, Riverside) for providing laboratory-reared houseflies and the larval medium.

LANNA CHENG
V. F. HODGE
R. A. LEWIN

Scripps Institution of Oceanography,
University of California,
La Jolla, California 92093

Received 11 July; accepted 15 August 1977.

1. Hodge, V. F., Hoffman, F. L. & Folsom, T. R. *Health Phys.* **27**, 29 (1974).
2. Wong, K. M., Hodge, V. F. & Folsom, T. R. *Nature* **237**, 460 (1972).
3. Zobel, C. E. *Nova Hedwigia* **32**, 269 (1971).
4. Dobson, T. in *Marine Insects* (ed. Cheng, L.) 447 (North-Holland, Amsterdam, 1976).
5. Oldroyd, H. *Discovery* **15**, 198 (1954).

Free-running activity rhythm in the natural environment

RHYTHMICAL activities of plants and animals in their natural habitats have, so far, always been found to be synchronised with cyclic environmental changes¹⁻³. In the absence of appropriate environmental timing cues (usually light-dark or tidal cycles) the rhythms free-run with endogenously controlled periods other than those of the environmental cycle. These may be longer or shorter than those observed in the presence of the entraining environmental cycles. We describe here an apparently unique example of an invertebrate which seems to free-run in its natural habitat. The animal is a predatory prostigmatid mite (*Bdella interrupta* Evans) which inhabits marine salt marshes and is exposed to regular tidal inundation during periods of spring tides. During periods of neap tides the salt marsh is not covered by the sea. The mites showed day and night peaks of locomotory activity on the soil surface at creek edges (Fig. 1a) and, also, in isolated experimental conditions (Fig. 1b). The narrow peaks of activity imply that the individual members of the population are closely synchronised with one another.

The peaks of locomotory activity in the natural habitat did not exhibit constant relationships to the times of dawn and dusk. The interval between day peaks of activity during a sequence of non-covering tides was less than 24 h. The slope of the regression line for the first 6 d illustrated in Fig. 2 corresponds to an average separation of 23.1 h between successive day peaks during this period of non-submerging tides. During

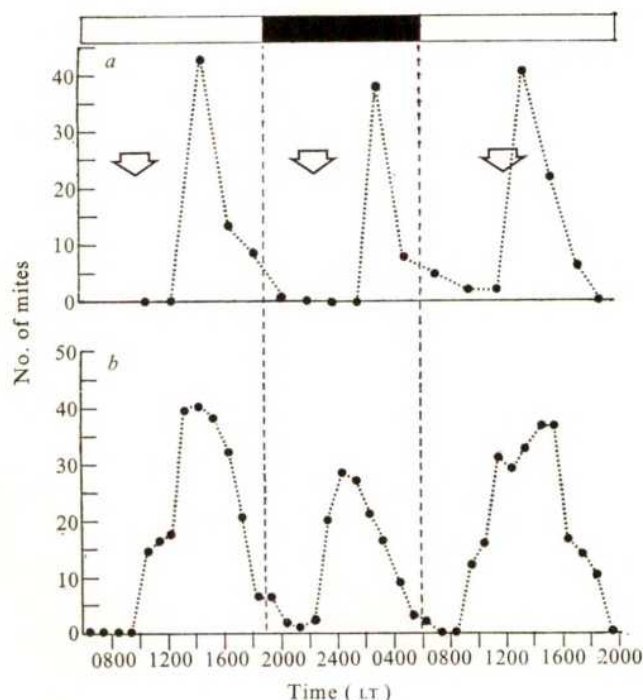


Fig. 1 *a*, Numbers of mites observed walking on the soil surface on the edges of a drainage channel on Hut Marsh, Scolt Head, Norfolk, UK. Observations were made on a 25-m length of bank during a period of tidal emergence (6-7 April 1976). The times of high tides are indicated by open arrows. Dawn and dusk are shown by broken lines. *b*, Surface activity of *B. interrupta* in isolated habitat portions (in five cylindrical containers of 15.2 cm diameter containing soil of 10 cm depth). The mites were maintained in natural light conditions.

the subsequent period of submerging tides the average separation between successive day peaks increased to 25.1 h, so that the surface locomotory activity of the mites effectively paralleled the times of the high tides for the period (Fig. 2).

Periodograms for the 1976 data illustrated in Fig. 2 yielded activity peaks at 22.9 h (for locomotory activity during non-submerging tides) and 24.9 h (during the subsequent period of submerging tides) (Fig. 3) for data collected during the daylight hours. Field observations made in 1977 confirmed these differences in periodicity observed during periods of tidal emergence and submergence. Since the activity peaks occur twice

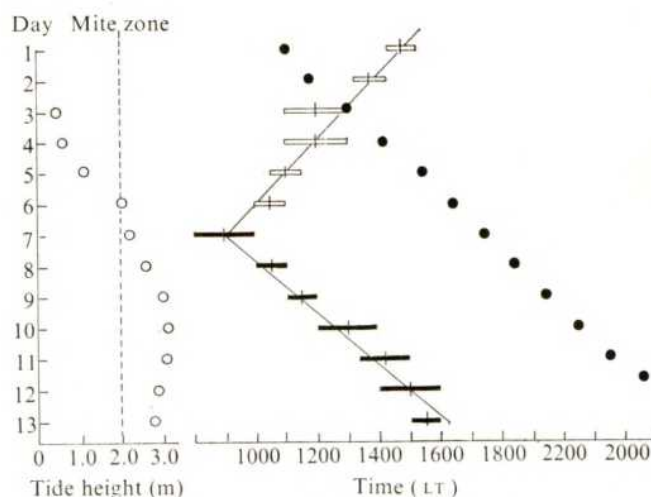


Fig. 2 The timing of day activity peaks related to the time (●) and the height (○) of high tides from 6 to 18 April 1976. The horizontal bars indicate the period during which mite activity was more than 50% of the maximum. The vertical lines are the midpoints of the bars and are taken as the phase reference point. The open bars represent activity during tidal emergence and the closed bars that during a subsequent period of submerging spring tides. The continuous lines are the calculated regression lines (for the period of tidal emergence: $r = 0.9720$; $n = 6$; $P < 0.005$; for the period of tidal submergence $r = 0.9923$; $n = 7$; $P < 0.001$). For these calculations the day sequence was used as the x axis.

daily and the rhythm entrains to the tidal frequency when the animal is exposed to tidal inundation the locomotory activity rhythm can be defined as a circatidal rhythm with a periodicity of about 12.5 h. As with circatidal rhythms in other organisms, the mite rhythm is insensitive to light-dark cycles. In this respect this salt-marsh mite is similar to an oribatid mite (*Ameronothrus marinus* Banks), which on rocky shores shows an apparently circatidal rhythm of 12.3-h periodicity⁴.

The novel feature revealed by this investigation is that during periods of non-submerging tides the period of the activity rhythm (11.5 h) is shorter than when it is entrained by the tides (12.5 h) and can be reasonably presumed to be a free-running circatidal rhythm. The maintenance of narrow peaks of activity throughout periods of tidal emergence indicates that the periods of the individuals are very similar whilst free-running. This contrasts with the breakdown of synchrony seen in free-running populations of other organisms in constant experimental conditions.

There is no conclusive evidence that the alternation of an apparently free-running rhythm, during tidal emergence, with an entrained rhythm, during periods of tidal submergence, is of adaptive value. It is conceivable, however, that this alternation could be a mechanism to achieve synchrony with the pattern of tidal coverage on the marine salt marsh. At the onset of periods of tidal coverage the activity peaks occur approximately midway between the times of successive high tides. This temporal separation is maintained during subsequent periods of tidal coverage by the circatidal rhythm of 12.5-h periodicity. The alternation between 11.5- and 12.5-h periodicities ensures

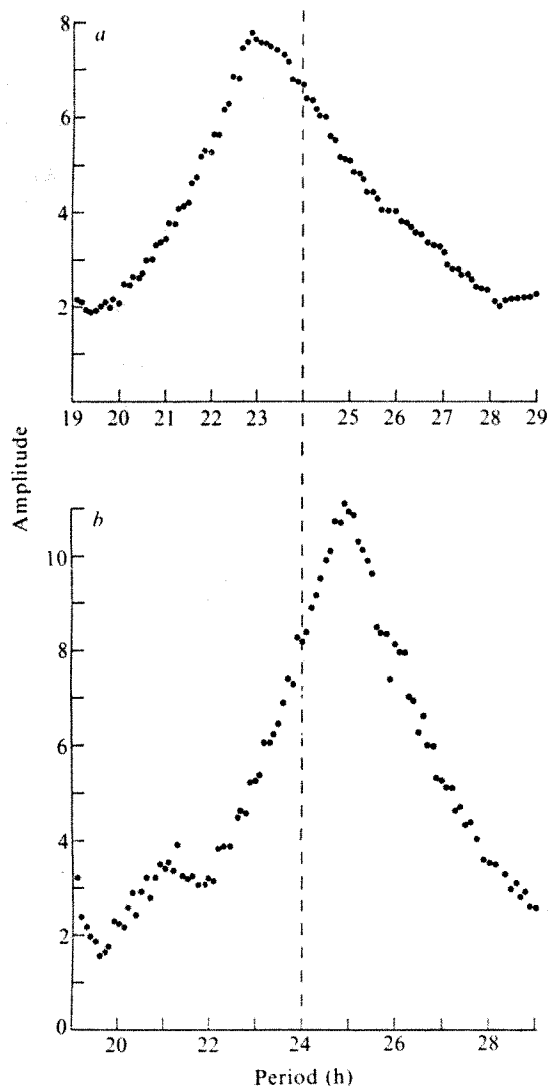


Fig. 3 Periodograms of data illustrated in Fig. 2, for the observed locomotory activity during periods of non-submerging (a) and submerging (b) tides.

that a peak of mite activity is continuously maintained in daylight hours. This would not occur if the circatidal periodicity (12.5 h) continued during tidal emergence. Calculations using data from the 1977 tide tables show that with an intermediate free-running period, for example 12.0 h, the activity peak is always within 2.5 h for the first, critical, tidal inundation in a series of rising spring tides. With a free-running period of 11.5 h, on the other hand, the activity peak is within 2.5 h of only 17% of the critical tides.

We thank Drs Jack Benson and J. Brady for helpful discussions. We also thank the Nature Conservancy for permission to work on the Scolt Head Nature Reserve.

J. E. TREHERNE
W. A. FOSTER
P. D. EVANS
C. N. E. RUSCOE*

Department of Zoology,
University of Cambridge, UK

Received 25 July; accepted 19 August 1977.

*Permanent address: ICI Plant Protection Division, Jealott's Hill Research Station, Bracknell, Berkshire, UK.

1. Bünning, E. *The Physiological Clock* (English Universities Press, London, 1973).
2. Enright, J. T. *J. comp. Physiol.* **77**, 141-162 (1972).
3. Palmer, J. D. *Biol. Rev.* **48**, 377-418 (1973).
4. Schulte, G. *Mar. Biol.* **37**, 265-277 (1976).

Sexual dimorphism, socionomic sex ratio and body weight in primates

BECAUSE the primates are a particularly well studied group they provide a rare opportunity to investigate the adaptive significance of species differences in sexual dimorphism in body size. We describe here an investigation of the relationship between the degree of sexual dimorphism and three variables which are predicted might affect it.

In many birds and mammals (with certain exceptions)^{1,2} the male is larger than the female. The commonest explanation for this trend, originally offered by Darwin in 1871 (ref. 3) is that increased body size confers advantages in contests between males for access to females (and there is now detailed evidence supporting this⁴⁻⁶). The basis of this argument is that males are able to enhance their breeding success greatly by maintaining exclusive breeding access to several mates³, whereas females cannot usually⁷ enhance theirs to the same extent by maintaining exclusive access to multiple males. For this reason, the argument runs, polyandry is rarer than polygyny. Once polygyny has developed, males will compete for access to females⁴ and individuals possessing traits which enhance their competitive ability (such as increased body size) will be selected. This argument assumes that increases in body size are constrained by ecological, perhaps energetic, disadvantages which can be outweighed by reproductive advantages.

There is another functional explanation of sex differences in body size—these may permit the sexes to utilise different kinds of food and consequently enhance the efficiency with which they can exploit their resources. These two theories are not mutually exclusive, but their predictions differ: the first predicts that sexual dimorphism should be most marked in strongly polygynous species where competition between males for access to females is intense since only a small proportion of males can breed; the second, that dimorphism should be most marked in monogamous species or in those with a relatively equal adult sex ratio where divergence between the sexes would be most effective^{8,9}. While there is reasonable evidence implicating the second explanation in particular cases⁸, the distribution of dimorphism across species generally fits the predictions of the first: monogamous species show least dimorphism and those with the most imbalanced socionomic sex ratios (the number of adult females per adult male in breeding groups) the most.

But, although a positive relationship between body size dimorphism and socionomic sex ratio has been suggested in reviews of birds^{1,10,11}, ungulates^{12,13} and primates¹⁴⁻¹⁶, none have offered formal statistical evidence for such a relationship. That such evidence is necessary is demonstrated by the extent to which species with similar breeding systems differ in their degree of body size dimorphism (see below). Also, it is necessary to investigate what other factors affect the degree of size dimorphism and to consider the possibility that the apparent relationship between dimorphism and socionomic sex ratio is the product of some third factor associated with these two variables.

In most orders, formal comparisons are impossible because of the paucity of accurate data about social organisation. This is not so in the primates, where the social behaviour of a large number of species has now been studied in detail. To examine this relationship, we extracted as many estimates of body weight and of breeding sex ratio from the literature as we could find (the sources and data used will be published elsewhere (refs 17, 18 and B. Rudder, in preparation), selecting weights of wild animals in preference to those collected from zoos wherever possible. Sexual dimorphism was then calculated by dividing male weight by female weight. Our measure of socionomic sex ratio was the mean number of adult females per adult male in breeding groups: where several estimates were available for the same species, we took the mean of these. Our sample included 42 primate species.

There is a significant relationship between sexual dimorphism and socionomic sex ratio (Fig. 1: $r_s = 0.610$, $t = 4.87$, 40 d.f., $P < 0.001$) but, this relationship is largely due to the fact that

monogamous species show consistently less dimorphism than polygynous ones (Kruskal-Wallis $H = 22.16$, $P < 0.001$) and, when monogamous species are excluded, no relationship exists ($r_s = 0.087$, $t = 0.44$, 27 d.f., not significant). Why should this be the case? There are probably two main reasons. First, socionomic sex ratio fails to provide a reliable index of inter-male competition. It would, for example, be likely to underestimate the degree of competition in multi-male troops (as in *Papio* or *Macaca* species) where a marked dominance hierarchy exists and breeding access is not shared equally among the males. Conversely, it would overestimate the degree of competition in species where access to females is strongly age-dependent and the majority of males pass through a short reproductive phase¹⁹. Also, our data will inevitably have included a proportion of cases where estimates of socionomic sex ratio were a typical, thus introducing error. Second, the data show that factors apart from socionomic sex ratio exert a strong influence on the degree of sexual dimorphism. Terrestrial species show consistently greater dimorphism than arboreal ones (Kruskal-Wallis $H = 8.96$, $P < 0.01$) probably because, in the former, increased weight does not restrict the male's access to food supplies (often located on fine terminal twigs) to the same extent as in the latter. In addition, sexual dimorphism is positively related to body weight (the mean of adult male and adult female body weight: see Fig. 2: $r_s = 0.484$, $t = 3.50$, 40 d.f., $P < 0.01$). This relationship is not a product of the inclusion of female weight in both parameters: if male weight is plotted against

female weight, the slope is significantly greater than 1 ($b = 1.063$, $t = 66.33$, $P < 0.001$). Nor is it the product of either of an association between large-size and terrestriality since the same relationship holds within arboreal species (Fig. 2: $r_s = 0.395$, $t = 2.27$, 28 d.f., $P < 0.05$) or of an association between size and polygyny (see below).

Since several monogamous species are of small size (such as *Callicebus* spp., *Saguinus* spp.) it is necessary to consider whether the overall relationship between dimorphism and socionomic sex ratio could be the product of that between the former and body weight. To do this, we linearised the relationships and used a partial regression analysis²⁰. This tests whether a dependent variable (in this case, sexual dimorphism) is significantly related to each of two or more independent variables (socionomic sex ratio and body weight) when the effect of the other has been removed. (In the description of results, b_1 refers to the regression coefficient for the first independent variable and b_2 to the second). The analysis showed that sexual dimorphism is significantly related to both variables in the sample as a whole ($b_1 = 0.111$, $t = 2.14$, $P < 0.05$; $b_2 = 0.078$, $t = 3.11$, $P < 0.005$, 39 d.f.) and that 56.3% of the variance in sexual dimorphism is accounted for by the two variables. The relationship to body weight was considerably stronger than that to socionomic sex ratio: standardised partial regression coefficients for sex ratio and body weight are 0.28 and 0.41 respectively. Similar results were obtained when we applied the same analysis to the data for arboreal primates only

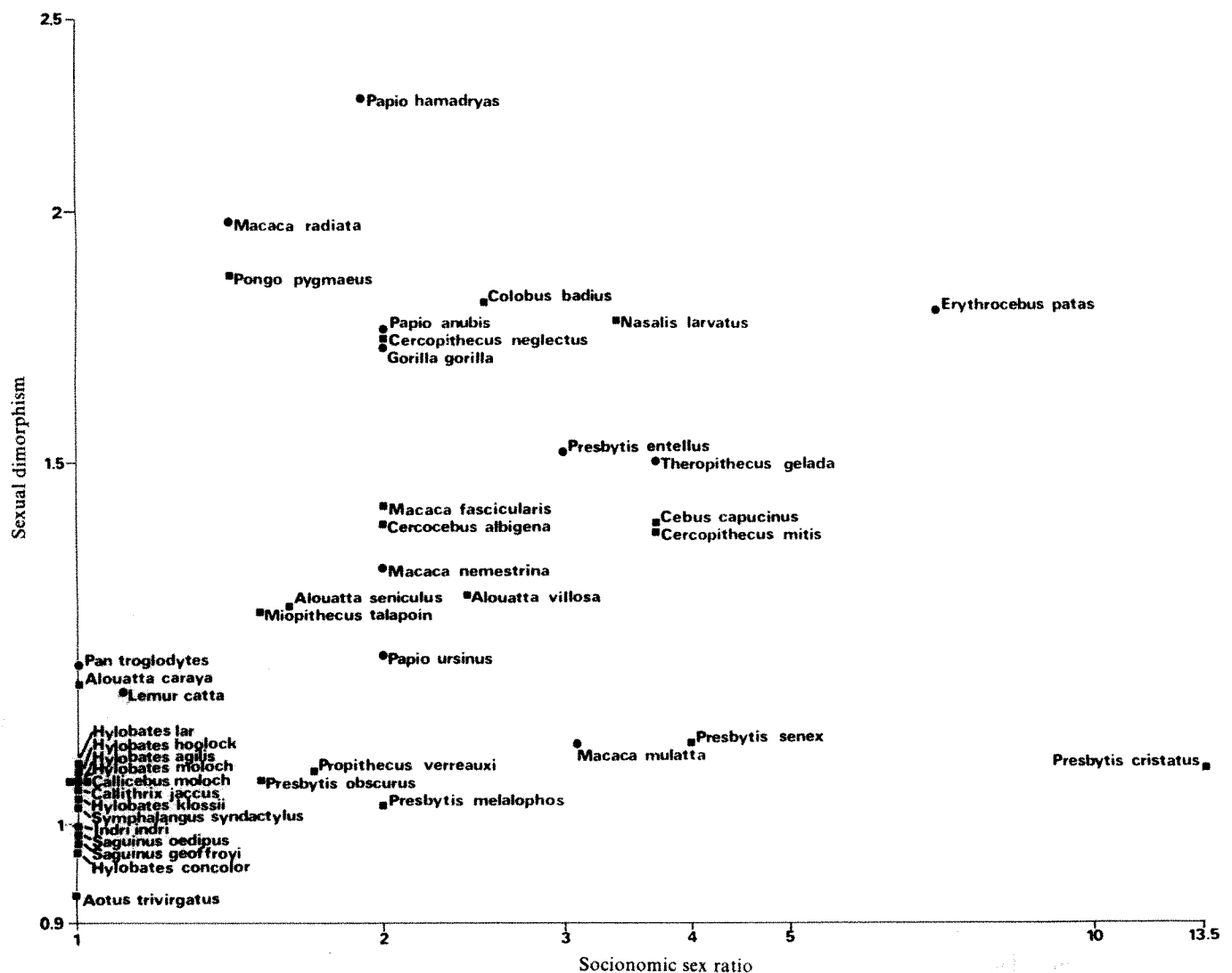


Fig. 1 Sexual dimorphism in body weight (weight of male/weight of female) plotted against socionomic sex ratio (females per male). ■, Arboreal; ●, terrestrial.

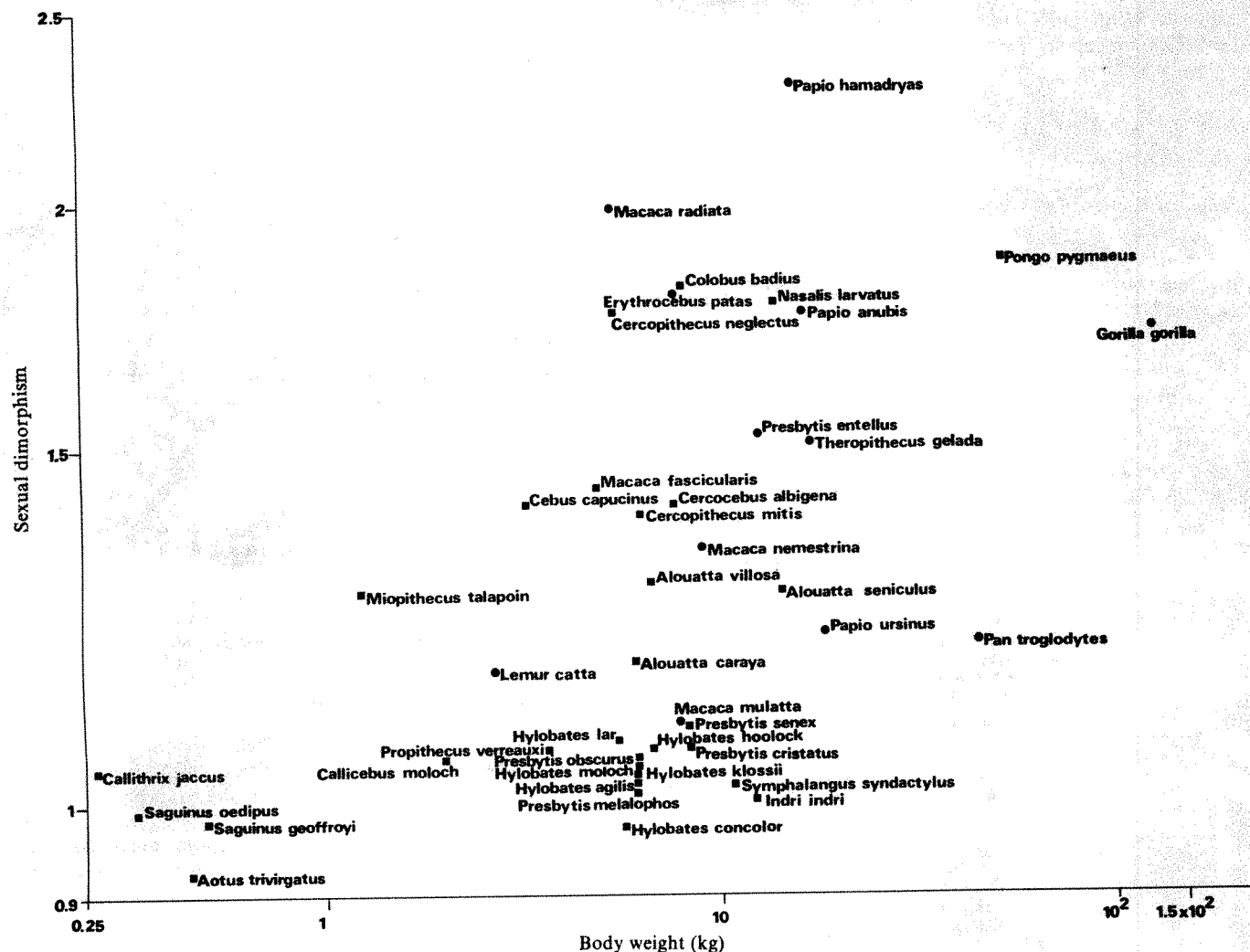


Fig. 2 Sexual dimorphism in body weight plotted against average of adult male and adult female body weight. ■, Arboreal; ●, terrestrial.

($b_1 = 0.097$, $t = 1.81$, $P < 0.1$; $b_2 = 0.064$, $t = 2.32$, $P < 0.05$, 27 d.f.).

Why should sexual dimorphism in body weight be positively correlated with absolute body weight? There is extensive evidence that this relationship is not confined to primates and occurs in invertebrates²¹, birds^{10,21,22}, rodents (G. Mace, personal communication) and carnivores. It was apparently first recognised by Rensch^{21,23} who attributed it to allometric growth: "Secondary sexual characters of many mammals usually represent structures of positively allometric growth because they usually become more conspicuous during a more advanced period of ontogenetic development (due in mammals to the increasing influence of sex hormones)".

There is, however, no obvious reason for supposing that allometry is inevitable. At least six functional explanations could be involved. It has been suggested that sexual selection for increased size in males may, as a side effect, have led to increased female size thus creating a relationship between dimorphism and average body size²⁴. This seems unlikely because it requires the assumption that increases in male size are more closely paralleled by increases in female size in small animals than in large. Moreover, selection for energetic efficiency probably imposes strict constraints on female size^{9,10,18,25}. Alternatively, it is generally true that large body size is associated with a reduction in the number of competing species^{26,27}. This could relax constraints on increasing male body size imposed by the presence of larger species in neighbouring niches, allowing sexual selection for male competitive ability to produce relatively large increases in male

size. There is evidence that in some birds sexual dimorphism in body size is commonly associated with sex differences in food choice (see above) and that the presence of competing species can constrain intraspecific variance in many characteristics, including size²⁶⁻²⁸. It is also possible that energetic constraints on increasing male body size may be relaxed in larger species since larger body size leads to a decrease in the surface area: volume ratio, metabolic rate and relative nutritional requirements (refs 30-32 and J. Kurland, personal communication) thus making further increases energetically 'cheaper'. Alternatively, where dimorphism permits the sexes to diverge in food choice, selection may favour increased feeding divergence (and thus increased dimorphism) in species which feed on low density food distributed in clumps²² since, in this situation, the effects of feeding competition are strongest. Mammalian species which feed on dispersed and unpredictable food supplies are often of large size^{12,32}. Finally, it is possible that evolutionary factors favouring the reduction of female size may be involved³³. It has been argued that, in fluctuating environments, smaller females may be able to breed at a younger age than larger ones³⁴ and it is conceivable that this pressure might be more acute in large species where the age at first breeding is relatively late, than in small species where it is generally early.

None of these explanations is wholly satisfactory and evidence to support them is almost totally lacking. Until they have been investigated systematically, however, it would be premature to assume that the relationship between body weight and sexual dimorphism is inevitable.

We thank Dr S. A. Altmann, Dr A. H. Harcourt, R. M.

Gibson, P. Greene, P. J. Greenwood, G. Mace, Professor J. Maynard Smith, Dr N. Owen-Smith and Professor R. L. Trivers for help, advice and criticism.

T. H. CLUTTON-BROCK

King's College Research Centre, Cambridge, UK

PAUL H. HARVEY

School of Biology, University of Sussex, Brighton, UK

B. RUDDER

Wellcome Institute of Comparative Physiology, Regent's Park, London, UK

Received 25 January; accepted 13 August 1977.

1. Lack, D. *Ecological Adaptations for Breeding in Birds* (Methuen, London, 1968).
2. Ralls, K. *Q. Rev. Biol.* **51**, 245–275 (1977).
3. Darwin, C. *The Descent of Man and Selection in Relation to Sex* (Murray, London, 1871).
4. Trivers, R. L. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) (Aldine, Chicago, 1972).
5. Wilson, E. O. *Sociobiology, the New Synthesis* (Belknap, Cambridge, Massachusetts, 1975).
6. Geist, V. *Mountain Sheep* (University of Chicago Press, Chicago, 1971).
7. Jenni, D. A. *Am. Zool.* **14**, 129–144 (1974).
8. Selander, R. W. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) (Aldine, Chicago, 1972).
9. Bourliere, F. in *Small Mammals, their Productivity and Population Dynamics* (eds Golley, F. B., Petruszewicz, K. & Ryszkowski, L.) (Cambridge University Press, Cambridge, 1975).
10. Wiley, R. H. *Q. Rev. Biol.* **49**, 201–227 (1974).
11. Crook, J. H. *Behav. Suppl.* **10**, 1–178 (1964).
12. Jarman, P. J. *Behaviour* **48**, 215–267 (1974).
13. Geist, V. *Am. Zool.* **14**, 205–220 (1974).
14. Crook, J. H. in *Sexual Selection and the Descent of Man* (ed. Campbell, B.) (Aldine, Chicago, 1972).
15. Gautier-Hion, A. *Mammalia* **39**, 365–374 (1975).
16. Clutton-Brock, T. H. & Harvey, P. H. in *Growing Points in Ethology* (eds Bateson, P. P. G. & Hinde, R. A.) (Cambridge University Press, Cambridge, 1976).
17. Clutton-Brock, T. H. & Harvey, P. H. *J. Zool.* **183**, 1–39 (1977).
18. Clutton-Brock, T. H. & Harvey, P. H. in *Primate Ecology: Studies of Feeding and Ranging Behaviour in Lemurs, Monkeys and Apes* (ed. Clutton-Brock, T. H.) (Academic, London, 1977).
19. Rowell, T. E. *Behav. Biol.* **11**, 131–154 (1974).
20. Bailey, N. T. J. *Statistical Methods in Biology* (English Universities Press, London, 1964).
21. Rensch, B. *Evolution Above the Species Level* (Methuen, London, 1959).
22. Selander, R. K. *Condor* **68**, 113–151.
23. Rensch, B. *Bonner Zool. Beitr.* **1**, 58–69.
24. Maynard Smith, J. *Anim. Behav.* **25**, 1–9.
25. Bell, R. H. V. *Sci. Am.* **225**, 86–93 (1977).
26. MacArthur, R. H. *Geographical Ecology* (Harper and Row, New York, 1972).
27. MacArthur, R. H. & Wilson, E. O. *The Theory of Island Biogeography* (Princeton University Press, Princeton, 1967).
28. Yom Tov, Y. & Ollason, T. G. *Oikos* **27**, 81–85 (1976).
29. Lack, D. *Ecological Isolation in Birds* (Blackwells, Oxford, 1970).
30. Kleiber, M. *The Fire of Life* (Wiley, New York, 1961).
31. Janis, C. *Evolution* **30**, 757–774 (1976).
32. Clutton-Brock, T. H. in *Primate Ecology: Studies of Feeding and Ranging Behaviour in Lemurs, Monkeys and Apes* (ed. Clutton-Brock, T. H.) (Academic, London, 1977).
33. Ralls, K. *Am. Nat.* (in the press).
34. Downhower, J. F. *Nature* **263**, 558–563 (1976).

Ultrastructure of the spermatophores of *Siboglinum ekmani* Jägersten (Pogonophora)

THE marine invertebrate phylum Pogonophora has attracted much attention since its discovery in 1914 because of its uncertain phylogenetic position and its unusual biological features, such as the lack of a digestive tract^{1,2}. Little is known of the spermatophores and the mechanism of fertilisation^{1,3}, although it has been suggested that the long filament of the spermatophore serves as a flotation device⁴. Because the expulsion of spermatophores into the sea would involve great wastage of sperm, however, some form of copulation has been said to be likely⁵. Expulsion of spermatophores by the tentacles of the male has been observed in one species³, but even in dense populations of 200 animals per m², which have been reported¹, the tentacles are too short to transfer the spermatophores to the females. Electron microscopy has now revealed that the spermatophoral filament is equipped with numerous electron dense 'threads', composed of tubular elements with a central electron dense core. Apparently the 'threads' which receive their strength from the tubular elements, play an important part as a floating or adhesive apparatus of the released spermatophores.

Several specimens of *Siboglinum ekmani* Jägersten were collected from the Skagerrak during tests of the German research vessel Poseidon (January 1977, 57°50'N, 8°05'E, 537 m deep, 3.4°C, salinity 32.6 ‰). The animals were removed carefully from their tubes using pointed watchmaker's

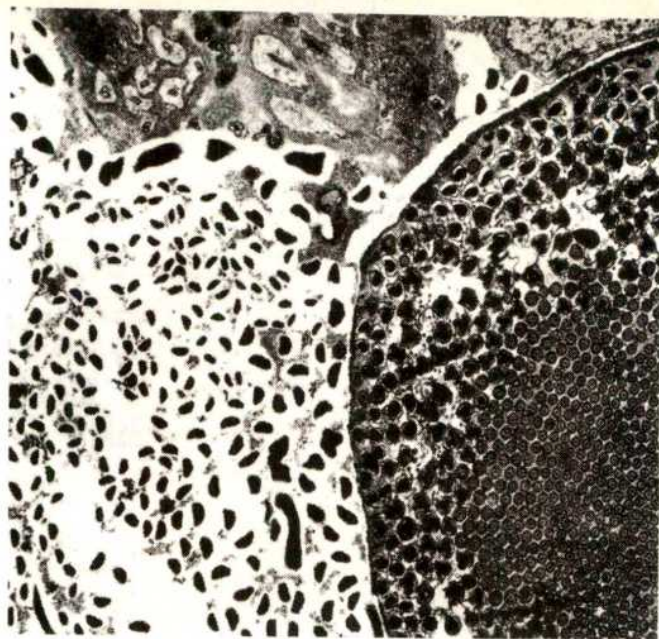


Fig. 1 Transverse section of sperm duct. Spermatophore containing mature spermatozoa cut at various levels (left), electron-dense threads (right) and parts of the filament (bottom). Phosphate-buffered glutaraldehyde (3.5%) and osmium (2%) fixation. Araldite embedding, uranyl acetate and lead citrate staining ($\times 6,080$).

forceps. Mature males were identified easily by the large numbers of spermatophores within the transparent sperm ducts.

Sections cut at various levels of the sperm ducts show the well known spindle-shaped spermatophores, portions of their coiled filaments and numerous electron-dense threads of unknown function (Fig. 1). These threads are about 0.5 μ m in diameter and several μ m long, their irregular shape preventing exact measurement. High magnifications reveal a new structural element, which I call 'dense core tubuli'. These tubuli constitute the major structural unit of the spermatophore wall as well as the long filament and the electron-dense threads (Fig. 2). The tubuli are embedded in an amorphous ground substance. They have an outside diameter of 150–170 Å, thus being slightly smaller than the microtubules of many plant and animal cells. In contrast to microtubules, they are clearly extracellular. Their length is indeterminate but tubules of several μ m have been

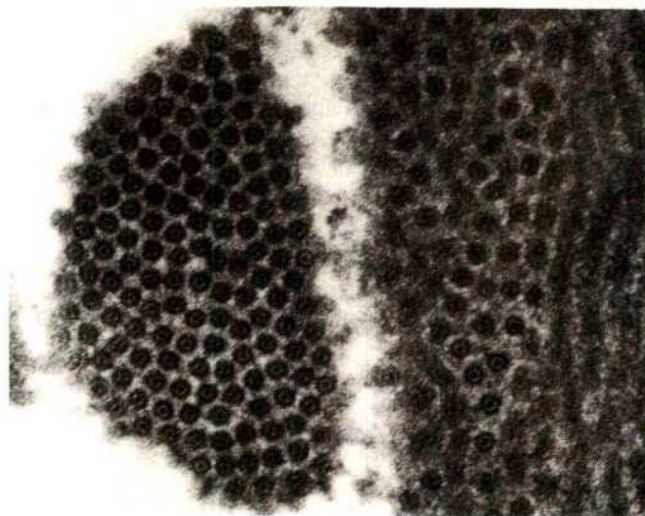


Fig. 2 Cross sections of electron dense thread (left) and spermatophore wall (right) reveal the dense core tubuli ($\times 152,000$).

observed. Occasionally the central core is composed of sub-units which also form a tubular structure. Dense core tubuli are linked in regularly spaced arrangements: 12–180 tubules are longitudinally orientated within the threads. In the spermatophore wall the arrangement resembles dense connective tissue of vertebrates.

Clusters of dense core tubuli also occur between developing male germ cells in the testes. Regularly linked tubuli are attached to groups of mature spermatozoa to mould the envelope of the spermatophores in the proximal region of the sperm ducts. Released spermatophores tend to stick firmly to the tube or trunk of the animal even after fixation, dehydration and embedding. This indicates remarkable adhesive properties. When spermatophores are exposed to seawater, the regular arrangement of the dense core tubuli is gradually lost and the spermatophore wall starts to disintegrate⁶. The filament

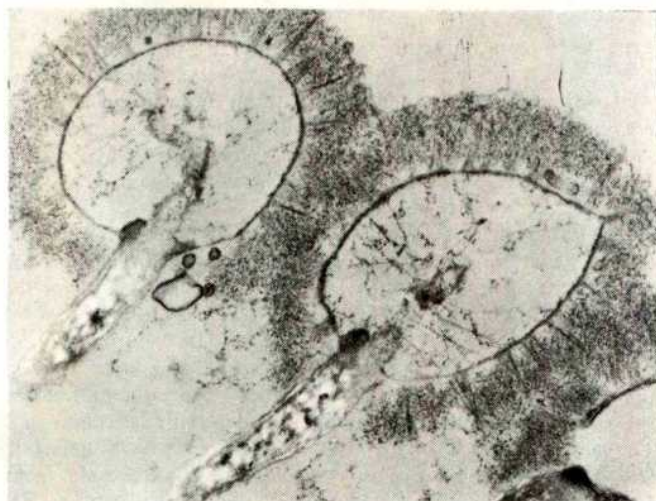


Fig. 3 Two stalked vesicles of the spermatophoral filament. Delicate spines project from the surface into the fibrous cover ($\times 30,500$).

uncoils, and at intervals it has areas of radiating stalked vesicles (Fig. 3). Within the sperm ducts these vesicles are smaller, from which I conclude that the filament uncoils through the swelling of the vesicles. They are probably identical with the capitate processes revealed by light microscopy^{3,7}. Fibrous, sticky material covers the surface of the vesicles, reinforced by delicate spines (Fig. 3). The dense threads found in the sperm ducts are also released and frequently appear near the spermatophores in sections. Undoubtedly, the stalked vesicles of the filament and the dense threads form an effective adhesive apparatus and floating device.

My investigation adds further support to the view that the released spermatophores of *Siboglinum* find their way passively without 'some form of copulation' to a receptive female^{1,5}. But further observations are required to elucidate the path of the released spermatozoa to the eggs.

H. FLÜGEL

Institut für Meereskunde,
Universität Kiel, 23 Kiel, FRG

Received 11 July; accepted 23 August 1977.

1. Southward, E. C. *Oceanog. mar. Biol. A. Rev.* 9, 193–220 (1971); in *Reproduction of Marine Invertebrates* (eds. Giese, A. C. & Pearse, J. S.) (Academic, New York, London, 1975); *Symp. Zool. Soc., Lond.* 36, 235–251 (1975).
2. Franzén, A. *Acta Zool.* 54, 179–192 (1973).
3. Webb, M. *Sarsia* 13, 45–49 (1963).
4. Ivanov, A. V. *Pogonophora* (Academic, New York, London, 1963).
5. Carlisle, D. B. in *Pogonophora* (ed. Ivanov, A. V.) (Academic, New York, London, 1963).
6. Southward, A. J. & Southward, E. C. *J. mar. biol. Ass. U.K.* 43, 57–64 (1963).
7. Ivanov, A. V. *Zool. Jb. Abt. syst.* 85, 430–500 (1957).

Blood-sucking flies and primate polyspecific associations

PRIMATE polyspecific associations are likely to be an effective means of reducing the number of fly bites individual monkeys receive¹. The probability of acquiring a vector-borne disease, and the severity of that disease increases with the number of fly bites an animal receives^{2–4}. Bait animals placed in groups are known to attract fewer individual mosquitoes per bait individual than are bait animals placed singly^{3,5}. Mean group size of the mangabey, *Cercocebus albigena* is about 13 in Kibale Forest, Uganda, and the mean group size of *Colobus badius* and *Cercopithecus ascanius* are 50 and 35 respectively⁶. Associations of groups of these species are likely to reduce significantly the number of dipteran bites individual monkeys receive^{1,7}. Other explanations proposed for primate polyspecific associations include increased food location abilities, and increased predator detection and avoidance^{8,9}. I report here the correlation of the temporal occurrence of *C. albigena* polyspecific associations with the activity of biting and sucking flies, *C. albigena* feeding and other activities, and the temporal occurrence of attacks by the crowned eagle-hawk (*Stephanoaetus coronatus*) (eagles are the only known predator of these monkeys). The study was carried out in the Kibale Forest, Ngogo Reserve.

On 5 d each month from January to September 1975, I followed a group of *C. albigena* from dawn to dark. Data on mangabey activity and polyspecific associations were recorded during four 5-min periods each hour (0–5, 15–20, 30–35 and 45–50 min). During each 5-min period as many individual *C. albigena* as possible were located, and the predominant activity of each during a 10-s interval was recorded. 'Moving', 'sitting', and 'feeding' are the activity categories used here. The location of each *C. albigena* observed was plotted on a map (4 cm = 100 m) of the study area. To record daily and hourly movements, net group displacements were measured from one 5-min period to the next. The actual distance measured was from the central focus of individuals in one 5-min period, to the central focus of the next. A polyspecific association was defined as the presence of more than one individual of another species within 20 m of the nearest mangabey. Times of eagle attack were recorded as they occurred. Attacks took the form of an eagle flying fast and low through the tree tops and branches, usually through the midst of the mangabey group. Activity of biting and sucking flies was determined using myself as bait. On 4 d I placed myself (naked arms and legs) on a platform 20 m above the forest floor, and remained there from dawn to dark. All flies (mosquitoes and others) biting me were recorded each hour, and when possible I killed all flies actually biting. Data from all days have been aggregated.

The *C. albigena* group took part in polyspecific associations on 43 of the 45 d of observation. Of a total 513 associations, 90.8% involved *C. ascanius*, 22.01% *C. badius*, 5.07% *Colobus guereza*, 1.75% *Papio anubis* and 0.59% *Cercopithecus thoeisti*. There was little interaction between species during the associations. On two occasions juvenile *C. albigena* attempted to join play groups of the other species. Other interactions were confined to adult *C. albigena*, displacing or occasionally chasing *C. ascanius*.

The occurrence of *C. albigena* in polyspecific associations was not equally distributed throughout the day ($\chi^2 = 19.71$, d.f. = 9, $P < 0.05$) (Table 1). There was a significant correlation between the hourly occurrence of polyspecific associations and the hourly activity of biting flies ($r_s = 0.63$, $N = 10$, $P < 0.05$) (Table 1). Because the data on Kibale fly activity are sparse, I also compared the temporal pattern of associations with the timing of mosquito activity in rainforest in Bwamba County, Uganda¹⁰. The correlation is significant ($r_s = 0.66$, $N = 10$, $P < 0.05$) (Fig. 1).

There were no significant correlations between the hourly occurrence of primate polyspecific associations and eagle attacks ($r_s = 0.119$, $N = 10$, $P > 0.05$), mangabey feeding activity ($r_s = 0.08$, $N = 10$, $P > 0.05$), sitting activity ($r_s = -0.08$, $N = 10$, $P > 0.05$), movement activity ($r_s = 0.42$, $N = 10$, $P > 0.05$), and the hourly distance travelled ($r_s = -0.05$, $N = 10$, $P > 0.05$) (Table 1).

Table 1 Polyspecific associations, feeding, moving and sitting activities, hourly distances travelled, eagle attacks and the number of biting flies of a group of mangabeys

Time	Total associations	Feeding observations	Sitting observations	Moving observations	Mean distance travelled (m)	Eagle attacks	Biting flies
0800-0900	57	308	170	200	107	3	12
0900-1000	41	367	123	209	133	1	5
1000-1100	42	410	145	187	100	4	5
1100-1200	35	400	157	196	118	4	1
1200-1300	24	298	178	241	141	1	9
1300-1400	54	364	167	168	95	2	11
1400-1500	36	321	158	218	164	2	11
1500-1600	42	408	105	225	121	0	8
1600-1700	46	452	108	198	147	1	31
1700-1800	49	338	126	211	118	0	61
r_s		0.08	-0.08	0.42	-0.50	0.119	0.63
P		NS	NS	NS	NS	NS	<0.05

The diurnal periodicity found in the occurrence of primate polyspecific associations is in agreement with studies on other African primates¹¹, and previous work on Kibale primates⁸. During the early mornings I noted that several species had frequently slept adjacent to one another. The dawn and dusk peaks in polyspecific associations probably represent the initiation and termination of night-long associations. As the *C. albigena* group slept in 45 different places on 45 different nights, there does not seem to be any shortage of sleeping sites.

These data do not support the possible existence of increased predator surveillance, increased food detection abilities or chance as causal to primate polyspecific associations. Predator detection may well be improved during the associations, but it does not

explain the temporal patterns. Feeding by two or more species in the same tree or in the same area was common, and one species may well use another as an aid in food location. But again, *C. albigena* feeding activities bore no significant relation to the temporal pattern of association. Predation, food and chance seem to do little more than add noise to a pattern that is probably due to another factor.

The importance of the significant correlation between polyspecific associations and biting fly activity is dependent on the importance of dipteran-borne disease to monkeys, and on the probability of polyspecific associations lowering the number of fly bites individuals receive. The size of the primate groups involved, and the published data on effects of number of bait animals suggest that polyspecific associations could easily reduce the number of bites received by individual monkeys. *C. albigena*, *C. ascanius*, and *P. anubis* all suffer from *Hepatoecystis kochi* ('monkey malaria') which is present in Kibale¹². *C. albigena*, and *P. anubis* suffer from *Dirofilaria* sp. and *C. ascanius* have been found with microfilaria¹². All Kibale monkeys suffer from yellow fever, and other arboviruses are probably present¹². Experimental infection of three *C. ascanius* with yellow fever resulted in fevers for 1-3 d, and the death of one due to an 'intercurrent infection'¹². Such effects are likely to have considerable impact on free-living monkeys. A mechanism exists for reducing the number of fly bites received per individual monkey. The temporal pattern of this mechanism correlates with the times of day when it could function to reduce the number of fly bites per individual monkey. Disease organisms using flies as vectors are prevalent among the Kibale monkeys, and seem capable of being a strong selective factor acting to promote primate polyspecific associations.

I thank the Department of Forestry and the National Research Council, Government of Uganda for allowing this study to take place and also my field assistants for their help. The New York Zoological Society, the NIH and the NSF provided financial support.

W. J. FREELAND*

Division of Biological Sciences,
University of Michigan, Ann Arbor,
Michigan 48109

Received 24 June; accepted 15 August 1977.

*Present Address: Department of Biological Sciences, Wright State University, Dayton, Ohio 45431.

- Freeland, W. J. *Biotropica* **8**, 12-24 (1976).
- Willett, K. C. *Ann. trop. Med. Parasitol.* **50**, 75-80 (1956).
- Reid, J. A. *Bull. ent. Res.* **52**, 43-62 (1961).
- Hewitt, R. *Bird malaria* (John Hopkins, Baltimore, 1940).
- Haddow, J. A. *Bull. ent. Res.* **33**, 91-142 (1942).
- Struhsaker, T. T. (in the press).
- Hamilton, W. D. *J. theor. Biol.* **31**, 295-311 (1971).
- Struhsaker, T. T. *The Red Colobus Monkey* (University of Chicago Press, Chicago, 1975).
- Gardlan, J. S. & Struhsaker, T. T. *J. zool. Soc. Lond.* **168**, 221-266 (1972).
- Haddow, A. J., Gillett, J. D. & Highton, R. B. *Bull. ent. Res.* **37**, 301-320 (1947).
- Gautier, J. P. & Gautier-Hion, A. *La Terre et la Vie* **116**, 164-201 (19xx).
- Haddow, A. J. *Proc. zool. Soc. Lond.* **122**, 297-394 (1952).

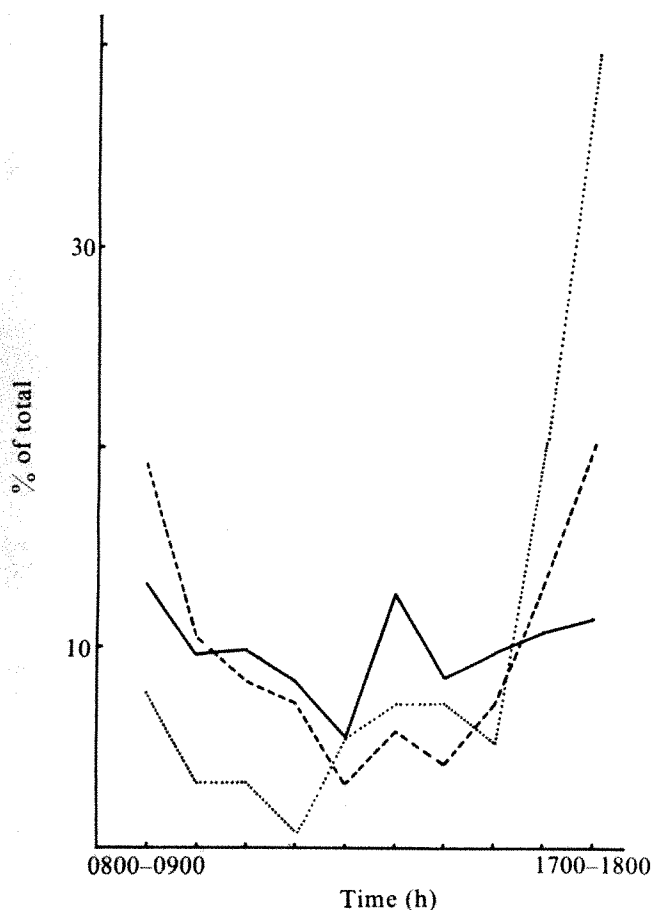


Fig. 1 *Cercopithecus albigena* polyspecific associations and the activity of Kibale biting flies and Bwamba mosquitoes. —, polyspecific associations; ---, Bwamba mosquitoes; Kibale biting flies.

Physiological correlates of an enzyme polymorphism

ATTEMPTS to demonstrate the action of natural selection at enzyme loci have relied mainly on *in vitro* biochemical techniques to detect differences between allelic forms of the enzymes¹⁻³. It is usually assumed that differences detected in this way will be relevant *in vivo*. The data on human inborn errors of metabolism do not support this view, however; in the majority of cases these are recessive. Heterozygotes, which usually have about 50% normal enzyme activity, rarely show clinical symptoms⁴. As any difference in activity between allozymes is likely to be much smaller, it cannot be assumed that such differences will necessarily be physiologically important. The properties of an enzyme molecule can only be biologically significant to the extent to which they affect the concentrations and flow of intermediates in its metabolic pathways. It was recently shown that changes in the concentration of most enzymes in a pathway at steady state will have little effect on flux^{5,6}. Therefore, to demonstrate that differences in fitness between strains possessing different genotypes at an enzyme locus are caused by that gene substitution, it is necessary not only to show that differences in kinetic parameters can be detected *in vitro*, but also that changes in the activity of the enzyme will affect flux through that pathway. I previously described⁷ significant changes in genotype frequency at a phosphoglucosylase locus in a wild population of field mice (*Apodemus sylvaticus*) which occurred at a time of food shortage and declining population numbers. I report here the results of some *in vivo* experiments on glycogen metabolism in animals of different genotypes at this locus, inducing glycogenolysis by fasting. The results parallel survival data from the wild population and suggest that the genotype of an individual at this locus is of physiological significance and may therefore be subject to natural selection.

The phosphoglucosylase isozyme pattern in field mice is similar to that in man⁷. Of the two loci that are active in erythrocytes, only the faster migrating one (PGM₂) is polymorphic. The two common alleles gave slow (*a*) and fast (*c*) bands on electrophoresis. This enzyme catalyses the interconversion of glucose-1-phosphate and glucose-6-phosphate—a reaction which links the pathways of glycogenolysis and glycolysis. In contracting muscle the flux passes directly from one pathway to the other but when liver glycogen is mobilised it is released primarily as free glucose. This pathway in liver is unusual in that it is short and has a clear beginning and end so that flux can be determined by measuring changes in stored glycogen concentration.

Liver glycogen content, estimated with the anthrone reagent^{8,9}, falls when field mice are fasted overnight. The mean concentration in 28 control animals was 28.9 mg per g wet weight (s.e., 2.41) while in 58 fasted animals it was 13.4 mg per g

(s.e., 2.41; $t_{85} = 5.4$, $P < 0.001$). PGM₂ genotype was known for 16 of the control animals. There was no significant difference between genotypes: eight animals of the *aa* genotype had a mean value of 26.1 mg per g (s.e., 3.8) while an equal number of *ac* animals had a mean of 22.2 mg per g (s.e., 3.7).

Table 1 shows the relationship between liver glycogen level and PGM₂ genotype in 48 fasted animals. The mean for the *aa* genotype is significantly lower than the combined mean for the other two groups ($t_{46} = 3.32$, $P < 0.01$). These data show a correlation between liver glycogen metabolism and PGM₂ genotype which is revealed by fasting, although genetic studies would be required to establish the causal nature of this relationship.

Table 2 shows recapture rates for each PGM₂ genotype in a natural population of *A. sylvaticus* at a time of high density, restricted food supply and falling population numbers⁷. There are significant differences in the proportion recaptured for the different genotypes and, as in Table 1, the heterozygotes give similar results to the *cc* homozygotes. Such data give a minimum estimate of survival rates¹⁰. Although errors in these estimates could arise from genetically non-uniform dispersal, they are unlikely to be large in this case as dispersal is uncommon in declining populations¹¹. The differences between genotypes in physiological responses to fasting may therefore lead to differences in survival rates at a time of food shortage.

Table 2 Recapture rate of field mice according to PGM₂ genotype

PGM ₂ genotype	<i>aa</i>	<i>ac</i>	<i>cc</i>
Total caught	22	36	18
Total recaptured	11	7	2
Proportion	0.50	0.19	0.11

Data from a natural population at Charnwood (Leicestershire, UK) giving the minimum survival rate of animals first caught in October 1974. The population was studied for 2 yr by mark-recapture trapping and gel electrophoresis⁷ and this sample represents a single cohort of non-reproductive animals. $\chi^2 = 7.32$, $P < 0.01$.

The impact of genotype at the PGM₂ locus on liver glycogen metabolism is unexpected as this locus provides less than 10% of total PGM activity in human liver¹¹. These results indicate that the two major PGM loci may have different roles *in vivo*; they have already been shown to differ in kinetic properties¹². This view is supported by several studies on the fate of labelled glucose and glucose phosphates *in vivo*¹³⁻¹⁵, which suggest that there may be two pools of glucose-6-phosphate in both liver and muscle, one of which is accessible to exogenous glucose phosphates and is involved in glycolysis while the other, from which glycogen is synthesised, is accessible only to glucose. Each of the two major PGM enzymes may be associated with only one of these glucose-6-phosphate pools, with the PGM₂ enzyme being the more important in glycogen mobilisation.

These results make it possible to visualise a physiological mechanism whereby *aa* animals are more fit than are *ac* and *cc* ones when food supply is restricted. If a higher rate of mobilisation of glycogen reserves is reflected in a higher potential for activity, *aa* individuals will have a competitive advantage when food reserves are few and widely scattered.

Considerable effort has been expended in attempts to demonstrate a selective basis for enzyme polymorphisms by means of *in vitro* biochemical studies¹⁶ although *in vitro* differences may have little physiological importance. The results of the *in vivo* experiments described here suggest that this approach may prove more rewarding.

I thank Dr J. S. Jones for discussions, Drs G. Bulfield and C. H. Langley for criticism of the manuscript and Dr R. Semeonoff for supervision. The project was supported by an SRC Research Studentship.

A. J. LEIGH BROWN*

Department of Genetics,
University of Leicester,
Leicester, UK

Table 1 Glycogen content of liver in fasted *A. sylvaticus* according to PGM₂ genotype

Genotype	<i>aa</i>	<i>ac</i>	<i>cc</i>
Mean	6.3	16.5	15.2
s.e.	1.66	4.02	2.25
N	14	18	16

Values are in mg per g wet weight. Electrophoretic methods were as described previously⁷. The genetic background of the experimental animals was standardised by taking F₁ offspring from crosses between two lines, both of which were segregating for the *a* and *c* alleles at the PGM₂ locus. Thus, all the experimental homozygotes had received one allele from each line. Food, normally freely available, was removed from the cages of fasted animals at the start of their night period. All animals were killed 15–16 h later. Liver glycogen was estimated with the anthrone reagent⁸ using sodium sulphate as a coprecipitant⁹. The recovery rate of a glycogen standard with this technique was 92% at an initial concentration of 5%. $P < 0.01$; $t_{46} = 3.32$.

Received 15 July; accepted 30 August 1977.

* Present address: Laboratory of Environmental Mutagenesis, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina 27709.

1. Koehn, R. K. *Science* **163**, 943-944 (1969).
2. Day, T. H., Hillier, P. C. & Clarke, B. *Biochem. Genet.* **11**, 141-153; 155-165 (1974).
3. Miller, S., Percy, R. W. & Berger, E. *Biochem. Genet.* **13**, 175-188 (1975).
4. Harris, H. *The Principles of Human Biochemical Genetics* 2nd edn (North-Holland, Oxford, 1975).
5. Kacser, H. & Burns, J. A. *Symp. Soc. exp. Biol.* **27**, 65-104 (1973).
6. Rapoport, T. A., Heinrich, R. & Rapoport, S. M. *Biochem. J.* **154**, 449-469 (1976).
7. Leigh Brown, A. J. *J. Zool., Lond.* **182**, 281-289 (1977).
8. Hassid, W. Z. & Abraham, S. *Meth. Enzym.* **3**, 34-50 (1957).
9. Van Handel, E. *Analyt. Biochem.* **11**, 256-265 (1965).
10. Flowerdew, J. R. *J. Anim. Ecol.* **43**, 499-511 (1974).
11. Krebs, C. J., Gaines, M. S., Keller, B. L., Myers, J. H. & Tamarin, R. H. *Science* **179**, 35-40 (1973).
12. McAlpine, P. J., Hopkinson, D. A. & Harris, H. *Ann. hum. Genet.* **34**, 169-175 (1970).
13. Quick, C. B., Fisher, R. A. & Harris, H. *Eur. J. Biochem.* **42**, 511-517 (1974).
14. Landau, B. R. & Sims, E. A. H. *J. biol. Chem.* **242**, 163-172 (1967).
15. Dully, C. C., Bocek, R. M. & Beatty, C. H. *Endocrinology* **84**, 855-860 (1969).
16. Das, I., Sie, H.-G. & Fishman, W. H. *Archs Biochem. Biophys.* **144**, 715-722 (1971).
17. Clarke, B. *Genetics* **79**, 101-113 (1975).

Activated macrophages induce vascular proliferation

MICROVASCULAR proliferation is essential to many biological processes, such as wound healing, but the mechanisms underlying this vascular response are poorly understood¹. Neovascularisation can be induced by extracts from various cell types, including malignant solid tumour cells², normal and viral-transformed (SV40) BALB/c 3T3 cells, and diploid human embryonic lung fibroblasts³, and neutrophils⁴. Extracts of mouse salivary gland⁵ and skin⁶ have been reported to induce vascular growth, but the relevance of these observations to the vascular proliferation that occurs in wound healing and chronic inflammation is unclear. Neovascularisation is also an important component of immunological reactions. Sidky and Auerbach reported increased vessel density in the skin during graft-versus-host reactions and attributed it to the lymphocyte⁷. Herman and associates⁸ and Anderson *et al.*⁹ found an increase in capillary density, and extensive proliferation of postcapillary venular endothelium, respectively, in lymph nodes undergoing strong immunological reactions. We have shown significant endothelial proliferation in delayed hypersensitivity reactions in the skin of guinea pigs at the time of maximal mononuclear cell infiltration¹⁰ and so we have investigated whether macrophages, an important component of immunological and non-immunological inflammatory reactions, might be involved in this vascular response. We report here that macrophages activated *in vivo* and *in vitro*, and media conditioned by these cells, induce vascular proliferation in the guinea pig cornea.

Neovascularisation was assayed in the normally avascular cornea of the guinea pig eye by introducing either cells or media into the stroma and observing the growth of vascular sprouts from the limbus into the cornea. The macrophages were obtained from the peritoneal cavities of untreated guinea pigs, or from guinea pigs or mice that had previously been injected with paraffin oil or thioglycollate, respectively. We have used the terms "non-activated" and "activated" to refer to macrophages from untreated or injected animals, respectively. The activated macrophages were richer in dense phase vacuoles and in pinocytotic vesicles. Macrophages (5×10^5) were injected into the corneal stroma 1.5 mm from the limbus in $10 \mu\text{l}$ of medium 199 (Table 1). Media conditioned by incubation with macrophages *in vitro* (legend to Table 2) were incorporated into Hydron (a polymer of hydroxyethylmethacrylate) as described by Langer and Folkman¹¹, and a 5- μl pellet was implanted in an aseptically created pocket in the corneal stroma¹². This polymer was used because it is non-inflammatory and permits a prolonged release of incorporated compounds¹¹. Corneas were examined daily with a slit-lamp stereomicroscope ($10-40\times$ magnification) for evidence of neovascularisation. After 7 d, just before killing, the animals were perfused intra-arterially with colloidal carbon, which filled the corneal blood vessels and provided a permanent record of the individual vascular response.

Excised corneas were fixed in buffered neutral formalin, flattened, photographed, and then processed for histological examination. A positive neovascular response was recorded when a dense brushwork of new capillary sprouts and hairpin loops grew from the adjacent limbal plexus, in a directional fashion towards the depot of test materials (Fig. 1a and b). When observed *in vivo*, proliferating capillaries were seen to grow $0.1-0.2 \text{ mm d}^{-1}$ and they underwent extensive branching as the response developed. Corneas with positive vascular responses did not become oedematous and histologically showed negligible infiltration by polymorphonuclear leukocytes. Responses were recorded as negative

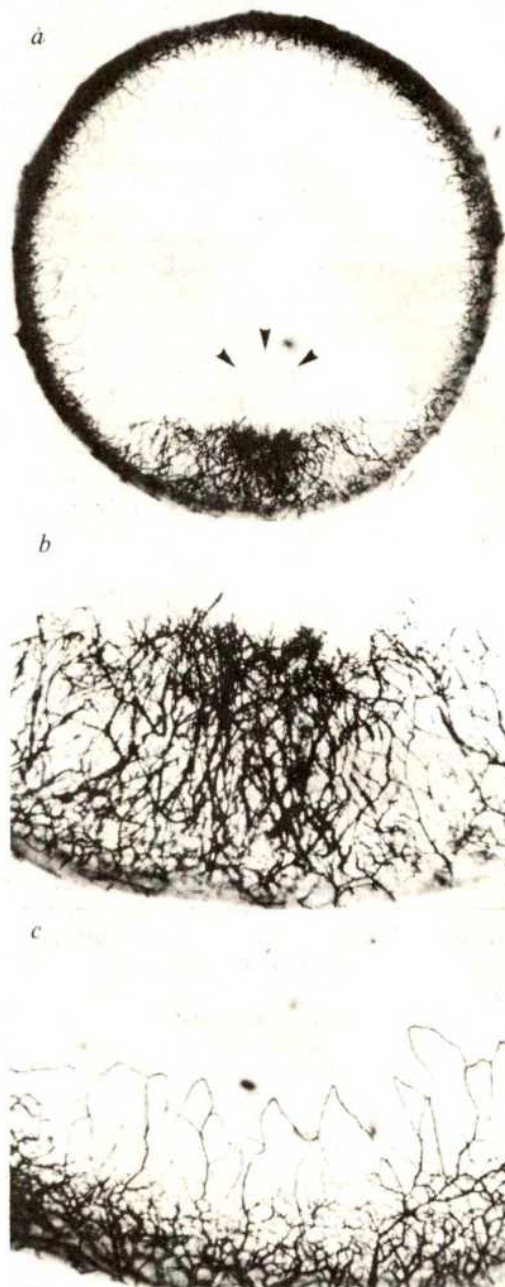


Fig. 1 Carbon-perfused vessels in Hartley albino guinea pig corneas 7 d after injection of macrophages or implantation of Hydron polymer. *a*, Positive neovascular response induced by homologous *in vivo*-activated (paraffin oil) peritoneal macrophages. Note directional ingrowth of small blood vessels from the limbal plexus adjacent to the injection site (arrows), and the normal vascular pattern around the remainder of the corneal circumference (whole mount preparation). *b*, Higher power view of positive response. The dense neovascular plexus consists of numerous small sprouts and dilated, tortuous loops, which have penetrated the corneal stroma in several planes. *c*, Typical negative response in test cornea implanted with Hydron plus unconditioned RPMI 1640 medium. Note the apparently normal limbal vessel pattern and the absence of both hairpin loops and sprouts (same magnification as *b*).

Table 1 Neovascularisation by various preparations of peritoneal macrophages injected into guinea pig corneas

Cell preparations	Total no. of corneas tested*	No. of negative	No. of positive	% Positive
Controls†	24	24	0	0
Homologous resident (non-activated)‡	12	11	1	8
Homologous activated§	18	4	14	78
Autologous activated¶	4	0	4	100
Isologous activated	8	2	6	75
Heterologous resident (non-activated)‡	12	11	1	8
Heterologous activated**	25	8	17	68
Heterologous macrophage incubated <i>in vitro</i> ††				
With latex	7	2	5	71
Without latex	4	3	1	25

*See text for criteria used in scoring neovascular responses and typical examples in Fig. 1.

†Inducing agents (paraffin oil, thioglycollate, peptone); collection media (M199); culture medium (RPMI 1640 \pm 5% foetal calf serum).

‡Resident peritoneal macrophages from Hartley albino guinea pigs and BALB/c mice were obtained from normal non-stimulated animals following lavage with serum-free medium 199 (M199).

§Homologous activated macrophages were obtained from peritoneal exudates in Hartley albino guinea pigs 3 d after an intraperitoneal injection of 30 ml of sterile paraffin oil. Whole exudates suspended in M199 were layered over Lymphocyte-Separation Media (Litton Bionetics) to remove contaminating red blood cells and granulocytes, washed twice and resuspended in M199 for intracorneal injection. These preparations were 85–95% viable (Trypan blue exclusion) and consisted of 85–90% macrophages, <15% lymphocytes, <5% granulocytes, <1% other cells.

¶In four Hartley albino guinea pigs, paraffin oil-induced peritoneal macrophages obtained from individual guinea pigs were tested in their own corneas.

||Paraffin oil-activated macrophages obtained from inbred (Strain II) guinea pigs were injected in the cornea of guinea pigs of the same strain.

**Activated macrophages obtained from BALB/c mice 3 d after intraperitoneal injection of 1 ml of 3% thioglycollate were collected, processed in the same manner as guinea pig exudates and injected in Hartley albino guinea pig corneas.

†† 4×10^6 cells in 1 ml RPMI 1640 \pm 5% foetal calf serum were plated in 35×10 mm Falcon plastic dishes and incubated at 37 °C with and without sterile latex particles (Dow Chemical, 1.09 μ m diameter, 50 beads per cell)¹⁶. After incubation for 1 h, non-adherent cells and latex were removed by washing, and adherent macrophages were detached by a 10-min treatment with 10 mM lidocaine, washed, suspended in RPMI 1640 and injected into test corneas.

when no growth, or only occasional capillary sprouts or hairpin loops, with no evidence of sustained directional growth were observed (Fig. 1c).

Tables 1 and 2 summarise the cumulative results of cells and media tested. Control materials (paraffin oil, thioglycollate, peptone; collection media, culture media incubated without cells; pellets of Hydron or Hydron and control media) showed activity in 3 out of 51 corneas tested (6%). Resident non-activated macrophages showed activity in 2 out of 24 corneas (8%). In contrast, *in vivo* activated macrophages (regardless of their source) induced a neovascular response in 41 out of 55 corneas tested (75%). In addition, macrophages stimulated *in vitro* by latex ingestion were more active (5 of 7 corneas) than unchallenged macrophages (1 of 4 corneas). Finally, incorporation of conditioned media from cultures of heterologous activated macrophages in Hydron pellets induced neovascularisation in 15 of 23 corneas (65%).

Our results demonstrate that macrophages can induce microvascular proliferation *in vivo* and they also point to some of the possible mechanisms for the reaction. First, from histological studies it seemed that the microvascular responses were not associated with an acute inflammatory response¹³. This was in contrast to other studies in which neovascularisation by macrophages was accompanied by acute inflammation¹⁴. Such a response complicates the issue of deciding which cell is

ultimately responsible for the reaction. Furthermore, in preliminary studies, we have been unable to produce corneal neovascularisation by implanting neutrophils or activated lymphocytes. Second, the neovascularisation could not be attributed solely to an immunological reaction since it was produced in a completely syngeneic cell system. These two points lead us to believe that the response is directly attributable to the macrophage, although some subtle interaction with other cell types cannot be strictly excluded. It is of interest that the responses to macrophages required their activation; namely, the macrophages either had to be obtained from peritoneal cavities following inflammation, or the cells had to be stimulated to phagocytose latex particles *in vitro*. There is ample evidence for increased macrophage function following various modes of stimulation¹⁵. Indeed, changes in phagocytic properties (for example, in secretion, in lysosomal contents) take place following stimulation of macrophages by various means. In the context of the possible *in vivo* significance of the phenomenon, it seems that the neovascularising properties of macrophages are inducible rather than constitutive. Finally, the results showing that macrophage-conditioned media contained an active material strongly suggests that the mechanisms responsible for the phenomenon may be by way of secretion of an active molecule. Macrophages can secrete a variety of macromolecules^{16–19} and characterisation of the active material is now in progress. Our

Table 2 Corneal neovascularisation induced by macrophage-conditioned media

Test material	Total no. of corneas tested	No. of negative	No. of positive	% Positive
Hydron and macrophage-conditioned media*	23	8	15	65
Hydron and control media†	12	11	1	8
Hydron carrier	15	13	2	13

*Macrophage-conditioned media were prepared by culturing peptone-induced BALB/c mouse peritoneal macrophages for 24 h in serum-free RPMI 1640 media¹⁶. After centrifugation, samples were dialysed for 72 h against RPMI 1640 to remove low molecular weight inhibitors of DNA and protein synthesis and concentrated on an Amicon UM05 filter. Conditioned medium (2.5 μ l; 0.5×10^6 – 2.5×10^6 cell equivalents) were then combined with an equal volume of sterile Hydron casting solution (prepared by dissolving the polymer in absolute alcohol at 37 °C)¹¹, and allowed to dry overnight before implantation.

†RPMI 1640 medium, incubated without cells and processed as described above.

observations therefore suggest that, among its diverse functions in the chronic inflammatory response, the macrophage may mediate microvascular proliferation.

We thank Dr S. Schlossman for his gift of Strain II guinea pigs, and Drs R. Langer and J. Folkman for assistance with the Hydron technique. This work was supported by a grant from the USPHS. M.A.G. is the recipient of an established investigatorship from the American Heart Association.

PETER J. POLVERINI

RAMZI S. COTRAN*

MICHAEL A. GIMBRONE, JR

EMIL R. UNANUE

Department of Pathology,

Peter Bent Brigham Hospital and Harvard Medical School,

721 Huntington Avenue,

Boston, Massachusetts 02115

Received 27 June; accepted 8 August 1977.

*To whom reprint requests should be addressed.

1. Folkman, J. & Cotran, R. S. *Int. Rev. exp. Path.* **16**, 207-248 (1976).
2. Folkman, J. *Cancer Res.* **34**, 2109-2113 (1974).
3. Klagsbrun, M., Knighton, D. & Folkman, J. *Cancer Res.* **36**, 110-114 (1976).
4. Fromer, C. H. & Klintworth, G. K. *Am. J. Path.* **82**, 157-167 (1976).
5. Hoffman, H., McAushan, B., Robertson, D. & Burnett, E. *Exp. Cell Res.* **102**, 269-275 (1976).
6. Wolf, J. E., Jr & Harrison, R. G. *J. invest. Derm.* **61**, 130-141 (1973).
7. Sidky, Y. A. & Auerbach, R. J. *J. exp. Med.* **141**, 1084-1100 (1975).
8. Herman, P. G., Yamamoto, I. & Mellins, H. Z. *J. exp. Med.* **136**, 697-714 (1972).
9. Anderson, N. D., Anderson, A. O. & Wyllie, R. G. *Am. J. Path.* **81**, 131-153 (1975).
10. Poverini, P. J., Cotran, R. S. & Sholley, M. M. *J. Immun.* **118**, 529-532 (1977).
11. Langer, R. & Folkman, J. *Nature* **263**, 797-800 (1976).
12. Gimbrone, M. A., Jr, Cotran, R. S., Leapman, S. B. & Folkman, J. *J. natn. Cancer Inst.* **53**, 413-427 (1974).
13. Clark, R. A. *et al. Surg. Forum* **27**, 16-18 (1976).
14. Fromer, C. H. & Klintworth, G. K. *Am. J. Path.* **81**, 531-544 (1975).
15. Unanue, E. R. *Am. J. Path.* **83**, 396-417 (1976).
16. Calderon, J., Williams, R. T. & Unanue, E. R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4273-4277 (1974).
17. Calderon, J. & Unanue, E. R. *Nature* **253**, 359-361 (1975).
18. Calderon, J., Kiely, J. M., Lefko, J. & Unanue, E. R. *J. exp. Med.* **142**, 151-164 (1975).
19. Leibovich, S. J. & Ross, R. *Am. J. Path.* **84**, 501-513 (1976).

Human virus-infected target cells lacking HLA antigens resist specific T-lymphocyte cytotoxicity

PRODUCTS of the major histocompatibility complex (MHC) are important as target structures in T lymphocyte-mediated cytotoxicity. In mice, virus (or hapten)-sensitized T cells are cytotoxic for specific virus-infected (or hapten-modified) target cells exclusively when syngeneic or at least identical for either the K or D region of the MHC¹⁻⁵. These findings led to the 'altered-self' hypothesis⁶ which assumed that cytotoxic T cells do not recognise specific viral antigens, but rather a modification of histocompatibility antigens induced by the viral infection. One prediction of this theory was that murine cells expressing viral antigens but lacking H-2 determinants would be resistant to T cell-mediated lysis. Indeed, this fact was established recently by Zinkernagel and Oldstone using murine F 9 teratoma cells⁷. There is still no evidence for the involvement of HLA antigens in the process of immune killing of virus-infected target cells by human T lymphocytes. But, Svedmyr and Jondal have shown that peripheral T cells from patients with infectious mononucleosis (IM)—and not from normal donors—killed specifically Epstein-Barr virus (EBV) genome-carrying B lymphoblastoid cell lines⁸ and Burkitt's lymphoma cells⁹. By contrast, these T cells were devoid of any cytotoxic activity against EBV negative cell lines. Similar data were obtained by other workers^{10,11}. Thus, peripheral T cells from patients with infectious mononucleosis seemed to be sensitised to viral coded determinants, and could presumably be used in a model to study T-lymphocyte killing of virus-infected cells in human. If, in man as well as mouse, the MHC is directly involved in the target structure recognised by virus-sensitized cytolytic T lymphocytes, HLA-lacking target cells

should be resistant to immune T-cell killing. We report here that Daudi cells, originating from a human Burkitt's lymphoma line and lacking HLA products¹²⁻¹⁴, are resistant to lysis by EBV-sensitized peripheral T lymphocytes from patients with infectious mononucleosis.

The experiments involved a ⁵¹Cr release assay according to Cerottini and Brunner¹⁵ with minor modifications. Targets were cells from several B-lymphoblastoid lines and effectors were peripheral T lymphocytes from patients with infectious mononucleosis. The EBV genome-carrying lines used were T-51 (ref. 16), 8866 (ref. 17) and Daudi, and the EBV-negative line was Ramos¹⁸. All lines were maintained in our laboratory. Effector cells were purified from the blood of six patients with infectious mononucleosis, as assessed by a positive Paul Bunnell-Davidson test. Peripheral lymphocytes were isolated on Ficoll-Hypaque gradients. As unpurified peripheral blood lymphocytes exerted nonspecific killing on cell lines⁹, further purification of T cells was required. This was achieved by removal of B cells either by EAC rosetting or by use of a cytotoxic anti-B antiserum¹⁹. These processes yielded 99% pure T lymphocytes as assessed by E rosette formation.

Peripheral T lymphocytes from patients with infectious mononucleosis were cytotoxic for cells from the two HLA-positive EBV genome-carrying lines T-51 and 8866, whereas they were unable to kill cells from the HLA-positive EBV genome-negative line Ramos (Fig. 1). These results are in complete agreement with the data of Svedmyr and Jondal⁸. Strikingly, Daudi cells, though containing EBV genome, were resistant to lysis by these EBV-sensitized T cells. The human cell line Daudi lacks HLA products as assessed by the absence

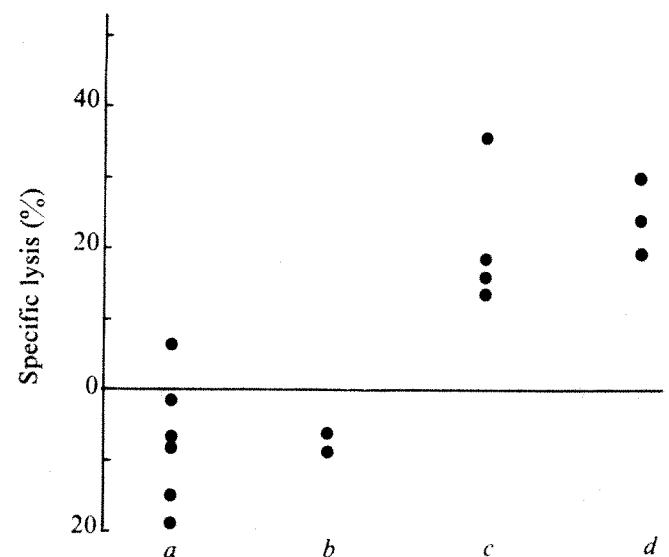


Fig. 1 Cytotoxicity of T lymphocytes from a patient with infectious mononucleosis towards target cell lines. Cytotoxicity tests were performed in U-shaped microtitre plates in a total volume of 0.2 ml RPMI 1640 medium supplemented with antibodies and 10% human AB serum (previously tested for absence of any cytotoxic activity). 2.5×10^4 labelled target cells and 1.25×10^6 or 2.5×10^6 effector cells were mixed (effector:target ratio of 50:1 or 100:1). Cultures were set up in triplicate. After 12-14 h of incubation at 37 °C in 5% CO₂ atmosphere, the supernatant of each culture was collected and ⁵¹Cr release was measured using a γ counter (Intertechnique). Simultaneously, spontaneous release in the presence of saponin was measured. Specific lysis was expressed according to the formula

$$\frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Total release} - \text{Spontaneous release}} \times 100$$

a, Daudi cell line; no HLA products, EBV genome positive. b, Ramos cells, HLA phenotype 3-5-X-Y, negative for EBV genome. c, T-51 cells, HLA phenotype 1-2-8-W27, EBV genome positive. d, 8866 cells; HLA phenotype 2-3-7; EBV genome positive.

of reactivity with anti-HLA antisera tested by cytotoxicity, absorption, immunofluorescence and immunoprecipitation¹²⁻¹⁴. Daudi cells express EBV-coded determinants, however, as shown by reactivity with relevant antibodies (anti-EBNA and MA)^{20,21}. Finally, Daudi cells are susceptible to antibody-mediated immune killing, since they could be lysed in ADCC assay using anti-EBV sera and normal lymphocytes²¹ and in complement-dependant cytotoxicity assay using different anti-B sera¹³. This is also established by our own data as shown in Table 1. Daudi cells were lysed by rabbit anti-human lymphocyte serum in presence of complement and not by anti-HLA serum. Also, ADCC was positive using anti-lymphocyte and anti-B cell sera, but negative using anti-HLA serum. According to results from murine models, it is tempting to assume that the resistance to lysis by EBV-sensitised T lymphocytes exhibited by Daudi cells is due to their lack of HLA products.

Table 1 Susceptibility of Daudi cells to lysis by antibodies

Target cell treated with*	% of specific cytotoxicity Antibody- and complement- dependent*	Antibody- dependent† cellular cytotoxicity (ADCC)
Normal AB serum	0.5	—
Normal rabbit serum††	0	2.9±0.6
Anti-HLA serum (Eluate from platelets)	0.5	8.5±1.2
Rabbit anti-human lymphocyte serum†	0	20.6±1.5
Human anti-B cell serum (Eluate from Daudi cells)	ND	26.0±0.9
Normal AB serum + guinea pig complement	1.8	—
Normal rabbit serum + guinea pig complement	2	—
Anti-HLA serum + guinea pig complement	1.3	—
Rabbit anti-lymphocyte serum + guinea pig complement	96.4±1.8	—

* 5×10^4 ⁵¹Cr-labelled Daudi cells were incubated with 150 µl of sera (1/30 dilution) for 30 min at 37 °C, then 100 µl of guinea pig serum (1/2 dilution) used as complement for 1 h. Cells were spun down from aliquots of cell supernatants and c.p.m. counted. % of specific cytotoxicity was calculated according to the formula

$$\frac{\text{c.p.m. total release} - \text{c.p.m. spontaneous release}}{\text{c.p.m. experimental release} - \text{c.p.m. spontaneous release}}$$

† 5×10^4 ⁵¹Cr-labelled Daudi cells were incubated with the sera for 30 min at 37 °C before addition of 10^6 human lymphocytes separated by gradient of Ficoll-Triosil. Final dilution of the sera was 1/500. After 16 h, percentage of cytotoxicity was calculated as above and results expressed as compared with the effect of AB sera (% of ⁵¹Cr-release in presence of experimental serum - % of ⁵¹Cr-release in presence of AB serum).

†† Absorbed on mouse cells.

This would imply that the HLA antigens are directly involved in the structure recognised by cytotoxic T lymphocytes at the surface of virus-infected target cells.

Analysis of the results of Svedmyr and Jondal⁸ shows a wide variability of the range of specific killing of each effector lymphocyte population towards different cell lines. This observation might be explained by a necessary requirement of HLA identity between target cells and effector cells, as suggested by murine models.

T lymphocytes from patients 3 and 4 did indeed exert higher specific killing for 8866 than for T-51 cells, while T lymphocytes from patient 6 were more cytotoxic for T-51 target (Table 2). This excludes a higher susceptibility to lysis of one cell line as compared with the other and may evoke a requirement of some HLA compatibility between effectors and targets. It should be noted, however, that T cells from patients 5 (HLA phenotype A.9 A.10 BW.35 BW.38) and 6 (A.9 A.19.2 B.14) were cytotoxic towards target cells with which they shared no HLA antigens.

Table 2 Cytotoxicity of T cells from individual patient with infectious mononucleosis cell lines

Patient	Target cells	Effector- target ratio	% ⁵¹ Cr release*	% specific lysis†
I	Daudi	—	32.1±2.15	—
		10:1	28.6±1.8	-5.1±2.7
		25:1	33.3±4.1	1.8±6.1
II	Daudi	—	38.5±0.1	—
		25:1	20.5±0.1	-29.2±0.15
		50:1	28.3±2.9	-16.5±4.7
III	Daudi	—	37.6±4.9	—
		50:1	25.8±1.3	-19.1±2.1
	T-51	—	27.6±1.2	—
		50:1	37.8±0.6	14±0.9
	8866	—	34.6±3.7	—
		50:1	50.5±2.2	24.3±3.4
	Ramos	—	28.7±1.3	—
		50:1	23.8±2.2	-6.8±3.1
IV	Daudi	—	37.6±4.9	—
		50:1	21.5±1.7	-25.8±2.7
		100:1	32.8±5.5	-7.8±8.8
	T-51	—	27.6±1.2	—
		50:1	27.9±1	0.9±1.9
		100:1	41±5.6	22.9±10.2
	8866	—	34.6±3.7	—
		50:1	43.3±0.9	-13.4±1.4
		100:1	54.1±0.2	29.7±0.3
	Ramos	—	28.7±1.3	—
		50:1	22.5±1.1	-8.6±1.5
		100:1	22.3±1.4	-9±1.9
V	Daudi	—	26.6±0.7	—
		50:1	31.9±2.3	7.1±3.1
	T-51	—	25.5±1.5	—
VI	Daudi	—	37.8±0.4	—
		50:1	39±2.2	2±3.5
		100:1	37.1±6.1	-1±9.8
	T-51	—	42.6±3.3	—
		50:1	63.7±2.1	36.7±3.6
		100:1	49.9±1.8	12.8±3.1
	8866	—	37.5±1.7	—
		50:1	44.1±0.5	10.6±0.8
		100:1	48.7±4.8	17.8±7.7

* %⁵¹Cr release (±s.e.m.) calculated according to the formula

$$\frac{\text{c.p.m. experimental release}}{\text{c.p.m. total release}} \times 100$$

† Values given ±s.e.m. methodology and specific lysis determination as in Fig. 1 legend.

Therefore in man, the histocompatibility identity did not seem to be as strongly required as in murine models.

But it is well established that series of irrelevant specificities can be detected on cell lines by usual HLA serotyping. For instance, T-51 cells additionally reacted with sera directed against HLA 10 and 21 and 8866 with anti-HLA 1-11-28-19-2-27-5-29-35-21 antisera. These irrelevant reactions could be due to alloantibodies directed to Ia-like B-cell antigens. It might be postulated that the expression of HLA antigens at the surface of cell lines allows a high degree of cross reactivity with alloantisera²², or that virus-infected cell possibly express new HLA specificities, as suggested in the mouse²³. If the latter were so, this would explain why cell lines would not apparently be submitted to strong allogeneic restriction when used as target cells. It remains possible that much higher specific killing could be observed if the target cells were autologous EBV-infected B lymphocytes from the infectious mononucleosis patient whose T cells are used as effectors. This possibility is presently under investigation.

But, regardless of the apparent lack of allogeneic restriction in this model, the fact that Daudi cells are resistant to lysis by EBV-sensitised T cells is the first evidence to suggest direct HLA involvement in anti-viral T-cell killing in man.

We thank Mrs Colette Chereau, Maryvonne Guillard, Marielle Dutheil and Yvonne Thies for technical assistance. This work was supported by grants from Inserm and Institut Gustave-Roussy, Villejuif, France.

Note added in proof: After submission of our paper, another example of implication of HLA in the target structure of T-cell killing was published (Goulmy, E., Termijtelen, A., Bradley, B. A. and van Rood, J. J. *Nature* **266**, 544-546, 1977) concerning the response to the H-4 antigens. In this case the authors found an allogeneic restriction to HLA-2.

T. TURSZA

Laboratoire d'Immuno-hematologie et Service de Medecine,
Institut Gustave Roussy, 94800 Villejuif, France

W. H. FRIDMAN

A. SENIK

Laboratoire d'Immunologie Cellulaire,
IRSC, 94800 Villejuif, France

A. TSAPIS

Laboratoire d'Immunochimie et d'Immunopathologie
(Inserm U108),
Institut de Recherches sur les Maladies du Sang,
Hôpital Saint-Louis

M. FELLOUS

Laboratoire de Genetique de la Transplantation Humaine
(Inserm U93),
Institut de Recherches sur les Maladies du Sang,
Hôpital Saint-Louis, 75010 Paris, France

Received 15 March; accepted 26 August 1977.

1. Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701-702 (1973).
2. Koszinowski, U. & Ertl, H. *Nature* **255**, 522-524 (1975).
3. Shearer, G. M. *Eur. J. Immun.* **4**, 527-533 (1974).
4. Schrader, J. W. & Edelman, G. M. *J. exp. Med.* **143**, 601-614 (1976).
5. Burakoff, S. J., Germain, R. N., Dorf, M. E. & Benacerraf, B. *Proc. natn. Acad. Sci. U.S.A.* **73**, 625-629 (1976).
6. Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. *Transplant. Rev.* **29**, 89-124 (1976).
7. Zinkernagel, R. M. & Oldstone, M. B. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3666-3670 (1976).
8. Svedmyr, E. & Jondal, M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1622-1626 (1975).
9. Jondal, M., Svedmyr, E., Klein, E. & Singh, S. *Nature* **255**, 405-407 (1975).
10. Hutt, L. M., Huang, Y., Dascomb, H. E. & Pagano, J. S. *J. Immun.* **115**, 243-248 (1975).
11. Royston, I., Sullivan, J. L., Periman, P. O. & Perlman, E. *New Engl. J. Med.* **293**, 1159-1163 (1975).
12. Fellous, M., Mortchelewicz, F., Kamoun, M. & Dausset, J. in *Histocompatibility Testings* (ed. Kissmeyer-Nielsen) 708-711 (Munksgaard Copenhagen, 1975).
13. Jones, E. A., Goodfellow, P. N., Bodmer, J. G. & Bodmer, W. F. *Nature* **256**, 650-653 (1975).
14. Reisfeld, R. A., Seives, E. D., Pellegrino, M. A., Ferrone, S. & Poulik, M. D. *Immunogenetics* **2**, 184-197 (1975).
15. Cerrotini, J. C. & Brunner, K. T. in *In Vitro Methods in Cell Mediated Immunity* (eds Bloom, B. R. & Glade, P. R.) 369, (Academic, New York and London, 1971).
16. Orkin, S. H., Buchanan, P. D., Ryount, W. G., Reisner, H. & Littlefields, G. W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2401-2405 (1973).
17. Pious, D., Bodmer, G. J. & Bodmer, W. F. *Tissue Antigens* **4**, 247-256 (1974).
18. Steinitz, M. & Klein, G. *Virology* **70**, 570-573 (1976).
19. Tursz, T., Preudhomme, J. L., Labaume, S., Matuchansky, C. & Seligmann, M. *J. clin. Invest.* **60**, 405-410 (1977).
20. Klein, G., Wiener, F., Zech, L., Zur Hausen, H. & Reedman, B. *Int. J. Cancer* **14**, 54-64 (1974).
21. Jondal, M. *Clin. exp. Immun.* **15**, 1-5 (1976).
22. Dick, H. M., Bodmer, J. G., Steel, C. M., Crichton, W. B. & Evans, J. in *Histocompatibility Testings* (ed. Kissmeyer-Nielsen) 671-676 (Munksgaard, Copenhagen, 1975).
23. Garrido, F., Schirmacher, V. & Festenstein, H. *Nature* **259**, 228-229 (1976).

Evidence for an H-2/viral protein complex on the cell surface as the basis for the H-2 restriction of cytotoxicity

CYTOTOXIC T lymphocytes generated in mice by immunisation with syngeneic Friend virus (FV)-induced tumour cells show H-2 restriction, that is, their cytotoxicity is specific not only for FV-associated antigenic determinants expressed on the surfaces of potential target cells but also for products of genes associated with the major histocompatibility complex, H-2 (ref. 1). We report here the results of studies concerning the role of molecules governed by genes in the K and D regions of the H-2 complex in the stimulation of FV-specific immune responses. Studies in other viral systems have shown that H-2-restricted T-cell

killing can be associated with either K or D region determinants, that is, cytotoxic T cells from virus-infected mice will kill virus-infected target cells which share either the K or D region with the infected strain². Our studies, however, indicate that in the FV system there is a selective participation of H-2 determinants in virus-specific killing by cytotoxic T cells: BALB.B mice immunised with cultured, syngeneic FV-induced tumour cells produce T cells with a cytotoxicity for other FV-induced tumour cells which depends only on identity at the H-2D (and not the H-2K) region of the H-2^b haplotype. This H-2D^b restriction in FV-specific cytotoxicity correlates with previous observations concerning: (1) partial co-capping of H-2D^b and not H-2K^b specificities following treatment with anti-FV antiserum³; (2) selective incorporation of H-2D^b and not H-2K^b specificities into mature FV particles⁴; and with our present observation of (3) blocking of T-cell cytotoxicity by anti-H-2D^b and not anti-H-2K^b antisera. We suggest that the basis for this correlation is the formation of H-2D^b/viral protein complexes on the surfaces of FV-infected cells, forming an 'altered self' molecule recognised by cytotoxic T cells as previously proposed for the mechanism of H-2 restriction⁵.

Mice for these studies were of the BALB/c strain (H-2^d) or of congenic strains differing at the H-2 complex: BALB.B (H-2^b from C57BL/10) or BALB.G (H-2^g, a recombinant haplotype originating from an H-2^b/H-2^d heterozygote, from HTG/Go). Cultured FV-induced tumour cell lines derived from mice of these strains were of the HFL series⁶: HFL/b from BALB.B, HFL/d from BALB/c, and HFL/g from BALB.G. Cells of each of these lines express both the FV-induced FMR cell-surface antigen and the virus envelope antigens associated with the gp70 protein of FV.

BALB/c, BALB.B and BALB.G mice were immunised by intraperitoneal inoculation of syngeneic HFL tumour cells. Peritoneal exudate cells (PEC) taken on day 5 after the second or third inoculation were assayed for lymphocyte-mediated cytotoxicity (LMC) by the method of Burke *et al.*⁷, using ⁵¹Cr-labelled HFL tumour cells as targets. Controls for the sensitivity of the LMC reaction to pretreatment with anti-Thy-1.2 antiserum and complement were included in most experiments and demonstrated that the killing was attributable to T cells.

Table 1 H-2 restriction of T-cell cytotoxicity

Mice immunised*	H-2 haplotype	Syngeneic immunising cells	Target cell cytotoxicity (% ⁵¹ Cr release)			
			HFL/b	HFL/g	HFL/d	HFL/k
BALB.B	b b b b	HFL/b	18	15	0	0
BALB.G	d d d b	HFL/g	20	37	1	0
BALB/c	d d d d	HFL/d	0	0	0	0

In the LMC, 2.5×10^6 immune PEC in 1 ml and 5×10^4 ⁵¹Cr-labelled tumour cells in 0.1 ml (PEC-target cell ratio, 50:1) were mixed and incubated at 37 °C in 5% CO₂ atmosphere for 6 h. The cells were suspended in Medium 199 plus 20% foetal calf serum. Specific release of radioactivity into supernatant was compared with that from tumour cells alone frozen and thawed four times.

*PEC were collected at previously determined peaks of activity, 5 d after the second or third immunisation.

One criterion used to define the extent of H-2 identity with target cells which would permit the T cells to exert their cytotoxicity was direct cross reactivity in the LMC assay (Table 1). The marked cross reactivity of BALB.B anti-HFL/b and BALB.G anti-HFL/g PEC indicated that H-2D^b identity was sufficient to permit killing to occur. Whether H-2K^b identity was also sufficient could not be tested by this technique, since no target cell was available which shared this genotype but differed in the H-2D region. The observation that BALB.G anti-HFL/g PEC were ineffective against HFL/d tumour cell targets suggested that H-2K^d, H-2I^d and H-2S^d region identity was not sufficient to permit killing.

Another criterion used to elucidate the precise requirements for *H-2* identity between immunising and target cells, was the capacity of high concentrations of specific anti-*H-2* antisera added to the reaction mixture to block T-cell killing of syngeneic target cells⁸. Accordingly, we tested the capacity of antisera specific for either *H-2K^b* or *H-2D^b* antigenic determinants to inhibit the cytotoxicity of BALB.B anti-HFL/b PEC for HFL/b target cells. The results (Fig. 1) showed that anti-*H-2D^b* antibodies were effective in blocking the reaction whereas anti-*H-2K^b* antibodies showed no greater inhibitory effect than did irrelevant anti-*H-2K^k* antibodies. Preliminary attempts to detect blocking in this same reaction by antibodies to specific FV proteins met with little success; of the various antisera tested, only one of three available pools of goat anti-gp70 antiserum demonstrated significant inhibitory activity (data not shown).

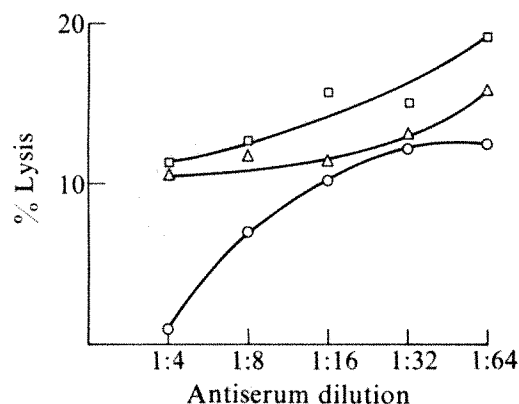


Fig. 1 Lysis of HFL/b target cells by peritoneal exudate cells (PEC) from BALB.B mice immunised with HFL/b tumour cells. PEC (2.5×10^6) in 0.2 ml and 5×10^4 ^{51}Cr -labelled tumour cells in 0.1 ml were mixed together with 0.1 ml of diluted anti-*H-2* antiserum and assayed for lymphocyte-mediated cytotoxicity as noted in Table 1. Antisera used were: \circ , anti-*H-2D^b* (BALB/c anti-BALB.G normal spleen cells); \triangle , anti-*H-2K^b* [(A \times BALB.G) F_1 anti-BALB.B normal spleen cells]; \square , anti-*H-2K^k* (BALB.B anti-BALB.K normal spleen cells).

These experiments indicate that T-cell recognition of foreign antigenic determinants on FV-induced *H-2^b* and *H-2^d* tumour cells involved specificities associated with both the *H-2D^b* molecule and a virus molecule, perhaps gp70. The *H-2K^b* and *H-2K^d* molecules did not seem to be involved in this recognition, and no assessment of the possible involvement of the *H-2D^d* molecule could be made, since cytotoxic activity has never been detected in any of several experiments with PEC from BALB/c mice immunised with HFL/d tumour cells. Our findings correlate with those of recent studies in which disrupted FV particles collected from the serum of infected (BALB/c \times BALB.B) F_1 mice (*H-2^b/H-2^d*) were examined for *H-2* antigen content and found to have incorporated *H-2D^b* but not *H-2K^b*, *H-2D^d* or *H-2K^d* determinants⁴. These two sets of experiments strongly imply that a physical association between a viral molecule and the *H-2D^b* molecule occurs on cell surfaces which is based on an affinity between the two molecules which does not exist between the viral molecule and the *H-2K^b*, *H-2D^d* or *H-2K^d* molecules.

Two further sets of experiments provide an indication that this molecular association may be of importance *in vivo*. First, the rejection of grafts of cultured, syngeneic HFL cells was correlated with the presence of *H-2D^b* molecules (Table 2). All BALB.B mice and 76% of BALB.G mice rejected HFL/b and HFL/g cell grafts, respectively, whereas only 8% of BALB/c mice rejected HFL/d cell grafts.

Second, we attempted to demonstrate that the antigenic specificities of cultured HFL tumour cells recognised by immune T cells were the same as those of primary FV-infected cells *in vivo* by carrying out adoptive transfer experiments (Table 3). Spleen

Table 2 Survival of mice challenged with syngeneic tumour cells

Recipient mice*	Tumour cells	Cell dose	No. survivors/no. inoculated
BALB.B	HFL/b	5×10^6	57/57
BALB.G	HFL/g	5×10^6	16/21
BALB/c	HFL/d	5×10^6	1/12
		5×10^5	2/14

*Mice were inoculated i.p. with syngeneic tumour cells and observed for 8 weeks.

cells from BALB.B and BALB.G donor mice which had rejected syngeneic HFL cell grafts were injected into irradiated (350 R) non-immune syngeneic recipient mice which then received a high dose of FV. Control recipients of non-immune spleen cells developed the pronounced splenomegaly characteristic of the FV disease, whereas recipients of immune spleen cells showed no splenomegaly. Pretreatment of the immune donor spleen cells with anti-Thy-1.2 antiserum and complement abolished their capacity to transfer immunity to the disease.

Table 3 Adoptive transfer of immunity to FV by syngeneic T cells from mice immunised with syngeneic HFL tumour cells

Irradiated recipients*	Mean spleen weights \pm s.d. (g)		
	A†	B	C
BALB.B	0.15 ± 0.01	2.31 ± 0.27	2.33 ± 0.07
BALB.G	0.10 ± 0.01	1.41 ± 0.21	

*Recipient mice were irradiated (350 R) and inoculated with 1.5×10^7 syngeneic spleen cells i.v.; 24 h later the mice received 3,000 FFU of FV i.v. Spleen weights were determined after 30 d or earlier if mice appeared moribund. Each group included 3–5 mice.

†Group A received immune syngeneic spleen cells pretreated with normal mouse serum and complement; group B received immune syngeneic spleen cells pretreated with anti-Thy-1.2 serum plus complement; group C received syngeneic spleen cells from unimmunised mice.

FV-infected cells which express the *H-2^b* or *H-2^d* but not the *H-2^k* haplotypes show cross-reacting virus-associated antigenic specificities capable of eliciting a cytotoxic T-cell response directed against specificities associated with the *H-2D^b* molecule. This immunogenicity seems to be the result primarily of the formation on the cell surface of an *H-2D^b*/viral molecule complex, but the capacity to form such a complex is not, in general, a property of other *H-2D* or of *H-2K* molecules. It seems likely that this phenomenon is, at least in part, the basis of the observation⁹ that *H-2* type is a major determinant of susceptibility or resistance to viral leukaemogenesis in mice.

This work was supported by a contract with the Virus-Cancer Program and by a grant, both from the National Cancer Institute. K.J.B. was supported by a training grant from the National Cancer Institute. We acknowledge the gift of some of the anti-MuLV protein antisera used from Drs M. Strand and J. T. August.

KENNETH J. BLANK

FRANK LILLY

Department of Genetics,
Albert Einstein College of Medicine,
Bronx, New York 10461

Received 21 July; accepted 22 August 1977.

- Blank, K. J., Freedman, H. A. & Lilly, F. *Nature* **260**, 250–252 (1976).
- Doherty, P. C. & Zinkernagel, R. M. *J. exp. Med.* **141**, 502–508 (1975).
- Bubbers, J. E., Steeves, R. A. & Lilly, F. *Proc. Am. Ass. Cancer Res.* **17**, 93 (1976).
- Bubbers, J. E. & Lilly, F. *Nature* **266**, 458–459 (1977).
- Zinkernagel, R. M. & Doherty, P. C. *Nature* **248**, 701–702 (1974).
- Freedman, H. A. & Lilly, F. *J. exp. Med.* **142**, 212–223 (1975).
- Berke, G., Sullivan, K. A. & Amos, D. B. *J. exp. Med.* **135**, 1334–1348 (1972).
- Shrader, J. W. & Edelman, G. M. *J. exp. Med.* **143**, 601–614 (1976).
- Lilly, F. *J. exp. Med.* **127**, 465–473 (1968).

Effect of protease inhibitors and substrates on deoxycorticosterone binding to its receptor in dog MDCK kidney cells

THE dog kidney MDCK cell line contains a protein in its cytosol that specifically binds deoxycorticosterone (DOC) and can concentrate DOC in its nuclei. A Scatchard analysis of ^3H -DOC binding shows that the K_d is about 8×10^{-8} M (refs 1, 2). We describe here a study of the effects of various protease inhibitors on DOC binding to its receptors. The DOC receptor in MDCK cells may contain a specific structure which recognises protease inhibitors.

The protease inhibitors, phenylmethyl sulphonylfluoride³ (PMSF), tosyl-lysine chloromethyl ketone⁴ (TLCK) and tosylamide-phenylethyl-chloromethyl ketone⁵ (TPCK) eliminate specific ^3H -DOC binding activity in the MDCK cytosol. This occurs both when these compounds are present in the homogenising medium and when added to the 104,000g cytosol just before ^3H -DOC. The elimination of ^3H -DOC binding could be due to these protease inhibitors either interacting with the DOC receptor or preventing a proteolytic process necessary for activation of pro-receptor for DOC. To distinguish between these possibilities, we incubated the 104,000g cytosol with 3×10^{-8} M ^3H -DOC for 2.5 h at 0 °C. By this time, equilibrium binding of the ^3H -DOC has been achieved. Then a 1,000-fold molar excess of nonradioactive DOC, 1 mM PMSF, 1 mM TLCK, or 0.5 mM TPCK was added to aliquots of the cytosol. In 1 h the three protease inhibitors eliminated specific ^3H -DOC binding; whereas the binding of ^3H -DOC by the untreated cytosol did not change (Table 1). We therefore conclude that these protease

Table 1 Effect of protease inhibitors on ^3H -DOC binding in MDCK cell cytosol

Sample	Bound ^3H -DOC (c.p.m. per mg protein)*
^3H -DOC (3×10^{-8} M) 2.5 h incubation	220,300
^3H -DOC (3×10^{-8} M) + DOC (3×10^{-5} M) 2.5 h incubation	19,300
^3H -DOC (3×10^{-8} M) 3.5 h incubation	214,900
^3H -DOC (3×10^{-8} M) + DOC (3×10^{-5} M) 3.5 h incubation	19,900
^3H -DOC (3×10^{-8} M) 2.5 h incubation, then	
PMSF (1 mM) 1 h	21,600
TLCK (1 mM) 1 h	22,000
TPCK (0.5 mM) 1 h	20,000
DOC (3×10^{-5} M) 1 h	19,800

MDCK cells were cultured in Dulbecco-Vogt modified Eagles medium with 10% foetal calf serum on 100-mm Falcon tissue culture dishes. Two days after cell growth had stopped, the cells were washed once with cold phosphate-buffered saline and once with the homogenising solution, 10 mM Tris pH 7.4 + 2 mM EDTA. The cells were removed from the dish with a rubber policeman and homogenised in a Dounce homogeniser. The homogenate was centrifuged at 104,000g for 1 h. The resulting cytosol was incubated at 0 °C with 3×10^{-8} M ^3H -DOC (48 Ci mmol, New England Nuclear). An aliquot was also incubated with 3×10^{-8} M ^3H -DOC + 3×10^{-5} M non-radioactive DOC. After 2.5 h at 0 °C, aliquots from the 3×10^{-8} M ^3H -DOC sample were incubated with 1 mM PMSF, 1 mM TLCK, 0.5 mM TPCK, or 3×10^{-5} M non-radioactive DOC for 1 h at 0 °C. Bound steroid was separated from unbound steroid on BioGel P-10 columns (1 × 10 cm). Samples were done in duplicate and the results averaged. Variation was less than 10%. Fractions were counted in a liquid scintillation cocktail containing Omnifluor (New England Nuclear), Triton X-100 and toluene. Thin-layer chromatography, 100 min using dichloromethane-acetone (80:20) as the developing solvent, shows that incubation of ^3H -DOC with PMSF, TPCK or D-TME does not alter the chromatographic behaviour of ^3H -DOC.

*220,300 c.p.m. per mg = 1.26×10^{-11} mol per mg protein.

Table 2 Dependence of inhibition of ^3H -DOC binding on the concentration of protease inhibitors

Compound	Concentration (M)	% Inhibition
TPCK	1.5×10^{-6}	97
	1.5×10^{-7}	88
	3.0×10^{-8}	38
PMSF	1.5×10^{-5}	50
	1.5×10^{-6}	34
TLCK	3×10^{-4}	47
	1.5×10^{-5}	28

MDCK cell cytosol (with 4 mM dithiothreitol in the homogenising buffer) was incubated with 3×10^{-8} M ^3H -DOC for 1.5 h. Aliquots were then incubated for 2.5 h with 6×10^{-6} M DOC, or the above compounds. Bound steroid was separated from unbound steroid on Sephadex G-25 columns. Per cent inhibition refers to the inhibition of specifically bound counts, determined by subtracting the amount of ^3H -DOC bound in presence of 6×10^{-6} M DOC from the amount of ^3H -DOC bound in the absence of 6×10^{-6} M DOC.

inhibitors interact directly with the DOC receptor. Since DOC binding was inhibited by concentrations of protease inhibitors that were several orders of magnitude greater than the concentration of ^3H -DOC (Table 1), we conducted more detailed studies at lower concentrations of inhibitor to determine if inhibition of DOC binding occurred at concentrations which would be indicative of stoichiometric reaction with the DOC receptor. We also sought to determine differences in the affinity of these compounds for the DOC receptor. As shown in Table 2, TPCK apparently has a high affinity for the DOC receptor, since only a fivefold excess concentration inhibits DOC binding by nearly 90%. Furthermore, PMSF, TPCK, and TLCK all have different affinities for the DOC receptor in MDCK cells.

Such inhibition of DOC binding by protease inhibitors could be due to interaction either with a specific structure in the DOC receptor that recognises protease inhibitors or of a nonspecific type that denatures the DOC receptor. One way in which TPCK or TLCK might denature the DOC receptor would be to bind the thiol groups⁶⁻⁸ on the receptor. This possibility is unlikely, however, since TLCK and TPCK can inhibit ^3H -DOC binding to MDCK cytosol in the presence of 4 mM dithiothreitol and since TPCK is effective at concentrations as low as 10^{-7} M.

Additional observations with protease substrates also support our postulate that the DOC receptor in MDCK cells contains a specific structure that recognises the protease inhibitors. Moreover, this specific structure may be similar to that recognised by the protease inhibitors in proteases. Table 3 shows that four protease substrates, *p*-toluenesulphonyl-L-arginine methyl ester (TAME), L-tryptophan methyl ester (TME), acetyl-L-tyrosine methyl ester (ATEE), and benzoyl-L-arginine ethyl ester (BAEE) inhibit ^3H -DOC binding to MDCK cell cytosol. A more detailed examination of the relationship of the structure of the substrate and its effectiveness as an inhibitor of DOC

Table 3 Effect of protease substrates on ^3H -DOC binding

Substrate	% Inhibition
1 mM L-TAME	88
1 mM D-TAME	95
10 mM L-TME	73
10 mM D-TME	78
1 mM D-TME	25
1 mM L-ATEE	43
5 mM L-BAEE	60

Inhibition of binding of 3×10^{-8} M ^3H -DOC to MDCK cytosol by protease substrates was measured as described in Table 1, except that 4 mM dithiothreitol was included in the homogenising buffer.

binding was conducted for arginine and derivatives (Table 4). D-TAME produced 76% inhibition at 1.5×10^{-6} M, while 6×10^{-5} M tosyl-L-arginine was required to produce 46% inhibition and 10^{-2} M L-arginine produced only 17% inhibition. These findings emphasise the importance of the ester structure in effecting inhibition of DOC binding.

We then determined if protease substrates and inhibitors reversibly inhibit ^3H -DOC binding to its receptor. MDCK cytosol was incubated with 3×10^{-8} M ^3H -DOC and D-TME to eliminate specific ^3H -DOC binding. The inhibited cytosol was filtered through a Sephadex G-25 column to remove unbound D-TME, and the filtrate could then bind ^3H -DOC (Table 5). Similar results were found for inhibition of ^3H -DOC binding to MDCK cell cytosol by D-TAME. We conclude that the inhibition of ^3H -DOC binding to MDCK cytosol by D-TME and D-TAME is reversible. In contrast, a similar experiment with the protease inhibitor TPCK showed that the inhibition of DOC binding by TPCK is not reversible (Table 5). This suggests that TPCK binds covalently to the DOC receptor, as would be expected since TPCK is a covalent inhibitor of chymotrypsin.

Table 4 Dependence of inhibition of ^3H -DOC binding on the concentration of protease substrates

Compound	Concentration in cytosol (M)	% Inhibition
L-TAME	1.5×10^{-5}	55
D-TAME	6×10^{-6}	95
	1.5×10^{-6}	76
Tosyl-L-arginine	1.5×10^{-4}	84
	6×10^{-5}	46
L-Arginine	1.0×10^{-2}	17
	5×10^{-3}	3

MDCK cell cytosol (with 4 mM dithiothreitol in the homogenising buffer) was incubated with 3×10^{-8} M ^3H -DOC for 1.5 h. Aliquots were then incubated for 2.5 h with 6×10^{-6} M DOC, or the above compounds. Bound steroid was separated from unbound steroid on Sephadex G-25 columns. Per cent inhibition refers to the inhibition of specifically bound counts, determined by subtracting the amount of ^3H -DOC bound in presence of 6×10^{-6} M DOC from the amount of ^3H -DOC bound in the absence of 6×10^{-6} M DOC.

Although the details of the mechanism of inhibition of ^3H -DOC binding are not known, several structural features might be anticipated from the well understood mechanism of inhibition of proteases by PMSF, TLCK and TPCK^{2-6,9}. PMSF inhibits a broad class of proteases by binding covalently to the active serine residue, while TLCK and TPCK inhibit by binding covalently to a histidine residue spatially adjacent to the serine. Since both protease substrates and inhibitors prevent DOC binding, it is possible that a structure similar to that of the serine proteases is present in the DOC receptor. The DOC receptor is unique, however, in binding both a trypsin substrate (TAME) and a chymotrypsin inhibitor (TPCK). This might be expected for a receptor that recognises a steroid nucleus rather than specific amino acid residues.

An important question is whether protease inhibitors and substrate recognise only the DOC receptor or a structural feature common to several steroid binding proteins. Our evidence suggests that the inhibition of steroid binding by protease inhibitors and substrates is a more general phenomenon. For example, PMSF, TLCK, TPCK, TME, and TAME inhibit the binding of ^3H -oestradiol to purified rat α foetoprotein (M.B. and D.F., in preparation). In addition, PMSF, TLCK, and TPCK inhibit the binding of ^3H -dexamethasone and ^3H -oestradiol

to rat kidney cytosol (M.E.B., N. Anderson, D. Vaughn, and D.F., unpublished results).

From our results, we cannot indicate whether the steroids and protease inhibitors and substrates bind to the same active site in the steroid receptor or if there is a specific catalytic function associated with the ability of steroid receptors to bind protease inhibitors and substrates.

Table 5 Reversibility of inhibition of DOC binding

Condition	% Inhibition by TME (15 mM) TPCK (1.5 μM)	
a, Inhibitors present with ^3H -DOC-receptor complex for 1 h*	92	100
b, Inhibitors in (a) removed by filtration on G-25 Sephadex; followed by re-equilibration with ^3H -DOC for 2 h†	9	95

*MDCK cell cytosol was incubated with 3×10^{-8} M ^3H -DOC for 2 h then aliquots were incubated for 1 h with 15 mM D-TME or 1.5×10^{-6} M TPCK. Bound steroid was separated from unbound steroid on Sephadex G-25 columns.

†Aliquots of 1 ml cytosol which had been incubated with 3×10^{-8} M ^3H -DOC for 3 h with or without 15 mM D-TME or 1.5×10^{-6} M TPCK for 1 h in (a) were filtered on Sephadex G-25 columns to remove unbound ^3H -DOC, D-TME, or TPCK. Then the filtrate was incubated with 3×10^{-8} M ^3H -DOC with or without 6×10^{-6} M DOC for 2 h. Bound steroid was separated from unbound steroid on Sephadex G-25 columns. % Inhibition refers to the inhibition of specifically bound counts, which were determined by subtracting the amount of ^3H -DOC bound in the presence of 6×10^{-6} M DOC from the amount of ^3H -DOC bound in the absence of 6×10^{-6} M DOC.

Nevertheless, two important applications of our results should be noted. First, the reversible inhibition of steroid binding by the D forms of TAME and TME suggest that it may be possible to purify steroid receptors by using the protease substrates as the ligand in affinity chromatography. To date, most attempts at purification of steroid receptors by affinity chromatography using steroid ligands have been unsuccessful, apparently because the high affinity of the steroid ligand for the receptor makes difficult the elution of the receptor in an active state or in high yield^{10,11}. With D-TAME or D-TME, however, the lower affinity of the steroid receptor for the protease substrate could improve the yield of receptor. Second, a new class of compounds which could react with steroid receptors *in vivo* is suggested. PMSF, TPCK, and TLCK enter cells, covalently, interact with proteins, and are unstable at pH 7.4 (37 °C)¹². Therefore, inhibitors could react with steroid receptors regionally with little toxicity or effect in areas of the organism distant from the point of application. Such compounds, or more selective protease inhibitors, might prove useful in treating localised steroid dependent tumours. The ability of protease inhibitors and substrates to block steroid binding to receptors may thus provide information on both structure and function of the steroid receptors.

We thank D. Vaughn for assistance and Dr N. Anderson for discussion of his model of formation of steroid receptors from proreceptors in cell cytosol. This work was supported by grants from the USNIH (USPHS AM 14915 and USPHS CA 15796).

MICHAEL E. BAKER
DARRELL D. FANESTIL

Division of Nephrology,
Department of Medicine, M-013,
University of California, San Diego,
La Jolla, California 92093

Received 7 October 1976; accepted 1 August 1977.

- ¹ Ludens, J. H., Vaughn, D. A., Mawe, R. C., Saier, M. H. & Fanestil, D. D. *Fedn Proc.* **34**, 663 (1975).
- ² Ludens, J. H., Vaughn, D. A., Mawe, R. C. & Fanestil, D. D. *J. Steroid Biochem.* (in the press).
- ³ Fahrney, D. E. & Gold, A. M. *J. Am. Chem. Soc.* **85**, 997-1000 (1963).
- ⁴ Shaw, E. M., Mares-Guia & Cohen, W. *Biochemistry* **4**, 2219-2224 (1965).
- ⁵ Schoellmann, G. & Shaw, E. *Biochemistry* **2**, 252-255 (1963).
- ⁶ Shaw, E. M. in *The Enzymes* **1**, 3rd ed. (ed. Boyer, P. D.) 91-146, (Academic, New York, 1970).
- ⁷ Stein, M. J. & Liener, J. E. *Biochem. biophys. Res. Commun.* **26**, 376-382 (1967).
- ⁸ Bender, M. L. & Brubacher, L. J. *J. Am. Chem. Soc.* **88**, 5880-5889 (1966).
- ⁹ Shaw, E. M. in *Proteases and Biological Control* (eds Reich, E., Rifkin, D. B. & Shaw, E.) 455-465 (Cold Spring Harbor Laboratory, New York, 1975).
- ¹⁰ Ludens, J. H., DeVries, J. R. & Fanestil, D. D. *J. biol. Chem.* **247**, 7533-7538 (1972).
- ¹¹ Kuhn, R. W., Schrader, W. T., Smith, R. G. & O'Malley, B. W. *J. biol. Chem.* **250**, 4220-4228 (1975).
- ¹² Gold, A. M. *Meth. Enzym.* **2**, 706-711 (1967).

Non-sequential expression of multiple immunoglobulin classes by isolated B-cell clones

As humoral immunity to a given antigen is generally thought to display an early IgM response which is later dominated by IgG synthesis, it has long been thought that clones of antibody-producing cells switched from μ to γ (refs 1-5). The sequence of appearance of immunoglobulin classes produced by the progeny of isolated precursors (B cells) to antibody-forming cells may answer the questions (1) does a clone of cells switch from μ to γ to α , and (2) do all clones switch in the same order following antigen stimulation? Results presented here show that there is no order of expression of immunoglobulin class with time by B-cell clones stimulated by antigen in the presence of carrier help, because any class can appear first or simultaneously with any other class.

Antibody polypeptides are encoded by separate variable (V) and constant (C) genes which are subsequently joined at the DNA level during lymphocyte differentiation to code for one polypeptide chain. Considerable chemical, genetic, and serological evidence has accumulated supporting the 'two gene-one polypeptide' theory proposed by Dreyer and Bennett in 1965⁶. Different V regions, as defined by subgroup-specific amino acid sequences, allotypic or idiotypic determinants, are found in association with a single C region for each of the κ and λ light (L) chain and heavy (H) chain loci⁷⁻⁹. Conversely, one V_H region can be shared by several different C_H regions¹⁰⁻¹⁵. In view of the finding by Hozumi and Tonegawa¹⁶ that V and C genes coding for L chains are on separate restriction fragments in embryonic DNA but are on the same restriction fragment in myeloma DNA, it is apparent that gene translocation may have a central role in immunoglobulin synthesis and cell differentiation¹⁷.

As there are multiple C_H genes that can be associated with a single V_H gene, the question arises as to whether the C_H genes are expressed in a sequential order following antigen stimulation. Several studies suggest that one cell or its clonal progeny can produce more than one immunoglobulin class. B cells have been shown to bear membrane receptor molecules of the IgM and IgD class with the same idiotype¹⁸. Using the splenic focus assay, we have previously demonstrated that a single antigen-stimulated B cell can produce clonal progeny making antibodies with different H chain classes that seem to have the same variable region by idiotypic analysis¹⁹. Thus, an isolated B-cell precursor specific for the phosphorylcholine (PC) determinant can be stimulated *in vitro* to generate daughter plasma cells which secrete antibody of three immunoglobulin classes, μ , γ 1, and α . Also, antibody molecules of each class have the same idiotype as that of the TEPC 15 (T15) myeloma. As shown here, an analysis of the sequence of appearance of constant regions within such T15 clones suggests that classes of immunoglobulin are produced in a random fashion following antigen stimulation in the presence of carrier help.

Ten million spleen cells from non-immune BALB/c mice were transferred intravenously into lethally irradiated BALB/c recipients that had been primed with 0.1 mg of *Limulus polyphemus* haemocyanin 2 months previously. Sixteen hours after transfer, spleen fragment cultures were prepared from the recipients and stimulated *in vitro* with 5×10^{-7} M 3-(azophenylphosphorylcholine)-N-acetyltyrosylglycylglycine haemo-

cyanin²⁰. The antigen was removed by day 4, and culture fluids were collected every 2 d until day 18. Anti-PC antibody was quantified by radioimmunoassay for heavy chain class and idiotype using T15 protein and monoclonal culture fluids as standards. The binding of antibody to antigen coupled to bromoacetylcellulose immunoadsorbant was detected with ¹²⁵I-labelled goat anti-mouse μ , γ 1, and α -chain antibody. Antibody with the T15 idiotype was detected in a radioimmunoassay using anti-T15 sera raised in A/He mice²¹. The monoclonal nature of antibody from these cultures has been demonstrated in previous experiments, indicating that each clone is derived from a single B cell^{19,22}.

The serial analysis of immunoglobulin class production by clones with the T15 idiotype indicates variation in time of appearance after stimulation, amount of antibody, and sequence related to other classes. The overall view of the onset of antibody production, shown in Fig. 1, suggests that any one of the three classes of immunoglobulin may be synthesised initially, and that IgG1 secretion persists longer than secretion of the other two classes. The apparently random expression of each immunoglobulin class is confirmed by data collected from individual clones which are presented in Table 1. B-cell clones, although making antibody with the same idiotype, display considerable variation in the rate of IgM, IgG1, and IgA production with time. Immunoglobulin class expression seems to be completely random in that any class of immunoglobulin can appear at any time, either singly or in combination with others. Variations in time of synthesis after stimulation and in amount of antibody in each immunoglobulin class produced by individual clones are observed. In an analysis of over 60 clones making antibody with the T15 idiotype, the early production of solely IgM and later solely IgG1 (or IgA) was not observed. Indeed, some clones made IgG1 before or without detectable IgM synthesis.

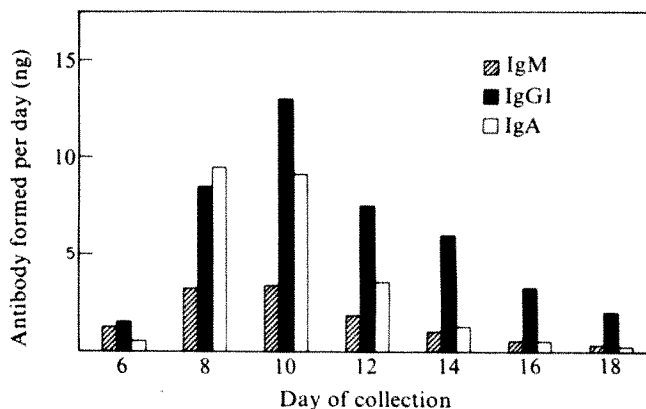


Fig. 1 Heavy chain class secretion by PC-specific clones with the T15 idiotype. The average amount of antibody produced by 43 clones in each immunoglobulin class is plotted as a function of time. Monoclonal fragment cultures were stimulated with antigen on day 1 and the antigen was removed on day 4. Culture fluids were collected every two days after that and analysed for idiotype and C_H determinants by radioimmunoassay.

The idea of an IgM to IgG 'switch' arose from observations that IgM is detected before IgG antibody in immune sera^{1,2}. Several explanations may be offered for these results. First, the assays used for IgM detection are more sensitive than those used for IgG detection. Second, it may be necessary to generate carrier-specific help to allow maximum expression of classes of immunoglobulin other than IgM; that is, IgM may appear first during the immune response because it requires less carrier-specific help. Thus, in physiological conditions, T cells or factors may influence the expression of heavy chain classes. Finally, subsets of B cells may have different developmental potentials for expressing C_H regions. For example, the clonal progeny of immature B cells produce predominantly IgM, whereas clones derived from immune B cells produce predominantly IgG²². Nonetheless, using a method which detects each heavy chain class equally well and provides

Table 1 Random production of immunoglobulin class by B-cell clones with time after antigen stimulation

Individual clones with T15 idiotype	Heavy chain class	Antibody formed on day of collection after antigen stimulation (ng)						
		Day 6	8	10	12	14	16	18
1	μ	0	6	4	1	0	0	0
	$\gamma 1$	0	0	0	0	0	0	0
	α	0	0	0	0	0	0	0
2	μ	0	0	0	0	0	0	0
	$\gamma 1$	1	12	6	2	0	0	0
	α	0	9	0	0	0	0	0
3	μ	0	0	0	0	0	0	0
	$\gamma 1$	0	0	0	0	0	0	0
	α	0	2	16	43	16	0	0
4	μ	0	0	1	2	0	0	0
	$\gamma 1$	2	4	15	10	9	2	3
	α	0	0	0	0	0	0	0
5	μ	0	14	16	5	2	2	0
	$\gamma 1$	0	1	2	0	0	0	0
	α	0	16	34	8	5	1	0
6	μ	2	3	9	3	1	0	0
	$\gamma 1$	11	60	75	103	31	23	8
	α	10	44	24	5	2	1	0
7	μ	7	11	11	8	6	2	1
	$\gamma 1$	1	6	9	7	8	3	2
	α	16	122	185	172	43	23	16
8	μ	0	0	11	2	0	0	0
	$\gamma 1$	0	0	28	18	5	8	6
	α	0	0	0	0	0	0	0
9	μ	2	7	7	2	0	0	0
	$\gamma 1$	1	12	10	3	0	0	0
	α	2	69	39	3	0	0	0
10	μ	1	4	11	5	2	2	2
	$\gamma 1$	0	3	19	34	6	2	0
	α	0	0	2	3	0	0	0

maximal carrier help for the stimulation of nonimmune B cells, the data presented here clearly indicate that the onset of expression of different immunoglobulin classes is completely random in that any class can appear singly or in combination with others. In this regard, no evidence was found for a sequential shift from $\mu \rightarrow \gamma 1 \rightarrow \alpha$ by stimulated clones.

There are two general models for the association of V_H genes with their corresponding C_H genes¹⁵. The simultaneous insertional model suggests that the V_H gene is copied multiple times and is associated with two or more C_H genes. The successive insertional model proposes that the V_H gene is successively translocated to various C_H genes during differentiation. The data presented here suggest that if the first model is correct, random expression of a V_H - C_H gene combination occurs, whereas if the second model is correct, a V_H gene can be translocated next to any C_H gene following antigen-triggered differentiation. These two models can probably only be tested by direct studies at the nucleic acid level.

This work was supported by USPHS grants from USNIH and NCI to Dr Norman Klinman at the University of Pennsylvania and a Helen Hay Whitney fellowship. I thank N. Klinman for encouragement and L. Hood for criticisms.

PATRICIA J. GEARHART

Department of Biology,
The Johns Hopkins University,
Baltimore, Maryland 21218

Received 27 June; accepted 15 August 1977.

1. Bauer, D. C. & Stavitsky, A. B. *Proc. natn. Acad. Sci. U.S.A.* **47**, 1667-1680 (1961).
2. Nossal, G. J. V., Szenberg, A., Ada, G. L. & Austin, C. J. *exp. Med.* **119**, 485-502 (1964).
3. Papermaster, B. W. *Cold Spring Harb. Symp. quant. Biol.* **32**, 447-460 (1967).
4. Lawton, A. R., Asofsky, R., Hylton, M. B. & Cooper, M. D. *J. exp. Med.* **135**, 277-297 (1972).
5. Herrod, H. G. & Warner, N. L. *J. Immun.* **108**, 1712-1717 (1972).

6. Dreyer, W. J. & Bennett, J. C. *Proc. natn. Acad. Sci. U.S.A.* **54**, 864-869 (1965).
7. Hood, L., Gray, W. R., Sanders, B. G. & Dreyer, W. J. *Cold Spring Harb. Symp. quant. Biol.* **32**, 133-146 (1967).
8. Hilschmann, N. Z. *physiol. Chem.* **349**, 945-951 (1968).
9. Cunningham, B. A., Pflumm, M. N., Rutishauser, U. & Edelman, G. M. *Proc. natn. Acad. Sci. U.S.A.* **64**, 997-1003 (1969).
10. Todd, C. W. *Biochem. biophys. Res. Commun.* **11**, 170-175 (1963).
11. Wikler, M., Köhler, H., Sinoda, T. & Putnam, F. W. *Science* **163**, 75-78 (1969).
12. Oudin, J. & Michel, M. J. *exp. Med.* **130**, 619-642 (1969).
13. Penn, G. M., Kunkel, H. & Grey, H. *Proc. Soc. exp. Biol. Med.* **135**, 660-665 (1970).
14. Wang, A. A., Wilson, S. K., Hopper, J. E., Fudenberg, H. H. & Nisonoff, A. *Proc. natn. Acad. Sci. U.S.A.* **66**, 337-343 (1970).
15. Sledge, C., Fair, D. S., Black, B., Krueger, R. G. & Hood, L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 923-927 (1976).
16. Hozumi, N. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628-3632 (1976).
17. Gally, J. A. & Edelman, G. M. *Nature* **227**, 341-348 (1970).
18. Fu, S. M., Winchester, R. J. & Kunkel, H. G. *J. Immun.* **114**, 250-252 (1975).
19. Gearhart, P. J., Sigal, N. H. & Klinman, N. R. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1707-1711 (1975).
20. Sigal, N. H., Gearhart, P. J. & Klinman, N. R. *J. Immun.* **114**, 1354-1358 (1975).
21. Gearhart, P. J., Sigal, N. H. & Klinman, N. R. *J. exp. Med.* **141**, 56-71 (1975).
22. Klinman, N. R. & Press, J. L. *Transplant. Rev.* **24**, 41-83 (1975).

Immunoprotection by embryonal carcinoma cells for methyl-cholanthrene-induced murine sarcomas

METHYLCHOLANTHRENE-induced sarcomas of mice (MCS) usually have transplantation antigens specific to each independently arising tumour¹. These antigens, defined by *in vivo* protection assays, are different from those defined serologically as viral² or embryonic antigens^{3,4}. The latter, common to most sarcomas, seem to contribute little in transplantation assays as cross protection is rare and weak^{1,5}. The occasional success in protecting against sarcoma growth by previous immunisation with cells of mid-term embryos^{4,6} encourages the search for more effective ways of using common embryonic antigens for this purpose. We

show here that effective immunisation against several different non-cross-reacting sarcomas, including one not immunogenic itself, can be achieved with embryonal carcinoma cells. These cells are the stem cells of a teratocarcinoma and show remarkable similarities to the pluripotent cells of early mouse embryos with regard to cell surface antigens⁷ as well as in morphological and biochemical properties⁸.

The sarcomas used were a set induced in female C57BL/10ScSn (B10) mice by subcutaneous injection of 0.5 mg 3-methylcholanthrene into the hind legs. Approximately 100 d later primary tumours were excised, passaged subcutaneously twice and then stored in liquid nitrogen. Tumours (*in vivo* passages 2–8) were characterised for immunogenicity in a rejection assay (Fig. 1). To assay immunogenicity, mice were immunised in two ways: by allowing tumour growth for 10 d followed by tumour excision or by three weekly immunisations with 10^7 irradiated MCS cells (legend Fig. 1). The mice were challenged a week later with 2×10^5 viable cells of the same or a different sarcoma and tumour diameter measured as a function of time.

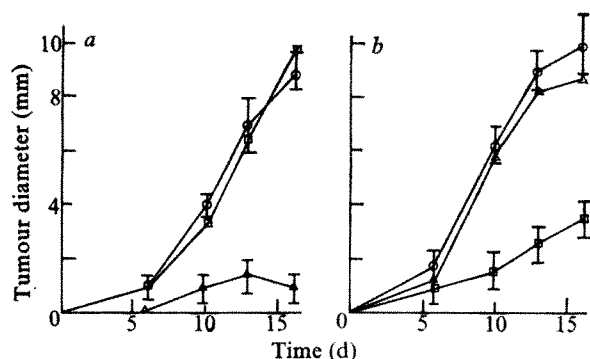


Fig. 1 Immunogenicity of methylcholanthrene-induced sarcomas MC6A and MC6B. Groups of 12 female B10 mice, 12–16 weeks old were immunised by three weekly subcutaneous injections of 10^7 MC6A or MC6B cells (irradiated with 10,000 rad from a ^{60}Co source) in 0.2 ml phosphate-buffered saline (PBS) into the right groin. Tumour cell suspensions were prepared from *in vivo* tumours chopped finely and stirred continuously at 37 °C in 2.5% trypsin in Eagles Ca^{2+} - and Mg^{2+} -free medium. One week following the last immunisation, groups of six mice were challenged with 2×10^5 viable MC6A (a) or MC6B (b) cells in 0.2 ml PBS into the right groin. Control groups of unimmunised mice were similarly challenged. Tumour diameter was measured in two perpendicular planes every 3 d using calipers for 16 d after challenge. Error bars represent \pm s.e.m. \circ , unimmunised; Δ , MC6A immunised; \square , MC6B immunised.

Figure 1 shows that MC6A and MC6B are strongly immunogenic; immunisation evokes good protection against subsequent challenge with the same methylcholanthrene sarcoma. A measure of the strength of immunisation is the antigenic ratio (AR) (ref. 9) (Table 1). The relative immunogenicity measured by AR ranks the tumours in the descending order MC6A, MC6B, MC9, MC5 with MC5 being non-immunogenic (Table 1). Data in Fig. 1 and Table 1 also demonstrate that MC6A and MC6B do not cross protect and thus apparently possess individually specific antigens.

Similar immunisations with cells of an embryonal carcinoma cell line Nulli-SCC.1, which do not differentiate *in vivo* or *in vitro*¹⁰, elicit protection against the strongly immunogenic MC6A and MC6B, the less immunogenic MC9, and the non-immunogenic MC5 (Table 1, Fig. 2a, b and c). The Nulli-SCC.1 cells are derived from the 129/J mouse strain which is not syngeneic with B10. To test whether alloantigens on Nulli were responsible for the protective effect against the sarcomas, 129/J spleen cells were used to immunise B10 mice; no protection was seen (Fig. 2d). In addition, immunisation with cells of C57BL/6 thymoma EL4 (ref. 11), a DBA/2 mastocytoma P815 (ref. 12), or a B10A Abelson lymphoma induced in our laboratory, gave no protection against subsequent challenge with MC6A cells (Fig. 2d). The Nulli-SCC.1 cells grow as homogeneous embryonal carcinoma *in vitro* and the cell line was derived from a spontaneous testicular teratocarcinoma (LS-402C-1684). These Nulli-SCC.1 cells resemble

Table 1 Relative immunogenicity of methylcholanthrene sarcomas and embryonal carcinoma with MCS challenge

Immunising tumour*	Challenge tumour†	Antigenic ratio‡
MC6A	MC6A	10.0
MC6B	MC6A	1.1
MC6A	MC6B	1.0
MC6B	MC6B	5.3
MC9	MC9	3.2
MC5	MC5	1.0
Nulli-SCC.1	MC6A	2.1
Nulli-SCC.1	MC6B	2.0
Nulli-SCC.1	MC9	1.9
Nulli-SCC.1	MC5	1.7

* 10^6 viable tumour cells prepared as in Fig. 1 were injected in 0.2 ml phosphate buffered saline (PBS) into the left flank of groups of four mice and tumours excised surgically 10–14 d later. Nulli-SCC.1 cells were grown as monolayers in tissue culture and cell suspensions prepared as previously described⁸.

† 2×10^5 viable cells were injected subcutaneously (s.c.) in 0.2 ml PBS into the right groin 10 d after the excision. Tumour diameter was measured in two perpendicular planes every 3 d by calipers and the mean calculated. A control group of unimmunised animals was challenged concurrently.

‡Antigenic ratio is calculated as

$$\frac{\text{mean tumour diameter in unimmunised mice}}{\text{mean tumour diameter in immunised mice}}$$

at 16 d after challenge. An antigenic ratio of greater than 1.0 indicates protection. Mean tumour diameter is computed from all mice in a group; no challenge dose failed to grow in these experiments.

early embryo cells in morphological, biochemical and antigenic properties¹³, but differ because they can no longer differentiate. Immunisation with irradiated homogeneous embryonal carcinoma cells from a pluripotent teratocarcinoma cell line¹⁰ also shows protection against challenge with MC6A tumour cells. This line was isolated from a 3-d embryo induced teratocarcinoma (OTT-5568) and retains the ability to differentiate *in vivo* and, in appropriate conditions, *in vitro*.

In addition to embryonal carcinoma, some protection against methylcholanthrene tumour challenge is also provided by immunisation with teratocarcinoma derived endodermal cells of the lines PSA5E (ref. 13) and PYS-2 (ref. 14) (Fig. 2e), though the

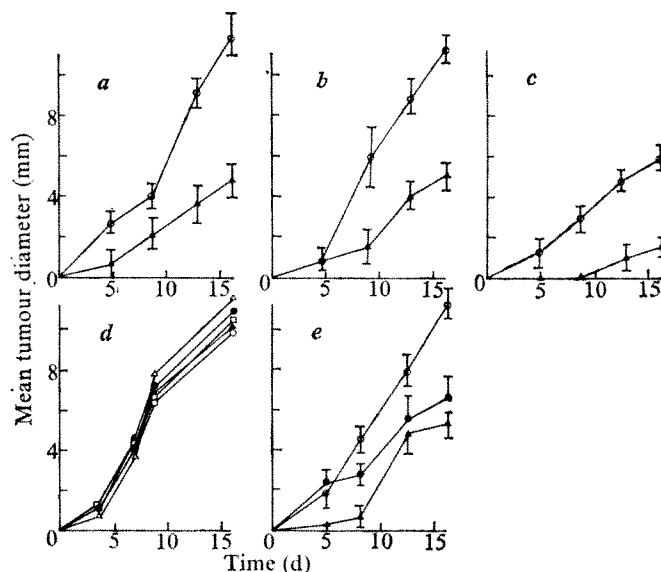


Fig. 2 Protection by various cells against MCS challenge. Groups of 3 B10 female mice were immunised by three weekly s.c. injections of 10^7 cells. A week following the last immunisation, the mice were challenged with 2×10^5 viable tumour cells as in Fig. 1. a, b, and c. Mice immunised with irradiated Nulli-SCC.1 (Δ); unimmunised (\circ). Challenge with MC6A, MC6B, MC5 respectively. d. Mice immunised with irradiated EL4 (Δ); B10.A.A2.P5 Abelson lymphoma (\bullet); P815 mastocytoma (\blacktriangle); and 129 spleen cells (\square); unimmunised (\circ). Challenge with MC6A. e. Mice immunised with PYS (\bullet), PSA5E endodermal cells (Δ); unimmunised (\circ). Challenge with MC6A.

degree of protection by PYS is weak. These lines were derived from the outgrowths of embryoid bodies *in vitro*.

How do embryonal carcinoma cells induce protection to tumours that do not protect against each other (MC6A and MC6B) and to a tumour that cannot protect against itself (MC5)? Nonspecific activation of the immune system by the embryonic cell lines, though not by the other tumour lines tested, might explain the observed cross protection¹⁵. A more interesting possibility is that there are antigens shared by all these cells and that presentation of these antigens on the embryonal carcinoma cells is particularly effective in inducing specific immune protection while presentation of these common antigens on the MCS is not effectively immunogenic. Indeed serological studies with teratocarcinoma have demonstrated antigen specificities present on these cells and which are shared by a wide range of tumour cell lines^{16,17}. Antisera prepared by us in syngeneic mice against MCS cross react with embryonal carcinoma cells. In addition, lymphocytes sensitised *in vitro* to syngeneic teratocarcinoma cells have been shown to inhibit growth of a wide range of tumour cells *in vitro* and *in vivo*, but do not affect untransformed cells¹⁸.

Why the MCS-embryonal carcinoma shared antigens might be immunogenic only on the latter cells is intriguing. It is perhaps relevant that both the embryonal carcinoma and the endodermal cells do not express the major histocompatibility antigens (H2) (refs 13, 19). Since there is increasing evidence that H2 plays an important part in directing the immune response²⁰, it is possible that in the absence of H2 expression, other antigens, not seen in the presence of H2, become immunodominant.

K.S. holds an MRC Training Fellowship. We thank Susan Baker for technical assistance.

KAROL SIKORA
PETER STERN
EDWIN LENNOX

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge, UK

Received 1 July; accepted 18 August 1977.

- Basombrio, M. A. *Cancer Res.* **30**, 2458-2462 (1970).
- Grant, J. D., Bigner, D. B., Fischinger, P. J. & Bolognesi, D. P. *Proc. natn. Acad. Sci. U.S.A.* **71**, 5037-5041 (1974).
- Brawn, R. J. *Int. J. Cancer* **6**, 245-249 (1970).
- Baldwin, R. W., Embleton, M. J., Price, M. R. & Vose, B. M. *Transplant. Rev.* **20**, 77-99 (1974).
- Reiner, J. & Southam, C. M. *Cancer Res.* **27**, 1243-1247 (1967).
- Coggin, J. H. & Anderson, N. G. *Adv. Cancer Res.* **19**, 105-165 (1974).
- Erickson, R. P. in *Immunobiology of Gametes* (eds Johnson, M. & Edidin, M.) 85-114 (Cambridge University Press, Cambridge, 1977).
- Martin, G. R. *Cell* **5**, 229-243 (1975).
- Bartlett, G. L. *J. natn. Cancer Inst.* **49**, 493-504 (1972).
- Martin, G. R. & Evans, M. J. in *Teratomas and Differentiation* (eds Sherman, M. I. & Solter, D.) 169-187 (Academic, London, 1975).
- Gorer, P. A. & Amos, D. B. *Cancer Res.* **16**, 338-343 (1956).
- Dunn, T. B. & Potter, M. J. *J. natn. Cancer Inst.* **18**, 587-595 (1957).
- Evans, M. J., Lovell-Badge, R. & Stern, P. L. in *European Teratology Soc. Meet. Symp.* (in the press).
- Lehman, J. M., Speers, W. C., Swartzendruber, P. E. & Pierce, G. B. *J. Cell Physiol.* **84**, 13-28 (1974).
- Smith, S. E. & Scott, M. T. *Br. J. Cancer* **26**, 361-367 (1972).
- Gooding, L. R. & Edidin, M. *J. exp. Med.* **140**, 61-78 (1974).
- Gooding, L. R., Hsu, Y. C. & Edidin, M. *Dev. Biol.* **49**, 479-486 (1976).
- Bartlett, P. F., Fenderson, B. A. & Edidin, M. *Fed. Proc.* **36**, 1290 (1977).
- Artzt, K. & Jacob, F. *Transplantation* **17**, 632-634 (1974).
- Doherty, P. C., Blanden, R. V. & Zinkernagel, R. M. *Transplant. Rev.* **29**, 89-124 (1976).

Chemical mutagenesis of mammalian cells can be quantified

STUDY of mammalian genetics has been traditionally limited to conventional breeding experiments, which are limited by high cost, long duration and the availability of well-characterised genetic markers. These factors have especially impeded rapid progress in mammalian mutagenesis studies. Many of the mechanisms underlying the process of mutation induction in mammals can be investigated through the use of mammalian cells in culture¹⁻³, but the use of cultured cells for quantitative analyses of mutagenesis has been hampered by the lack of a suitable mutation-induction system, particularly with chemical mutagens. We show here that quantitative dosimetry of chemical mutagenesis can be studied in a mammalian cell culture system. This will allow

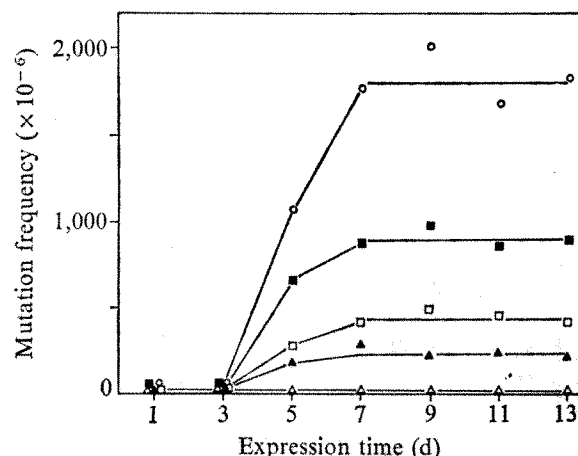


Fig. 1 Expression time of HGPRT⁻ (TG-resistant) mutant phenotype. CHO cells (clone K₁-BH₄) were plated at 5×10^5 cells per 100-mm plate in Ham's F12 medium containing 5% dialysed foetal calf serum and were incubated at 37 °C in 5% CO₂ in air in a 100% humidified incubator. After 24 h (cell no. $1-1.5 \times 10^6$ cells per plate), EMS was added (zero time), and the cultures were incubated for 16 h. The cultures were washed three times with saline, the cells were removed by trypsinisation, and approximately 10^6 cells were subcultured. For selection of the mutant phenotype, cells were plated at 2×10^5 cells per 100-mm plate (5 plates = 10^6 cells) in hypoxanthine-free F12 medium containing 5% dialysed foetal calf serum and 10 µM TG. For cloning efficiency, 200-1,000 cells were plated in triplicate in the same medium without TG. After 7 d of growth colonies were fixed, stained, and counted. Cultures were also subcultured and plated for mutant selection every 2 d thereafter. Mutation frequency was calculated as the number of mutant colonies per 10^6 cells (corrected by cloning efficiency at time of selection). Cells treated with 0 (Δ), 50 (▲), 100 (□), 200 (■), or 400 (○) µg ml⁻¹ of EMS. Spontaneous frequency in the range $2-3.6 \times 10^{-6}$.

not only studies of the mechanisms of chemical mutagenesis, but also an estimate of the genetic hazard of chemical agents in the environment.

The mutation assay system we have developed uses cultured Chinese hamster ovary (CHO) cells and the purine analogue 6-thioguanine (TG) to quantitate mutation induction at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus⁶. This CHO/HGPRT system is based on the facts that TG is converted to the nucleotide by the HGPRT activity of wild-type cells and that the nucleotide or its metabolites are lethal. Mutant cells lacking this enzyme activity are able to survive and grow in the presence of TG. We have defined optimal conditions for the selection of the mutant phenotype, including phenotypic expression time, concentration of TG and medium composition for the selection step, and optimal cell density in the selection to permit maximum mutant recovery. With our system the TG-resistant mutation seems to affect the HGPRT locus almost exclusively, since more than 98% of the resistant clones show greatly reduced incorporation of radiolabelled hypoxanthine into macromolecules, are sensitive to the anti-metabolite aminopterin, and contain highly reduced or undetectable HGPRT activity (ref. 6 and O'Neill, J. P., Brimer, P. A., Machanoff, R., Hirsch, G. P., and Hsie, A. W., submitted for publication). Using the CHO/HGPRT system, we have studied mutagenesis with a common chemical mutagen, ethyl methanesulphonate (EMS), in a quantitative fashion.

An important aspect to consider in any mutation induction is phenotypic delay, the time required for mutants to express the altered phenotype. For TG resistance, a delay would be expected due to the time necessary for the dilution and decay of pre-existing HGPRT enzyme and its mRNA before the mutant phenotype could be expressed. This important parameter was investigated with cultures incubated with various concentrations of EMS for 16 h;

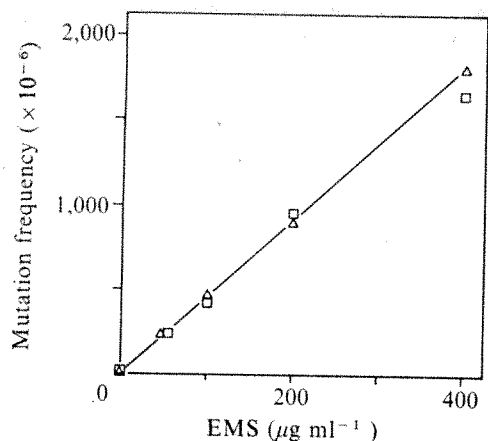


Fig. 2 Dose response of EMS-induced mutagenesis. Data from two separate experiments are presented: the average of the mutation frequencies at days 7, 9, 11, and 13 from Fig. 1 (Δ) (average spontaneous frequency 3.1×10^{-6}); and the frequencies for 24-h incubation time from Fig. 3 (□).

thereafter, the cells were subcultured and plated for selection every 2 d. Mutant colonies were found after 5 d of expression, and the mutation frequency reached a maximum value at day 7 with all EMS doses (Fig. 1). The expression time was independent of the mutagen dose, and there was no loss of mutants with longer periods of expression, as the mutation frequency remained at a constant value in cells subcultured for an additional 6 d. There was a linear dose-response relationship between mutation frequency and EMS concentration (Fig. 2). Since the mutation frequency reached a stable maximum value at 7–9 d of expression, cells were routinely plated for selection on day 9 in all subsequent experiments.

The dose-response data of Fig. 2 were obtained with long incubation times with EMS (16–24 h). It would be difficult to compare such chemical mutagenesis with that induced by physical agents like X rays because of complicating factors such as the stability of EMS in the medium, rate of cellular uptake, and intracellular stability. To investigate these aspects, cultures were incubated with EMS for

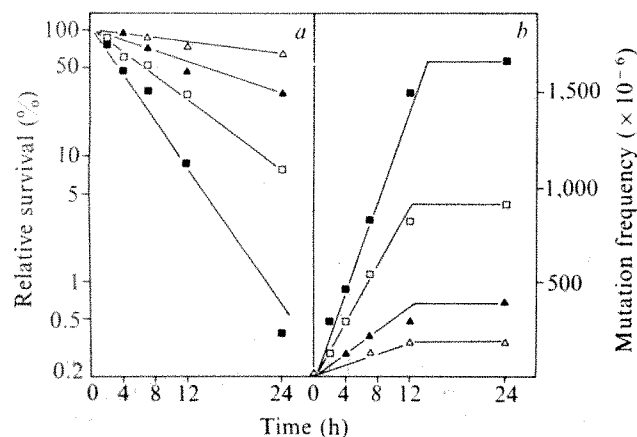


Fig. 3 Initial cell survival (a) and EMS-induced mutation frequency (b) as a function of incubation time. Cells were plated at 5×10^5 cells per plate as described in Fig. 2. After 24 h, EMS was added, cultures were incubated for the indicated time interval and then washed, and fresh medium was added. After a total interval of 24 h the cultures were trypsinised, and single-cell survival was determined (absolute cloning efficiency of untreated control cultures was 84%). Cultures were subcultured every 2 d and plated for mutant selection on day 9 of expression as described in Fig. 1. EMS at concentrations of 50 (Δ), 100 (▲), 200 (□), and 400 $\mu\text{g ml}^{-1}$ (■). Spontaneous mutation frequency 4.2×10^{-6} .

intervals of 2–24 h. A linear increase in mutation frequency with incubation times of up to 12–14 h was found with EMS concentrations of 50–400 $\mu\text{g ml}^{-1}$ (Fig. 3b). But cell survival decreased exponentially with time over the entire 24-h period (Fig. 3a). This difference in the time courses of cellular lethality and mutagenicity might be due to the formation of toxic, non-mutagenic breakdown products in the medium with longer incubation times or might reflect a difference in the mode of action of EMS in these two biological effects. The increases in both cell lethality and induced mutation with incubation times of up to 12 h allow a study of the dosimetry of this chemical mutagen. For better defined dosimetry studies, cultures were incubated with various concentrations of EMS (0.05–3.2 mg ml^{-1}) for 2–12 h. As shown in Fig. 4 the decrease in cell survival was exponential with dose ($\text{mg ml}^{-1} \text{ h}$), and the increase in mutation frequency was linear with dose. From these studies the mutagenic potential of EMS can be described as 310×10^{-6} mutants ($\text{cell mg ml}^{-1} \text{ h}$) $^{-1}$.

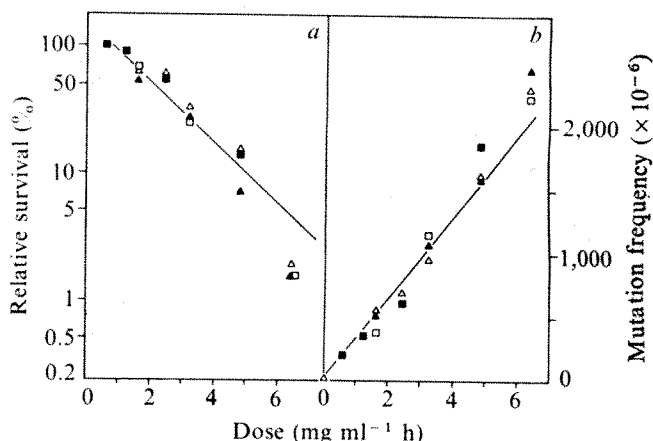


Fig. 4 Dosimetry for EMS cellular lethality (a) and induced mutation frequency (b). Cells were treated with various concentrations (mg ml^{-1}) of EMS for 2 (Δ), 4 (▲), 8 (□), or 12 h (■). After treatment the cultures were washed, and single-cell survival was determined after a total time of 24 h. Cells were plated for selection on day 9 of expression as in Fig. 2. Spontaneous mutation frequency = 2.3×10^{-6} . In (a) the linear fit of the survival curve was found over 1.6–6.4 $\text{mg (of EMS) ml}^{-1} \text{ h}$ through fitting to the 'multitarget model'

$$R_i = 1 - (1 - e^{-Bx})^N$$

where B and N are fitted coefficients. The extrapolation number (N) is 1.74 with 95% confidence limits of 1.18, 2.58; the slope (B) is -0.248 with 95% confidence limits of -0.308 , -0.188 . In (b) the straight-line fit for the pooled data on mutation induction over the dose range 0.64–6.4 $\text{mg (of EMS) ml}^{-1} \text{ h}$ is $f(x) = 10^{-6} (5.58 + 310.77x)$. The 95% confidence limits for the slope are 283.68, 337.87. All confidence limits are estimated based on the residual sum of squares. Statistical analyses were performed as in ref. 6.

These results demonstrate (1) that the mutagenicity of EMS in mammalian cells follows a linear dosimetry relationship, and (2) that a quantitative study of chemical mutagenesis in mammalian cells is possible if an adequately defined selection protocol is developed.

We thank M. H. Hsie, D. G. Gosslee and T. J. Mitchell for help with statistical analysis and D. B. Couch and E. I. Shaw for reviewing the manuscript. This work was supported by the US National Center for Toxicological Research, EPA and ERDA under contract with the Union Carbide Corporation.

J. P. O'NEILL
A. W. HSIE

Biology Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

Received 27 March; accepted 18 July 1977.

- ¹ Puck, T. T. *The Mammalian Cell as a Microorganism* (Holden-Day, San Francisco, 1972).
² Thompson, L. H. & Baker, R. M. in *Methods in Cell Biology* (ed. Prescott, D. B.) 209-281 (Academic, New York, 1973).
³ Chu, E. H. Y. *Genetics* 78, 115-132 (1974).
⁴ Siminovich, L. *Cell* 7, 1-11 (1976).
⁵ DeMars, R. *Mutat. Res.* 24, 335-364 (1974).
⁶ Hsie, A. W., Brimer, P. A., Mitchell, T. J. & Gosslee, D. G. *Somat. Cell Genet.* 1, 247-261 (1975); 1, 383-389 (1975).

Increasing ovulation rate in sheep by active immunisation against an ovarian steroid androstenedione

ALTHOUGH low rates of ovulation constitute a major limitation to increased productivity of sheep and cattle little is known about the mechanisms that control ovulation either within or among species. Androstenedione, a weak androgen secreted by ovaries¹ and adrenals¹, is quantitatively the major steroid secreted by the ovary before ovulation in several species² and as such it might have a role in determining ovulation rate; it is also an important precursor for the biosynthesis of oestrogens by the ovary³. Although androstenedione can act as a prehormone by virtue of its peripheral metabolism to oestrogens⁴, a direct hormonal action has only been demonstrated in the female rhesus monkeys⁵ where it seems to be important for sexual behaviour. We report here that by actively immunising sheep against androstenedione a slight but significant increase in ovulation rate can be induced.

Two series of experiments were carried out on 10 Welsh mountain ewes. Half of the sheep were immunised against androstenedione conjugated in the 11 position to bovine serum albumin (BSA)⁶ and the other half were immunised against BSA alone and served as controls. All of the ewes immunised against androstenedione-11-BSA produced antibodies with a high degree of specificity for androstenedione⁶, and the titre of antibody was maintained at elevated levels by periodic booster immunisations⁶.

During the normal sheep mating season (October-March) the ewes were kept with a harnessed vasectomised ram and observed daily for signs of oestrus⁷. In March 1975, and again in November 1975, the ewes were subjected to a laparotomy and the number of corpora lutea and visible Graafian follicles of greater than 4 mm diameter were recorded. The pooled results are presented in Table 1 and show that there was a significant increase in the ovulation rate (Fisher's exact test, $P < 0.01$, February 1975; $P < 0.07$, November 1975) but the length of the oestrous cycle and the duration of oestrus were unaffected (t test). The increase in ovulation rate was accompanied by an increase in follicular growth, the control ewes having 1.6 ± 0.2 Graafian follicles per animal in contrast to 4.2 ± 0.7 per ewe in the immunised group ($P < 0.05$, Wilcoxon rank sum test).

Table 1 Length of oestrous cycle, duration of oestrus and ovulation rate in sheep immunised against androstenedione-11-BSA

Group	Oestrous cycle (d) (mean \pm s.e.m.)	Duration of oestrus (h) (mean \pm s.e.m.)	Ovulation rate (mean \pm s.e.m.)
Control	16.7 ± 0.2 ($n = 56$)	40.4 ± 1.3 ($n = 10$)	1.11 ± 0.11 ($n = 9$)
Immunised	17.5 ± 0.8 ($n = 70$)	45.1 ± 1.9 ($n = 7$)	2.13 ± 0.12 ($n = 8$)

These experiments clearly show that ovulation rate can be increased by immunisation against androstenedione-11-BSA. This technique has the additional advantage that excessive superovulation such as is often produced by the use of exogenous gonadotrophins is avoided: all of the immunised ewes had either two or three ovulations, while all the control ewes had one or two ovulations. In subsequent experiments on larger groups of sheep only 1 out of 15 immunised ewes had an ovulation rate of greater than 3.

Measurement of the peripheral levels of luteinising hormone

(LH) (ref. 8) and progesterone⁶ in samples collected daily throughout one complete oestrous cycle are shown in Fig. 1. The ewes immunised against androstenedione-11-BSA had elevated peripheral concentrations of both hormones throughout most of the oestrous cycle. An additional series of blood samples was collected from both groups of ewes, the collections beginning at 0800 LT on day 16 of the oestrous cycle and continuing at 4-h intervals until 36 h after the onset of oestrus. The plasma samples were assayed for the LH content, and the results show that the preovulatory LH discharge reached its maximum concentration 24 ± 4.0 h (mean \pm s.e.m. $n = 3$) after the onset of oestrus in the immunised ewes as compared with 8 ± 1.3 h ($n = 5$) in the control group ($P < 0.001$; unpaired t test).

These experiments suggest that androstenedione has a role (possibly direct) in the control of ovulation rate and, furthermore, that androstenedione might provide a manifestation of the genetic expression of ovulation rate. The mechanisms involved in

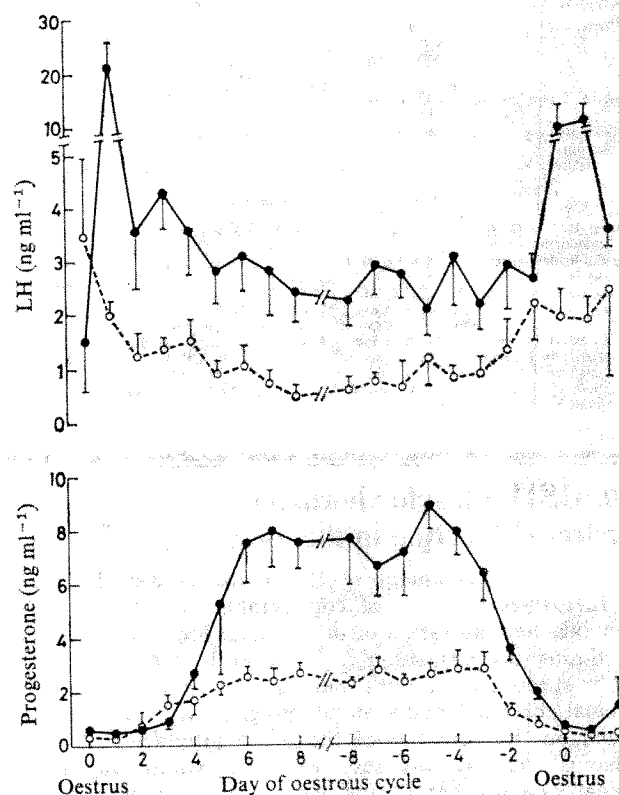


Fig 1 The mean \pm s.e.m. peripheral concentrations of progesterone (lower) and LH (upper) throughout the oestrous cycle of ewes immunised against androstenedione-11-BSA (\bullet , $n = 5$) and control ewes immunised against BSA (\circ , $n = 5$). The oestrous cycle is dated 8 d forward and 8 d back from successive oestrous periods. Note the split vertical scale for LH.

producing the increased ovulation rate and peripheral concentrations of progesterone and LH are speculative. Neutralisation of the biological activity of androstenedione by antibody binding could interfere with oestrogen synthesis in the ovary, or with the conversion of androstenedione to oestrogen in peripheral tissues and hypothalamus. Alternatively, androstenedione itself may have a direct inhibitory action on the hypothalamo-hypophyseal system. It is even possible that the androstenedione antibody cross reacts to a minor degree with oestrogens, although this cross reaction was shown to be less than 0.1% in an *in vitro* test⁸. Whatever the explanation, our results show that the ovarian steroid hormone(s) involved in the feedback control of LH have a reduced effectiveness after immunisation against androstenedione-11-BSA, and that this exposes the animal to higher tonic secretions of gonadotrophin which in turn leads to increased follicular development, multiple ovulations and enhanced progesterone secretion. A similar mechanism is thought

to operate when synthetic anti-oestrogens such as clomiphene are used to induce ovulation in anovulatory women⁹. The ability to produce a modest increase in the ovulation rate in sheep following a simple immunisation procedure could be of practical significance, provided that the increased ovulation rate leads to an increased number of live births per sheep mated.

We thank Dr G. Woods (Organon Laboratories, Newhouse, Scotland) and Dr F. Rutherford MRC Radioimmunoassay Team, Edinburgh) for preparing the androstenedione-11-BSA, and Mr J. B. Donnelly, CSIRO Division Mathematics and Statistics, for statistical advice. P. Van Look was supported by the National Fonds voor Wetenschappelijk Onderzoek, Brussels, Belgium.

R. J. SCARAMUZZI*
W. G. DAVIDSON
P. F. A. VAN LOOK†

MRC Unit of Reproductive Biology,
2 Forrest Road,
Edinburgh, UK

Received 11 January; accepted 9 August 1977.

*Present address: C.S.I.R.O., Division of Animal Production, P.O. Box 239, Blacktown, N.S.W., 2148, Australia.

†Present address: Vrouwenklinik, Academisch Ziekenhuis Leiden, Leiden, The Netherlands.

1. Baird, D. T., McCracken, J. A. & Goding, J. R. in *Pregnancy and Parturition* (ed. Pierrepont, C. G.) 5-21 (Alpha Omega, Cardiff, 1973).
2. Baird, D. T. in *The Ovary* 2nd edn (eds Zuckerman, Sir S. & Weir, B.) (Academic, New York, London, in the press).
3. Rado, A., McCracken, J. A. & Baird, D. T. *Acta endocr., Copenh.* **65**, 244-260 (1970).
4. Baird, D. T., Horton, R., Longcope, C. & Tait, J. F. *Perspect. Biol. Med.* **11**, 384-421 (1968).
5. Everitt, B. J. & Herbert, J. J. *Endocr.* **51**, 575-588 (1971).
6. Scaramuzzi, R. J., Corker, C. S., Young, G. & Baird, D. T. in *Steroid Immunoassay* (eds Cameron, E. H. P., Hillier, S. G. & Griffith, K.) 111-122 (Alpha Omega, Cardiff, 1975).
7. Radford, H. M., Watson, R. H. & Wood, G. F. *Aust. vet. J.* **36**, 57-66 (1960).
8. Martensz, N. D., Baird, D. T., Scaramuzzi, R. J. & Van Look, P. F. A. *J. Endocr.* **69**, 227-237 (1976).
9. Vaitukaitis, J. L., Bermudez, J. A., Cargille, C. M., Lipsett, M. B. & Ross, G. T. J. *clin. Endocr., Metab.* **32**, 503-508 (1971).

Is α MSH a trophic hormone to adrenal function in the foetus?

FUNCTIONAL development of the pituitary-adrenal axis in the foetus seems to be of considerable importance in influencing the maturation of other foetal organ systems, and the timing of parturition^{1,2}. In several species including man^{3,4}, sheep⁵ and rabbits⁶, there is evidence for an increase in foetal adrenal function in late pregnancy, which seems to depend on the activities of the foetal pituitary and hypothalamus⁷. The factors responsible for stimulating foetal adrenal development during late gestation remain poorly understood, however. From detailed studies in foetal sheep, it is apparent that the prepartum acceleration of adrenal growth and glucocorticoid secretion is not preceded by elevated ACTH concentrations in the foetal plasma^{8,9}. But, prostaglandin E₂ (PGE₂) administered intra-arterially into the foetus stimulated an increase in the concentration of cortisol in foetal plasma⁸ at a stage of pregnancy when the adrenal gland was essentially unresponsive to endogenous or exogenous ACTH (refs 2, 9). Experiments in newborn lambs suggest that the pituitary or brain rather than the foetal adrenal gland was the likely site of the PGE₂ action¹⁰, raising the possibility that other pituitary peptides might mediate this effect. The ACTH related peptides, α -melanocyte stimulating hormone (α MSH) and corticotropin-like intermediate lobe peptide (CLIP) have been identified in human foetal pituitaries, and it has been suggested that the ratio of these peptides to ACTH might be critical to maturation of foetal adrenal function¹¹. We have examined the ability of one of these peptides, α MSH, to influence adrenal function in the rabbit foetus. We present here evidence that the foetal adrenal secretes cortisol in response to α MSH at stages in pregnancy when it is unresponsive to ACTH, but as the sensitivity to ACTH increases, the relative response to α MSH is diminished.

Pregnant New Zealand white rabbits were purchased from Canadian Hybrid Farms, Quebec on day 17 of gestation and were housed individually. The animals had been mated once with a fertile buck, and the day of mating was designated as day 0. Experiments were performed on foetuses on day 22 post coitum and day 28 post coitum and on newborn rabbits, 4-6 d-old after spontaneous delivery at full term.

Foetal experiments were performed under local (1% Xylocaine) anaesthesia. A laparotomy incision was made, the uterus exposed, and ACTH (10 μ g Synacthen, ACTH₁₋₂₄, CIBA), α MSH (10 μ g, Bachem) or saline was injected intramuscularly (i.m.) into the foetus through the uterine wall. All injections were made in 100 μ l saline. After injection, the uterus was replaced, and the abdomen closed with surgical clips. After 60 min the foetuses were delivered, decapitated, and the blood collected into chilled heparinised tubes and immediately frozen. Newborn rabbits, handled gently with gloves, were injected i.m. with ACTH, α MSH or saline as above, and returned to their mothers. After 60 min the newborns were removed and blood (0.5-1.0 ml) collected rapidly by cardiac puncture. All samples were frozen at -20 °C until analysis for cortisol using a previously described antibody⁴ and radioimmunoassay procedure^{8,12}.

An initial study in newborn rabbits examined the time course of the blood cortisol response to ACTH injection. The concentration of cortisol increased from 2.97 ng ml⁻¹ at the time of injection to a maximum concentration (10.9 ng ml⁻¹) within 60 min (Table 1). This time interval was therefore adopted in subsequent studies.

Table 1 Blood cortisol concentrations in foetal and newborn rabbits in response to exogenous α MSH or ACTH

Age	Control t_0	Cortisol (ng ml ⁻¹)		
		Saline t_{60}	Synacthen t_{60}	α MSH t_{60}
22 d p.c.	14.65 \pm 1.02	18.35 \pm 2.85	21.39 \pm 3.24	32.45 \pm 3.17**
28 d p.c.	6.60 \pm 0.82	6.13 \pm 1.57	7.94 \pm 0.83	10.54 \pm 0.80*
4-6 d p.p.	2.97 \pm 1.05	2.74 \pm 0.76	10.91 \pm 1.45***	6.93 \pm 1.36*

The values are the mean \pm s.e. of 10-18 foetuses or newborn from 2-13 mothers in each group. Control observations (t_0) were made before injections. Measurements were then made 60 mins (t_{60}) after saline, Synacthen, or α MSH. p.c., Post coitum; p.p., post partum. Significant differences from 'saline t_{60} ' are indicated as follows: *** P < 0.001, ** P < 0.005, * P < 0.05. Other mean values within any age group are not statistically significantly different from the corresponding saline t_{60} group.

There was no significant effect (P > 0.05) of saline injection on the blood cortisol concentration in any of the three groups of animals (Table 1). In foetuses 22 and 28 d post-coitum, Synacthen produced no significant change in the blood cortisol concentration, whereas a significant increase in cortisol (P < 0.001) was found 60 min after Synacthen injection in the newborn. In marked contrast, α MSH caused a significant increase in the concentration of cortisol in the blood of foetuses at 22 and 28 d post-coitum (Table 1). α MSH also increased the concentration of cortisol in the blood of newborn rabbits but the response relative to that achieved with Synacthen was less marked than earlier in gestation.

Although cortisol and corticosterone are present in similar concentrations in the blood of foetal rabbits⁵, cortisol binds more strongly than corticosterone to liver, kidney and lung receptor sites¹³, and in adults its secretion is stimulated preferentially by ACTH (ref. 14). We therefore measured cortisol as an index of foetal adrenal glucocorticoid production. The control (t_0) levels we have measured are similar to those reported by Mulay *et al.*⁵, who also observed a decrease in the plasma cortisol concentration between day 22 and 28. In view of the significant increase in body weight and blood volume of the rabbit

foetus between day 22 and 28, comparison of blood cortisol levels between these times are difficult to interpret, and a twofold concentration decrease may not necessarily reflect a decrease in total production rate. Total and unbound cortisol in plasma increase significantly by day 30 (ref. 5), providing evidence for a pre-partum increase in adrenal steroid secretion.

At days 22 and 28 post coitum there was no increase in cortisol in response to ACTH injection into the foetus. Albano *et al.*¹⁵, failed to stimulate adrenal cyclic AMP with ACTH at day 22, implying that the appropriate tropic hormone receptors are poorly developed at that time. A similar insensitivity of the foetal adrenal to ACTH has been reported in the sheep^{2,9} and rhesus monkey¹⁶. It is of interest that while ACTH did not produce short-term changes in the concentration of cortisol in foetal blood, it will maintain adrenal weights in hypophysectomised rabbit foetuses¹⁷, showing that any deficiency of ACTH receptor is not absolute. In contrast to the effects in the foetus, we have shown that in the newborn rabbit, as in the newborn primate¹⁸, ACTH will stimulate an increase in the blood concentration of cortisol.

In striking contrast to the ineffectiveness of ACTH, α MSH provoked a significant increase in the concentration of cortisol in the blood of rabbit foetuses at day 22. The response to α MSH, both absolute and proportional was less marked at day 28, although this could relate to the dose injected. In the newborn the response to α MSH relative to that with Synacthen was reduced further. Previous work has shown that adrenal cells from adult rabbits *in vitro* do not respond to hyperphysiological amounts of α MSH, although they do secrete cortisol in response to ACTH¹⁸. The possible role of hormones other than ACTH as trophic agents to the adrenal gland in the foetus has been suggested from studies in sheep^{8,9,10} and in man^{11,19}, where the ACTH-related peptides, α MSH and CLIP have been isolated from foetal pituitaries. Although the concentrations of these peptides relative to ACTH decreased during late gestation and after birth, it is apparent that they are the dominant peptides of foetal life^{8,11}.

While one cannot conclude from our study that α MSH is 'the' trophic hormone to foetal adrenal function in the rabbit, it is apparent that the foetal adrenal responds to it by day 22 of pregnancy, at a time it does not respond to ACTH. The observation that the foetus may respond to a trophic hormone which is ineffective in the adult is an interesting one, and suggests that it may be inappropriate to extrapolate from adult control mechanisms to those in the foetus. In that sense, it may be necessary to revise some of our concepts of underlying control mechanisms in foetal endocrinology.

We thank Drs F. Naftolin and D. K. Lee for helpful comments. The work was supported by the Fraser Memorial Trust of the Royal Victoria Hospital, the Quebec Research Council and the MRC of Canada. J.R.G.C. is a Canada MRC Scholar.

JOHN R. G. CHALLIS*
JAMES D. TOROSIS

McGill University,
Departments of Obstetrics and Gynaecology
and Physiology,
Montreal, Quebec, Canada

Received 26 July; accepted 26 August 1977.

*To whom correspondence should be addressed. Present address: Royal Victoria Hospital, Women's Pavilion, 687 Pine Avenue West, Montreal, Quebec, Canada.

1. Avery, M. E. *Br. med. Bull.* **31**, 13–18 (1975).
2. Thornburn, G. D., Challis, J. R. G. & Robinson, J. S. in *Biology of the Uterus* (ed. Wynn, R. M.) 653–732 (Plenum, New York, 1977).
3. Murphy, B. E. P., Patrick, J. E. & Denton, R. L. *J. clin. endocr. Metab.* **40**, 164–167 (1975).
4. Fencil, M. de M. & Tulchinsky, D. *New Engl. J. Med.* **292**, 133–136 (1975).

5. Mulay, S., Giannopoulos, G. & Solomon, S. *Endocrinology* **93**, 1342–1348 (1973).
6. Rees, L. H., Jack, P. M. B., Thomas, A. L., Nathanielsz, P. W. *Nature* **253**, 274–275 (1975).
7. Jones, C. T., Boddy, K. & Robinson, J. S. *J. Endocr.* **72**, 293–300 (1977).
8. Louis, T. M., Challis, J. R. G., Robinson, J. S. & Thornburn, G. D. *Nature* **264**, 797–798 (1976).
9. Jones, C. T., Boddy, K., Robinson, J. S. & Ratcliffe, J. G. *J. Endocr.* **72**, 279–292 (1977).
10. Challis, J. R. G., Carson, G. D. & Naftolin, F. *J. Endocr.* (in the press).
11. Silman, R. E., Chard, T., Lowry, P. J., Smith, I. & Young, I. M. *Nature* **260**, 716–718 (1976).
12. Challis, J. R. G., Davies, I. J., Benirschke, K., Hendrickx, A. G. & Ryan, K. J. *Endocrinology* **96**, 185–192 (1975).
13. Giannopoulos, G., Hassan, Z. & Solomon, S. *J. biol. Chem.* **249**, 2424–2427 (1974).
14. Ganjam, V. K., Campwell, A. L. & Murphy, B. E. P. *Endocrinology* **91**, 607–613, (1972).
15. Albano, J. D. M., Jack, P. M., Joseph, T., Gould, R. P., Nathanielsz, P. W. & Brown, B. L. *J. Endocr.* **71**, 333–341 (1976).
16. Jaffe, R. B., Seron-Ferre, M., Huhtaniemi, I. & Korenbrot, C. *J. Steroid Biochem.* (in the press).
17. Jost, A. in *Foetal Autonomy* (eds. Wolstenholme, G. E. W. & O'Connor, M.). *Ciba Fdn Symp.* 79–89 (Churchill, London 1969).
18. Lowry, P. J., McMartin, C. & Peters, J. J. *Endocr.* **59**, 43–55 (1973).
19. Chard, T., Silman, R. E. & Rees, L. H. in *The Foetus and Birth* (eds. Knight, J. & O'Connor, M.). *Ciba Fdn Symp.* 354–370 (Elsevier, Amsterdam, 1977).

Local anaesthetic benzyl alcohol increases membrane thickness

WE present here evidence that the local anaesthetic, benzyl alcohol, causes a large expansion in the thickness (an increase of about 25% at 7.5 mM concentration) of the hydrocarbon region of bimolecular lipid membranes. Such a change in the bilayer fabric of nerve membranes would provide a new insight into the molecular basis of anaesthesia. While the primary process involved in the mode of action of the anaesthetic is apparently different from the possible effect on the boundary lipids around the sodium channel, recently suggested by Lee¹, the main features remain the same; the sodium channel becomes inoperative (closes?) due to a stress resulting from changes in the membrane's bilayer structure in the presence of the anaesthetic.

The effect of the benzyl alcohol on the thickness of the artificial lecithin bilayer membranes was studied using four-terminal measurements of the very low frequency (0.1–100 Hz) capacitance and conductance of artificial lecithin bilayers with the aid of a digital impedance measuring technique elsewhere^{2–4}.

The effect of benzyl alcohol on the hydrocarbon region capacitance is shown in Fig. 1a. At concentrations of over 7.5 mM, benzyl alcohol decreased the hydrocarbon region capacitance by more than 25%. The conductance of this region also increased in the presence of the anaesthetic (Fig. 1b).

In the absence of the anaesthetic, with a dielectric constant $\epsilon_H = 2.1$ for the hydrocarbon region², the bilayer has a hydrocarbon region thickness $\delta_H = 3.7$ nm. This is considerably less than twice the fully extended length of the hydrocarbon tails of the predominant lecithins in egg phosphatidylcholine. In the presence of the anaesthetic at 7.5 mM concentration the capacitance data yields a thickness^{2,4} of 4.9 nm for the hydrocarbon region. This is a little larger than twice the fully extended length of the hydrocarbon tails. In the absence of the anaesthetic the hydrocarbon tails therefore seem to be much folded, coiled, kinked (double-gauche forms may predominate) or even somewhat interdigitated.

The anaesthetic apparently has the effect of straightening out the hydrocarbon tails. This process is accompanied by an increase in the conductance of the membranes and, from independent nuclear magnetic resonance studies⁵, an increase in fluidity.

Although the presence of cholesterol in the membrane was found itself to decrease the hydrocarbon region thickness, we found that the effect of benzyl alcohol on lecithin-cholesterol membranes was similar to that reported here for pure lecithin bilayers when the electrolyte was 1 mM KCl.

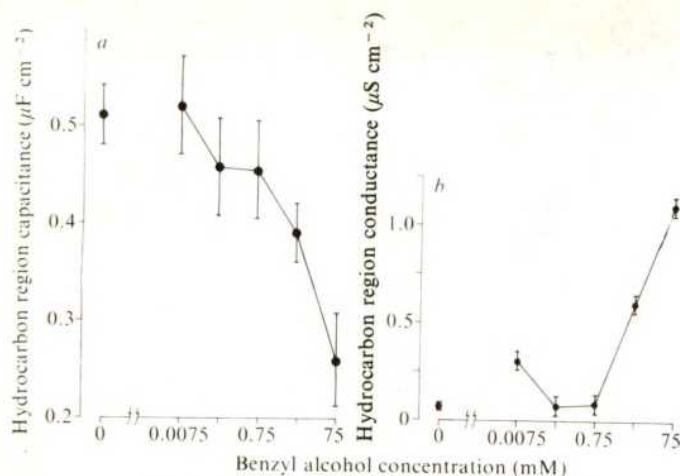


Fig. 1 *a*, The effect of benzyl alcohol on the capacitance of the hydrocarbon layer of bimolecular lipid (lecithin) membranes. The membranes were formed in a 1 mM KCl solution to which the anaesthetic was added. *b*, The conductance of the hydrocarbon region for various concentrations of the anaesthetic. The general increase in the conductance of this region was associated with an increase also in the apparent, mechanical, fluidity of the membranes. The points shown are mean values from several sets of measurements on different membranes (average of 12 measurements per point). The vertical bars indicate the total experimental scatter.

The lecithin bilayer membranes were generated from films of lecithin in *n*-tetradecane and at high concentrations of benzyl alcohol it seems from the low values of the hydrocarbon region capacitance that significant quantities of the *n*-tetradecane may then have been retained in the membrane.

The change in the thickness on addition of the benzyl alcohol at 7.5 mM concentration amounts to ~ 1.2 nm. This is probably $> 15\%$ of the total length of the excitation conduction modules for sodium ions, which span the membrane. Such a strain could readily lead to a closure of the channel. This is shown schematically in Fig. 2. For illustration purposes the conduction channel is depicted with an outer ring of hydrophobic material which is somewhat thicker than the normal width of the hydrocarbon region

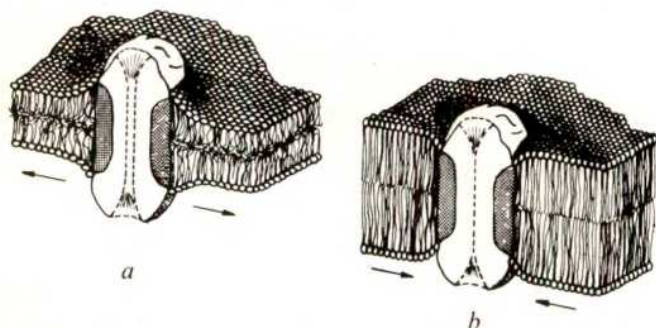


Fig. 2 Schematic representation of the proposed effect of benzyl alcohol on the structure of the bilayer membrane near a hypothetical sodium conduction channel embedded in it. For illustrative purposes, the latter is taken to have a hydrophobic portion (shown shaded) which is somewhat larger than the thickness of the hydrocarbon region of the unperturbed bilayer. The distortion of the bilayer to accommodate such a module together with the requirement of close packing of the fluid-like hydrocarbon portion necessitates the straightening of the fatty acid chains and a closer packing of the polar heads near the conduction module and leads to a radially outward tension on the module. When the hydrocarbon layer thickens in the presence of the anaesthetic, the bilayer is dimpled inwards around the module; this sets up a radial compressive stress on the module⁷. It is suggested that such changes in surface stress are associated with the loss of excitation by the membrane. Alternatively, the accompanying changes in the longitudinal stress in the conduction channel could also lead to inactivation of the gating mechanism.

of the bilayer. Israelachvili *et al.*^{6,7} and Israelachvili (personal communication) have shown from packing geometry and free energy considerations that this will of necessity lead to a distortion outwards of the bilayer system around the conduction module as indicated in Fig. 2*a*. As pointed out by Israelachvili, the extension of the hydrocarbon chains of the lipids adjacent to the protein module restricts their motion and hence reduces their configurational entropy which leads to surface stress as indicated by the arrows in the diagram.

We now propose that this stress would tend to strain the conduction module radially outwards, which would assist in keeping the sodium channel through this module open. When benzyl alcohol is present we have found that the bilayer hydrocarbon region thickness is increased by at least 25% (somewhat more if the benzyl alcohol increased the dielectric constant of this region). In nerve cells this would have the effect shown in Fig. 2*b*. The hydrocarbon region of the bilayer is now wider than the hydrophobic region of the conduction module, and the bilayer has become distorted inwards around the sodium channel. The surface forces now lead to a radial compression of the channel (see Fig. 2*b*) thus blocking conduction by the channel.

On the other hand, if the conduction module itself stretches to accommodate the changes in the hydrocarbon region of the bilayer, the resulting longitudinal strain could by itself lead to a radial closure, or otherwise interfere with the gating mechanism by way of a deleterious change in the conformation of the protein of the module. Alternatively cardinal sites on it would become buried (if only in the polar head region of the bilayer) as the hydrocarbon region expands. Both these possible effects could readily interfere with the function of many of the proposed excitation mechanisms (for example refs 8, 9).

We thank Dr J. N. Israelachvili of the Australian National University for a stimulating discussion of the packing of proteins in lipid bilayers and for a preprint of his publication on this subject. After submission of this paper we learnt of a manuscript submitted by D. A. Haydon *et al.* in which, on the basis of the effects of alkanes, they postulated a mechanism similar to that we propose here for the molecular basis of anaesthesia.

R. G. ASHCROFT
H. G. L. COSTER
J. R. SMITH

Biophysics Laboratory, School of Physics,
The University of New South Wales,
Kensington, Sydney, Australia 2033

Received 15 March; accepted 22 June 1977.

- Lee, A. G. *Nature* **262**, 545–548 (1976).
- Coster, H. G. L. & Smith, J. R. *Biochim. biophys. Acta* **373**, 151–164 (1974).
- Bell, D. J., Coster, H. G. L. & Smith, J. R. *J. Phys. E* **8**, 66–70 (1975).
- Ashcroft, R. G., Coster, H. G. L. & Smith, J. R. *Biochim. biophys. Acta* **469**, 13–22 (1977).
- Metcalf, J. C. in *Permeability and Function of Biological Membranes* (eds Bolis, L., Katchalsky, A., Keynes, R. P., Loewenstein, W. B. & Pethica, B. A.) (North-Holland, Amsterdam, 1970).
- Israelachvili, J. N., Mitchell, D. J. & Ninham, B. W. *J. Chem. Soc., Far. Trans. II* **72**, 1525–1568 (1976).
- Israelachvili, J. N. *Biochim. biophys. Acta* **469**, 221–225 (1977).
- Hille, B. *J. gen. Physiol.* **66**, 535–560 (1975).
- Coster, H. G. L. & Smith, J. R. *Bioenergetics Bioelectrochem.* **3**, 214–215 (1976).

Quantum amplitude distributions point to functional unity of the synaptic 'active zone'

THE exact nature of the quantum is still not known. Several observations^{1–6} support the idea that transmitter is released from synaptic vesicles. Analyses based on the quantum hypothesis using Poisson^{7,8} or binomial statistics^{9,10} cannot

provide direct evidence for or against this idea, since quanta might equally well be produced by the opening and closing of membrane shutters allowing release of cytoplasmic transmitter. All these considerations rest on the assumption that a single quantum is due to the discharge of a single vesicle or a single shutter action. Recent reports have pointed to a remarkable heterogeneity of the quantum in the mouse and frog neuromuscular junction^{11,12} and in some cases subunits are apparent. In this investigation, evidence is provided from the frog neuromuscular junction that the 'quantum' has subunits.

Spontaneous depolarisations of the postsynaptic cell were first observed in the neuromuscular junction of the frog¹³. Since the amplitude histograms of these depolarisations were well described by a single Gaussian curve they were consequently^{14,15} considered to be due to the liberation of the smallest possible amount of transmitter substance and thus named quanta, single units, and so on. In subsequent experiments (for example, refs 7 and 8) it was found that evoked transmitter release occurs in multiples of these unit packages.

In the present investigation conventional intracellular recording techniques were used to monitor spontaneous miniature endplate potentials (m.e.p.ps) from small muscle cells (20–40 μm diameter) of frog cutaneous pectoris muscles (*Rana temporaria* and *Rana esculenta*) at room temperature.

To rule out systemic errors in measuring the signal amplitudes two independent procedures were used in most experiments. Both involved electronic computing devices (with fast AD converters) which were programmed to recognise the typical signal shape. In the on-line version (PDP8E) a 1-mV signal at the cell corresponded to 231 digital points (thus measuring in steps of $\approx 4 \mu\text{V}$); peak amplitudes were obtained by gliding averaging of the signal trace. In the off-line (PDP10) version, signals were stored on analogue tapes (10 kHz) and later peak amplitudes were evaluated by fitting an idealised theoretical curve to each signal. Both methods produced the same results. Class width and starting point of the histograms were chosen such that the subunits were most clearly visible. Records were carefully investigated for a change in the average amplitude of the m.e.p.ps with time. In the present investigation only stable periods were used for further examinations.

Figure 1 shows amplitude histograms of two different cells. The overall appearance of the distributions is normal. Peaks are, however, apparent at regular distances from one another (dots). To analyse these peaks, a statistical test was adopted which is basically binomial and similar to the methods in use for the classic quantum analysis^{8,10}. It is assumed that there is a constant number of independent subunits (n_v) in each 'active site' (see below), each one having the same probability (p_v) of being discharged when the site is activated. Subunit size (SU = mean amplitude, b = coefficient of variation) is similar in each 'active site' and subunits sum linearly. The size of the subunits as well as the binomial parameters and the predicted curves were estimated by two means.

Method A: Estimation of SU from the amplitude histogram by eye; the multiples have to fit as many as possible of the visible peaks and the first theoretically possible SU must start at 0 mV. The binomial parameters n_v and p_v were then estimated using conventional methods¹⁰. To compare the observed and expected distributions the 'geometrical' method described in detail by Boyd and Martin⁸ was used. b was chosen to give the best fit.

Method B: A non-linear least-squares fit¹⁶, which takes into account all the above assumptions, was used in a computer program to find values for SU , b , n_v and p_v which would provide the best fit to the observed histogram.

Figure 2 shows observed and fitted curves for two

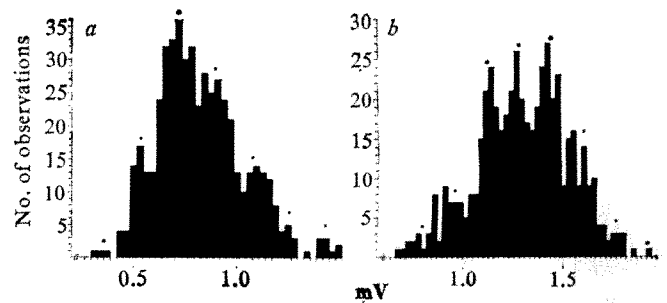


Fig. 1 Intracellular amplitude histograms of spontaneous m.e.p.ps recorded from two different muscle fibres. The total number of observations was 512 in a and 510 in b. Resting membrane potential was 85 mV and 65 mV, respectively. Note peaks at regular distances (dots). The amplitude of the subunits (SU) was estimated using method A. SU is 0.178 mV in a and 0.160 mV in b.

experiments. In all cases there is a good fit thus providing evidence for the existence of subunits. In most experiments (14 out of 18), subunits were apparent, whereas in others no evidence for subunits could be obtained.

Theoretically, the observed subunits could be produced at both the pre- and postsynaptic level or even in the synaptic cleft. Any such mechanism, however, is less plausible than the simple explanation, that spontaneous transmitter release occurs in multi-package units.

In electron micrographs, gatherings of synaptic vesicles are seen at the regularly spaced 'active zones'^{15,17,18}, and it seems that there is a strictly ordered double row with a total of about 10–30 vesicles at each 'active zone'¹⁷. From simultaneous measurements of individual quantal discharges with three micro-electrodes, it has been inferred that spontaneous release of transmitter occurs exclusively at discrete sites ('active sites'), which most likely correspond to the 'active zones'¹². Recent statistical estimations of the number of 'units in the nerve terminal capable of (quantal) release (n)' indicate that in the frog neuromuscular junction, n is small (about 1 per micron length of the nerve terminal). These numbers roughly correspond to the number of 'active zones' in the activated length of the nerve terminal, which in turn suggests that not more than one quantum is discharged at each 'active zone'.

The numbers of subunits in the amplitude histograms were found in several experiments to be between 2 and 15 which are somewhat less than, but still in accord with, the number of vesicles lined up in an 'active zone'. Considering the evidence for a special function of the 'active zone'^{10,12,17}, therefore, we find it plausible that a quantum might be due to the simultaneous discharge of several or all vesicles in one 'active zone'. No temporal resolution of subunits has as yet been observed during a quantal discharge, so a mechanism must be postulated which triggers release from several vesicles virtually simultaneously. In Fig. 2, at some peaks observed frequencies deviate from the expected ones (for example, Fig. 2a, second and third peak of the theoretical distribution). Such deviations merely violate the simple binomial assumption. Indeed, it is more realistic to assume a threshold mechanism such that all vesicles 'available' at an active site are released ($p_v=1$), n_v is changing in time and is different from site to site. The number of subunits observed in the amplitude distributions then shows the range in n_v .

Since the coefficient of variation (b) of the subunits is very small, the releasable transmitter content of individual vesicles must be very similar.

Based on amplitude measurements of a large number of spontaneous m.e.p.ps and application of a statistical test, evidence is presented that quanta consist of about 2 to 15

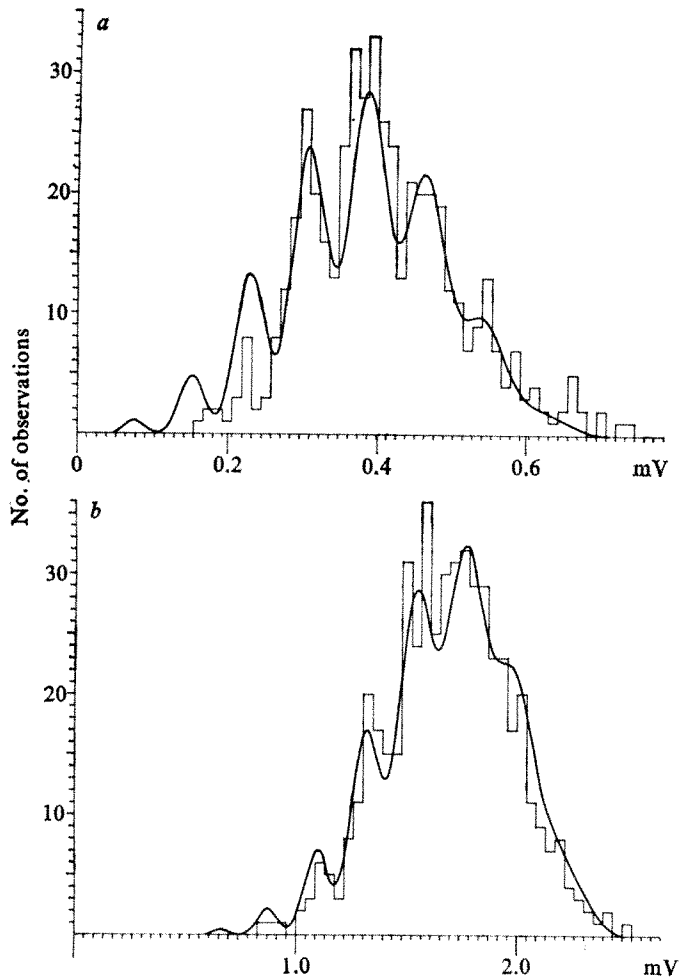


Fig. 2 Observed and fitted amplitude distributions for spontaneous m.e.p.s. from two experiments. Method B was used in *a* and method A in *b*. The amplitude of the subunit (*SU*) was 0.0768 mV in *a* and 0.2177 mV in *b*. The coefficient of variation was 0.11 (*a*) and 0.05 (*b*). The binomial parameter *n*, was calculated as 8 (*a*) and 10 (*b*). The total number of observations (*N*) was 489 (*a*) and 507 (*b*). The predicted curve starts at 0 mV. Note deviation from the observed distribution especially in *a* (for example, second and third peak of the predicted distribution).

subunits. It is proposed that the quantum is due to the simultaneous discharge of several transmitter quantities at one site. Taking into account other evidence^{10,12,17}, it is postulated that the 'active zone' is a functional unit. Its activation leads to the simultaneous discharge of synaptic vesicles lined up there: this creates a single quantum.

Some of the equipment used in this study was provided for A.W. by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung.

A. WERNIG
H. STIRNER

Department of Neurophysiology,
Max-Planck-Institut für Psychiatrie,
D-8000 München 40, Kraepelinstrasse 2,
Federal German Republic

Received 18 May; accepted 1 September 1977.

- Palade, G. E. & Palay, S. L., *Anat. Rec.* **118**, 335-336 (1954).
- De Robertis, E. & Bennet, H. S., *J. biophys. Biochem. Cytol.* **1**, 48-58 (1955).
- De Robertis, E., Rodriguez de Lores Arnaiz, G., Salganicoff, L., Pellegrino de Iraldi, A. & Zieler, L. M., *J. Neurochem.* **10**, 225-235 (1963).
- Whittaker, V. P., Michaelson, I. A. & Kirkland, R. J. A., *Biochem. J.* **90**, 293-303 (1964).
- Couteaux, M. R. & Pecot-Dechavassine, M., *C. r. hebdom. Séanc. Acad. Sci., Paris*, **271**, 2346-2349 (1970).
- Weinshilboum, R. M., Thoa, N. B., Johnson, D. G., Kopin, I. J. & Axelrod, J., *Science* **174**, 1349-1351 (1971).
- Del Castillo, J. & Katz, B., *J. Physiol., Lond.* **124**, 560-573 (1954 b).
- Boyd, I. A. & Martin, A. R., *J. Physiol., Lond.* **132**, 74-91 (1956).
- Johnson, E. W. & Wernig, A., *J. Physiol., Lond.* **218**, 757-767 (1971).

- Wernig, A., *J. Physiol., Lond.* **244**, 207-221 (1975).
- Kriebel, M. E., Llados, F. & Matteson, D. R., *J. Physiol., Lond.* **262**, 553-581 (1976).
- Wernig, A., *Brain Res.* **118**, 63-72 (1976).
- Fatt, P. & Katz, B., *Nature* **166**, 597-598 (1950).
- Fatt, P. & Katz, B., *J. Physiol., Lond.* **117**, 109-128 (1952).
- Del Castillo, J. & Katz, B., *J. Physiol., Lond.* **124**, 553-559 (1954 a).
- Marquardt, D., *J. Soc. Ind. appl. Math.* **11**, 431-441 (1963).
- Couteaux, M. R. & Pecot-Dechavassine, M., *C. r. hebdom. Séanc. Acad. Sci., Paris* **278**, 291-293 (1974).
- Dreyer, F., Peper, K., Akert, K., Sandri, C. & Moor, H., *Brain Res.* **62**, 373-380 (1973).

Light-activated GTPase in vertebrate photoreceptors

In studies of the regulation of cyclic GMP levels by light we found that rod outer segment disk membranes contain a cyclic GMP phosphodiesterase (PDE) with a molecular weight of 240,000, whose activation depends on the photoisomerisation of rhodopsin in the presence of a nucleoside triphosphate¹⁻³. We report here an additional light-dependent enzyme activity, the hydrolysis of GTP to form GDP and inorganic phosphate, which functions as another enzymatic component in the regulation of photoreceptor guanosine nucleotide levels by light. This GTPase has the action spectrum of rhodopsin and is half-maximally activated by bleaching only one in 2,000 rhodopsins. The enzyme has an apparent *K_m* for GTP of 0.5 μ M. GTPase activity can be quantitatively removed from the disk membranes and the activity can be fully restored to the disk membranes by a partially purified heat labile protein with an apparent molecular weight of 32,000.

In studies of the activation of photoreceptor cyclic GMP PDE by light and nucleoside triphosphate¹⁻³, we attempted to catalogue all possible reactions of the phosphoryl donors (γ -³²P)GTP and (γ -³²P)ATP. We have observed that between 0.01 and 5 μ M nucleoside triphosphate, light can stimulate the hydrolysis of GTP, but not ATP, in frog (*Rana catesbiana*) outer-segment disk membranes.

The photoreceptor GTPase shows remarkable sensitivity to light; half-maximal activation is observed when only 1 in 2,000 rhodopsins is photoisomerised (Fig. 1). In our conditions (only 0.05% of rhodopsin photoisomerised, 0.5 μ M GTP and 1.2 mg per ml protein), 95% of the radioactivity from (γ -³²P)GTP is found as inorganic phosphate within 10 min and less than 3% covalently labels disk membrane protein (as monitored in sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis). The sensitivity of this GTPase to light is identical to that reported for the light-activated photoreceptor PDE (ref. 3).

We next examined the dependence of the activation of GTPase on the wavelength of the exciting light. We arranged conditions where we could compare the efficacy of GTPase activation by equal numbers of photons at wavelengths ranging from 425 to 600 nm. This action spectrum for GTPase corresponds perfectly to the absorption spectrum of the rod photopigment rhodopsin (Fig. 2).

The light activation of photoreceptor GTPase can be accomplished in one of two ways. We can either randomly photoisomerise a small fraction of the total disk membrane rhodopsins (with a flash of light) or we can produce an activation of more than sixfold by adding a 1% admixture of fully photoisomerised membranes to a reservoir of a fully dark-adapted, unwashed suspension of disk membranes. This activation by the phenomenon of mixing was also observed for the light-activated photoreceptor PDE³. This activation is seen in conditions where the contribution of GTPase activity by the 1% admixture is negligible (see Table 1). It is not yet clear whether activation by mixing depends on the fusion of vesicles containing bleached rhodopsin with dark membranes, or whether the bleached material contributes some soluble cofactor in the activation sequence.

The light-activated GTPase activity can be quantitatively removed from the disk membranes by lysing unilluminated rods in 1.0 mM EDTA (pH 7.0), and collecting the disk membranes by centrifugation (35,000 r.p.m., Beckman SW56 rotor, 1 h,

Table 1 Light activation of GTPase by a flash of light or by the combining of dark and fully bleached membranes

	GTPase
0.05% bleached* membranes	300
Dark membranes	48
Dark membranes plus fully bleached membranes† (99 : 1 protein)	302
Fully bleached component of above mixture	0

GTPase activities are expressed as pmol GTP hydrolysed per mg protein per min. GTP assays were done in the conditions stated in Fig. 1.

*0.05% 'bleached' membranes are disk membrane suspensions in which 0.05% of the rhodopsin is photoisomerised.

†The bleached component of the mixture was washed (by sedimentation and resuspension) three times before full illumination with 100 mM Tris-HCl (pH 7.4) containing 5 mM MgSO_4 and 1 mM dithiothreitol. This washing procedure removes 90% of the GTPase activity.

4 °C). These disk membranes, after one additional wash in the same buffer, showed no GTPase or PDE activities. Both PDE and GTPase activities can be restored to the washed disk membranes by addition of light, 5 mM Mg^{2+} and the supernatant obtained by centrifugation of the lysed rods. This supernatant alone does not show GTPase activity. The supernatant was subsequently applied to a Sepharose 6B column which was equilibrated and eluted with 10 mM Tris (pH 7.5) containing 5 mM MgSO_4 , 1 mM dithiothreitol and 50 mM KCl. Column fractions were assayed by recombination with the washed and illuminated disk membranes described above. A single peak of GTPase activity is associated with a heat-labile molecule which does not display PDE activity and which gives a sharp (Coomassie blue positive) band with an apparent molecular weight of 32,000 in SDS-polyacrylamide gel electrophoresis⁶.

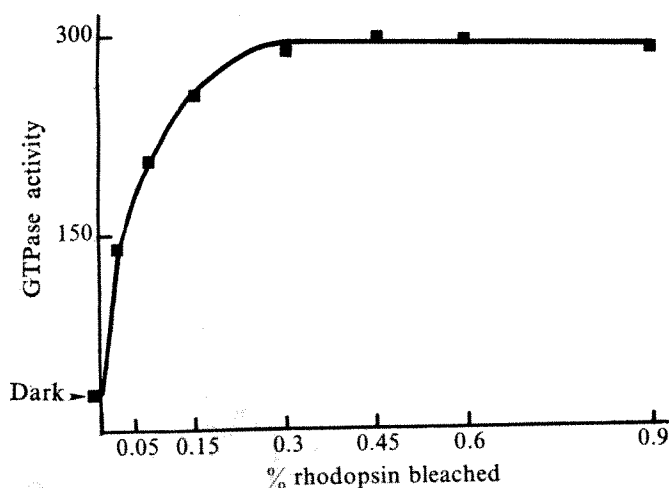


Fig. 1 Light activation of GTPase as a function of the extent of rhodopsin bleaching. Varying degrees of rhodopsin photoisomerisation were obtained by exposing disk membrane suspensions to attenuated (neutral density filtered) light for various times. The filter array was calibrated to permit half-maximal activation with a 30-s exposure. The % rhodopsin photoisomerised was directly measured in 1% hexadecyltrimethylammonium bromide for bleaches in excess of 5% and linearly extrapolated to determine fractional % bleaches accomplished at shorter exposure times. GTPase activity was measured (37 °C at pH 7.4) by the method of Neufeld and Levy⁴ using 0.5 μM (γ - ^{32}P)GTP with specific activity of 20 Ci mmol^{-1} in a reaction volume of 100 μl containing 90 μg of disk membrane protein. Activities are expressed as pmol GTP hydrolysed per mg protein per min. Alternatively, GTPase was assayed by measuring the formation of GDP from (β , γ - ^{32}P)GTP with descending thin-layer chromatography⁵. Rod outer-segment disk membranes were isolated from *Rana catesbiana* by sucrose flotation³. All manipulations and GTPase assays (except intentional illumination) were performed in infrared light using image converters³.

We emphasise that the range of GTP concentrations over which light activation is observed is from 0.01 to 5 μM GTP. The K_m for the light-activated reaction is approximately 0.5 μM ($\pm 0.4 \mu\text{M}$) GTP. The maximum velocity achieved by the light-activated GTPase is approximately 500 pmol GTP hydrolysed per mg protein per min. Above 5 μM GTP, however, no light activation of GTPase is seen but an additional GTPase activity is observed which has a K_m of 90 μM GTP and a V_{max} of 12 nmol GTP hydrolysed per mg protein per min.

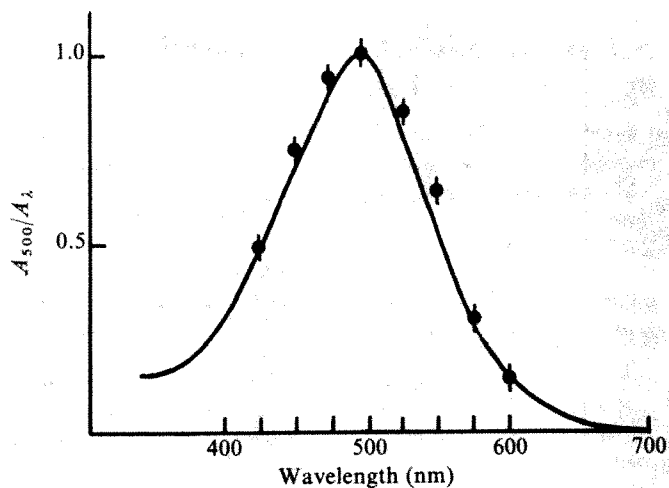


Fig. 2 Action spectrum of light-activated GTPase. Points represent the fraction of highest (A_{500}) GTPase activity above basal. The highest activity observed (at 500 nm) was 80% of maximum possible activation by light. (Basal activity was 30 pmol GTP hydrolysed per mg protein per min). The solid curve is the absorption spectrum of rhodopsin in 1% hexadecyltrimethylammonium bromide. Exposure of disk membranes to equal photon numbers of monochromatic light was accomplished using a light-shielded Gilford 2000 spectrophotometer. The sample holder and photomultiplier were replaced with a camera shutter positioned after the exit slits to allow timed flashes of light. Frosted glass placed in front of the shutter aperture provided a uniformly diffuse source of light. A 100- μl membrane sample was contained in a self-masking cuvette with a 5-mm path length, which was situated after the frosted glass. The intensity of the tungsten lamp at each wavelength was first measured with a YSI radiometer. The intensity profile was used to compute a sample exposure time to normalise photon number at each wavelength. GTPase activities were assayed using the conditions described in Fig. 1.

It is not yet clear whether the high- K_m GTPase activity resides in a second enzyme or reflects changes in the low- K_m enzyme which are brought about by higher (possibly non-physiological) levels of GTP. Interpretation of the physiological roles of the light-activated (low K_m) and non-light-activated (high K_m) GTPase will depend on further studies of GTP concentration *in vivo*.

Finally, we are intrigued by the extraordinary similarity in the light requirements shown by both the GTPase and PDE of vertebrate outer-segment disk membranes. This similarity embraces activation both by a flash of light and by the phenomenon of mixing, suggesting that these enzymes may be functionally, if not topologically, linked. After submission of this manuscript we were informed of a report of similar findings published in the abstracts of the 21st Biophysical Society meeting⁷.

G. L. WHEELER
Y. MATUO
M. W. BITENSKY

Yale University School of Medicine,
Department of Pathology,
New Haven, Connecticut 06510

Received 18 July; accepted 29 August 1977.

1. Bitensky, M. W. *et al. Adv. Cyclic Nucleotide Res.* **5**, 213-240 (1975).
2. Miki, N., Baraban, J. M., Keirns, J. J., Boyce, J. J. & Bitensky, M. W. *J. biol. Chem.* **250**, 6320-6327 (1975).
3. Keirns, J. J., Miki, N., Bitensky, M. W. & Keirns, M. *Biochemistry* **14**, 2760-2766 (1975).
4. Neufeld, A. & Levy, H. *J. biol. Chem.* **244**, 6493-6497 (1969).
5. Keirns, J. J., Wheeler, M. A. & Bitensky, M. W. *Analyt. Biochem.* **61**, 336-348 (1974).
6. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. *Biochemistry* **10**, 2606-2617 (1971).
7. Robinson, W. E. & Hagins, W. A. *Biophys. J.* **17**, 196A (1977).

Salt-sensitive *in vitro* protein synthesis by a moderately halophilic bacterium

EXTREMELY halophilic bacteria, such as *Halobacterium cutirubrum*, grow only in high NaCl concentrations (2.5–5 M) and have very high (5 M or higher) internal concentrations of salts, mainly KCl^{1,2}. The ribosomes and protein-synthesising systems of these organisms seem especially adapted to function in such concentrations³. In contrast, moderately halophilic bacteria, such as *Vibrio costicola*, grow over a much wider range of NaCl concentrations (at least 0.5–3.5 M)⁴. In *V. costicola*, though not necessarily in all moderate halophiles, the cell-associated monovalent cations are at least as concentrated as those of the external medium. For example, cells growing exponentially in medium containing 1 M NaCl and 0.008 M KCl can have internal Na⁺, K⁺, and NH₄⁺ concentrations of about 0.6, 0.7 and 0.4 M respectively, as well as 40 mM Mg²⁺ (ref. 5 and our unpublished results). Ribosomes from *V. costicola* differ from those of both extremely halophilic and non-halophilic bacteria in their ability to maintain a 'standard' sedimentation pattern (30, 50 and 70S) over a wide range of salt concentrations. This pattern is not changed by the NaCl concentration in which the cells are grown⁶. Such properties, however, do not really tell us how well the ribosomes function at different salt concentrations. Studies of *in vitro* protein synthesis, reported here, suggest that ribosomes may function at much lower salt concentrations than measurements of total cell-associated ions indicated to be present in the cell.

Extracts were prepared from cells grown in medium containing 1 M NaCl, washed, and suspended in mixtures of NaCl, KCl, NH₄Cl and MgCl₂ approximating the cell-associated concentrations of these cations. Cells were broken by osmotic lysis or by grinding with alumina. After centrifugation at 30,000g for 30 min to remove intact cells and membrane fragments, the supernatants (S-30) were incubated with the ingredients used to demonstrate *in vitro* protein synthesis in other bacterial extracts (see legends to Figs 1 and 2), but with the final salt concentrations adjusted to that in which the cells were broken. No activity was found, as measured by incorporation of radioactive amino acids into trichloroacetic acid-precipitable material. But, if no salt was added to the assay mixture other than that contained in the extract (final concentration about 0.2 M) protein synthesis occurred. If the concentration of any salt was increased, activity fell. At concentrations of 1 M or higher, no activity could be measured.

Figure 1 shows the effects of various concentrations of salts of monovalent cations and MgCl₂ on poly U-dependent phenylalanine incorporation. Figure 2 gives comparable results for incorporation using 'endogenous messengers'—that is, incorporation of a mixture of ¹⁴C-labelled amino acids from a *Chlorella* hydrolysate. In both systems activity was highest in NH₄Cl, followed by KCl, then NaCl. Optimal activities occurred in 0.1–0.2 M salts, and higher concentrations were inhibitory. This was especially noticeable in the effects of NaCl on the 'endogenous' system. Magnesium ions were needed for activity, maximal activity being reached at about 18 mM Mg²⁺ in both systems.

We considered the possibility that high concentrations of monovalent cations inhibited protein synthesis because they were displacing the essential divalent cation, Mg²⁺. If this were so, it should be possible to overcome the inhibitory effect of the former by increasing the latter. In both systems, however, increasing the MgCl₂ concentration two- or fivefold (from 20 mM) did not reverse the inhibition caused by higher concentrations of the other salts (up to 1 M). Higher concentrations of Mg²⁺ were just as inhibitory in the presence of higher as in lower concentrations of the other salts.

One of the problems with establishing a protein synthesising system in a 'new' bacterium, is showing that the measured incorporation represents polypeptide synthesis. This was shown by the following findings. Incorporation is energy dependent. In the absence of poly U phenylalanine incorporation was reduced by 90%, and ¹⁴C-leucine was incorporated to about the same extent as phenylalanine. Leucine incorporation was not stimulated by poly U. Treatment of the system with ribonuclease A (Sigma, 40 µg ml⁻¹ in assay mixture) abolished all activity. Chloramphenicol (15 µg ml⁻¹) and puromycin (25 µg ml⁻¹) strongly or completely inhibited activity. Aurintricarboxylic acid (105 µg ml⁻¹) inhibited incorporation in both the poly U-dependent

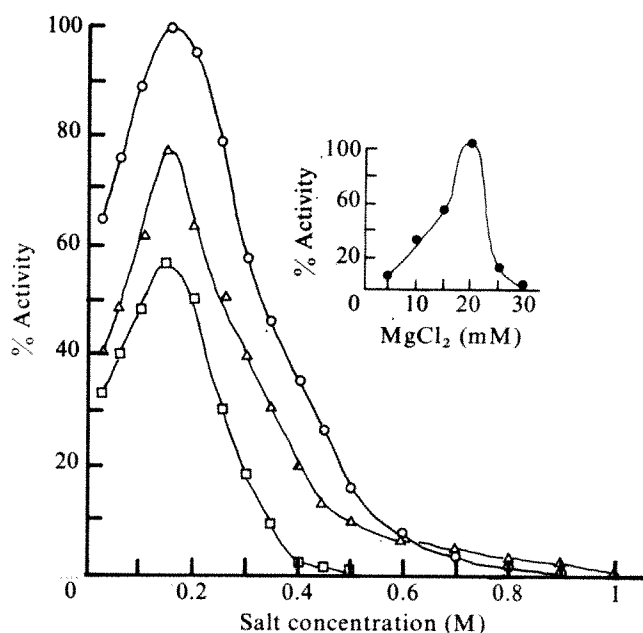


Fig. 1 Effects of NaCl (□), KCl (△), NH₄Cl (○) and MgCl₂ (insert) on the poly U-dependent ¹⁴C-phenylalanine incorporation. *V. costicola* cells, grown in 1 M NaCl, were broken by grinding with twice the wet cell weight with alumina. After centrifugation at 30,000g for 30 min to remove whole cells and membrane fragments, small volumes (1–2 ml) of the extracts were dialysed with rapid stirring for 1 h against 1–2 l of a solution of 100 mM NaCl, KCl, or NH₄Cl, depending on the salt to be studied, plus 20 mM MgCl₂ and 10 mM Tris-HCl, pH 7.6. This dialysed extract was then centrifuged at 30,000g for 30 min and the resulting supernatant was termed the S-30 extract. 90 µl of the S-30 extract was incubated with 210 µl of the reaction mixture so that the final concentrations were: 15 mM phosphoenol pyruvate; 2 mM adenosine triphosphate; 1 mM guanosine triphosphate; 18 mM MgCl₂ (or as indicated, insert); 30 µg ml⁻¹ pyruvate kinase; 50 mM Tris-HCl, pH 7.6; 7.5 mM reduced glutathione; 9.6 µM ¹⁴C-phenylalanine (522 mCi mmol⁻¹); 270 µg poly U; and 100 mM NH₄Cl, KCl, or NaCl, or the concentration as indicated. The reaction was incubated at 30 °C and 50 µl aliquots taken at 0, 2, 4, 6, and 8 min. The aliquots were precipitated with 3 ml of cold 5% (w/v) trichloroacetic acid, heated at 90 °C for 20 min, then collected onto 0.45-µm filters (Millipore). The filters were dried and counted in a liquid scintillation counter with 5 ml of Scintiverse (Fisher Scientific). An activity of 100% represents 22.2 pmol of phenylalanine incorporated per min per A₂₆₀ unit of the S-30 extract.

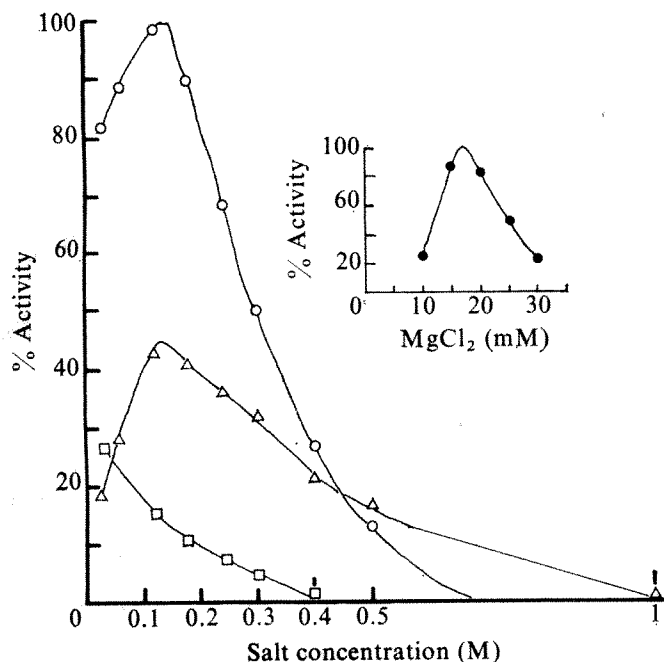


Fig. 2 Effects of NaCl (○), KCl (△), NH₄Cl (□) and MgCl₂ (insert) on the amino acid incorporation dependent on 'endogenous messenger'. *V. costicola*, grown in 1 M NaCl, cells were lysed osmotically by suspending the cell pellets in buffer containing: 200 mM KCl; 10 mM Tris, pH 7.6; and 5 mg ml⁻¹ lysozyme. After 2–3 min incubation on ice, the ionic concentration and composition was restored to: 100 mM KCl; 10 mM Tris, pH 7.6; and 18 mM MgCl₂. S-30 extracts were prepared and dialysed as in Fig. 1. The assay conditions are as in Fig. 1 except that poly U and ¹⁴C-phenylalanine were omitted and ¹⁴C-labelled *Chlorella* hydrolysate (54 mCi per mAtom of carbon) was substituted. The values are expressed as % of maximum activity, maximum activity being 300 c.p.m. per min per A₂₆₀ unit.

and the endogenous system. Analysis of the end products of ¹⁴C-phenylalanine incorporation by the method of Pestka *et al.*⁷ showed that 58% was in the form of diphenylalanine, 25% in the form of triphenylalanine, and 17% in the form of higher oligo- or polypeptides.

Salts had a different effect on the stability of the protein synthesising system than on its activity (Table 1). Concentrations of all monovalent salts which inhibited protein synthesis could stabilise the system. Usually, the system was more stable at lower temperatures. Only in the highest NaCl concentration (2 M) did rapid inactivation of the system occur (not shown in Table 1). We cannot assign the effects of the salts to any specific component of the protein synthesising system, but it has been shown that certain salts

Table 1 Effects of Na⁺, K⁺, and NH₄⁺ chlorides on the stability of the poly U-dependent incorporation of ¹⁴C-phenylalanine

Salt	30 °C	<i>t</i> _{1/2} values (min)		3 °C
		20 °C	20 °C	
0.1 M NH ₄ Cl	22	160	510	
1.0 M NH ₄ Cl	63	∞	∞	
0.1 M KCl	40	57	405	
1.0 M KCl	20	∞	∞	
0.1 M NaCl	30	45	∞	
1.0 M NaCl	48	58	1,095	

400 μl of the S-30 extract, dialysed against 100 mM of the appropriate salt was incubated with solid salt to raise the concentration to the desired level. Aliquots of 90 μl of this mixture were removed at various times and assayed as described in Fig. 1 except that for the 1 M salt concentrations no monovalent ions were added to the reaction mixture. This resulted in a final concentration of 0.3 M salt. The *t*_{1/2} is the time required for the extract to lose half of the original activity. The ∞ symbol indicates that the extract lost no activity over the test period (usually 8 h).

have different effects on the stability and the activity of individual enzymes of halophilic bacteria^{2,8}.

It is very difficult to predict the state of ions in the cytoplasm of a cell from knowledge of the total concentration alone. Certain physiological functions might provide a better estimate of the actual internal state of these ions. It is probably significant that many of the internal enzymes of the halobacteria function better in KCl than in NaCl^{2,8}. It is more significant that protein synthesis, a process dependent on many enzymes and on the proper configurations of ribosomes and their interactions with mRNA, also functions in the conditions that are suggested to exist in these cells by total ionic analysis. This is not so for *V. costicola*. Rather, protein synthesis occurs in much lower salt concentrations than those found in the cell as a whole. Protein synthesis would be completely inhibited by the salt concentrations found in the bacteria, if all were free in the cytoplasm. Our results suggest that they are not free, but rather that they are sequestered within the cell. Possibly Mg²⁺ is bound to the cell envelope (optimum Mg²⁺ level for protein synthesis is less than half of total cellular concentration). Many bacterial envelopes can bind divalent cations⁹. It is less easy to envisage a binding site for the monovalent cations. A binding of such cations to envelopes has been reported for other moderately halophilic and non-halophilic bacteria, however (discussed in ref. 5). We are investigating this possibility for *V. costicola*.

This work was performed when T.H. was a visiting scientist at Ottawa University.

R. M. WYDRO

Department of Biology,
University of Ottawa,

W. MADIRA

Department of Biochemistry,
King's College,

T. HIRAMATSU

Department of Bacteriology,
Osaka City University Medical School,
Osaka, Japan

M. KOGUT

Department of Biochemistry, King's College,
Strand, London WC2, UK

D. J. KUSHNER

Department of Biology, University of Ottawa,
Ottawa, Ontario, K1N 6N5, Canada

Received 17 May; accepted 6 September 1977.

1. Kushner, D. J. *Adv. Appl. Microbiol.* **10**, 73 (1968).
2. Brown, A. D. *Bact. Rev.* **40**, 803 (1976).
3. Bayley, S. T. in *Extreme Environments: Mechanism of Microbial Adaptation* (ed. Heinrich, M. R.) 119 (Academic, New York, 1976).
4. Forsyth, M. P. & Kushner, D. J. *Can. J. Microbiol.* **16**, 253 (1970).
5. Shindler, D. B., Wydro, R. M. & Kushner, D. J. *J. Bact.* **130** (1977).
6. Wydro, R., Kogut, M. & Kushner, D. J. *FEBS Lett.* **60**, 210 (1975).
7. Pestka, S., Scolnick, E. M. & Heck, B. H. *Analyt. Biochem.* **28**, 376 (1969).
8. Lanyi, J. K. *Bact. Rev.* **38**, 272 (1974).
9. Beveridge, T. J. & Murray, R. G. E. *J. Bact.* **127**, 1502 (1976).

Carbon dioxide governs the oxygen affinity of crocodile blood

OXYGEN affinity of haemoglobin inside red blood cells is decreased to a level where oxygen unloading can proceed at sufficiently high partial pressures in the tissue capillaries. This adaptation of oxygen affinity is accomplished mainly by the interaction of haemoglobin with intraerythrocytic phosphates¹. These comprise 2,3-diphosphoglycerate (DPG) in most mammals, ATP and GTP in fish², and *myo*-inositol pentaphosphate (IP₅) in mature birds^{3–5}. No haemoglobin system has been described in higher vertebrates which is devoid of functional interaction with the respective intracellular phosphates. Recently, however, Sullivan⁶ has questioned the role of phosphates for the control of oxygen affinity in crocodile

red cells (ATP and inorganic phosphate have been identified^{3,7,8}). He remarks that "the substance regulating oxygen affinity in alligators is very efficient, but unknown". We show here that the oxygen affinity of haemoglobin from a member of the order crocodiles (*Crocodylus porosus*) is not affected by any of the regulating allosteric cofactors known with the exception of molecular CO₂ and protons.

A specimen of *C. porosus*, about 5-yr-old, was anaesthetised with Rompun and Kataral and blood obtained by heart puncture. Red cells were washed in saline and lysed by addition of distilled water. The ghosts were removed by centrifugation. The clear supernatant was passed over Sephadex G-25 columns at 4 °C equilibrated with Tris buffer pH 8.1 to remove all phosphates, and subsequently dialysed against 0.1 M NaCl. The oxygen affinity of haemoglobin solutions was determined as described earlier⁹.

The partial pressure of oxygen at which purified haemoglobin solution is half saturated with oxygen (P_{50}) was 3.9 Torr (20 °C, pH 7.3) and increased to 28.8 Torr on addition of CO₂ ($p\text{CO}_2 = 40$ Torr, pH 7.3). This value is very near to the one found in whole blood ($P_{50} = 30.3$ Torr at 20 °C, extracellular pH 7.47, $p\text{CO}_2 = 40$ Torr). At more alkaline pH the effect of CO₂ on P_{50} is even more pronounced (Fig. 1). Note that the concentration of ATP was low in crocodile erythrocytes (0.6 mM) and that UTP, CTP, ITP, GTP and DPG could not be detected enzymatically¹⁰ or by thin-layer chromatography². Apart from ATP the following phosphates were

tested with respect to their possible influence on P_{50} of haemoglobin solutions: IP₅ prepared from chicken blood¹¹, DPG, fructose-1,6-diphosphate, and cyclic AMP, each used in a 10–20-fold excess of phosphate over Hb₄. None of these led to a significant shift of P_{50} . Similarly, inorganic phosphate (0.1 M) had no effect on the oxygen affinity of crocodile haemoglobin. Apart from CO₂ only protons led to heterotropic interactions with the oxygen binding: $\Delta \log P_{50}/\Delta \text{pH} = -0.43$ in the alkaline range (Fig. 1). When the change in pH was produced by varying $p\text{CO}_2$ at constant HCO₃⁻ concentration $\Delta \log P_{50}/\Delta \text{pH}$ increased to -0.66 due to the additional action of CO₂ on P_{50} . Cooperativity of oxygen binding was not significantly influenced by CO₂: the exponent n in Hill's equation¹² was 2.1 in the absence and 2.0 in the presence of CO₂.

The question arises then as to how this remarkable effect of CO₂ on the oxygen affinity of crocodile haemoglobin can be explained on a molecular basis. As reptile haemoglobins polymerise in certain conditions^{6,13} it is conceivable that the action of CO₂ leads to increased aggregation with consecutive decrease in oxygen affinity. Gel filtration experiments on Sephadex G100 columns operated with *bis*-Tris buffer pH 7.3 at 20 °C yielded two components in equal proportions, one which is probably 'octameric' (apparent molecular weight 110,000) and one 'tetrameric' species (apparent molecular weight 43,000). This proportion was very much the same when the experiment was carried out with bicarbonate buffer equilibrated continuously with CO₂ so to give the same pH and ionic strength. Furthermore, oxygen binding curves of the isolated components showed that CO₂ produced the same decrease in oxygen affinity in the 'octameric' and in the 'tetrameric' pigment as in the unfractionated haemolysate. Additional control experiments on Sephadex G100 were carried out to check that the respective components had not interconverted during the oxygen binding experiments. Both the 'octameric' and the 'tetrameric' component appeared as one major band in isoelectric focusing experiments with isoionic pH values of 7.24 and 7.26, respectively.

Thus we conclude that a reversible aggregation-dissociation phenomenon is not the cause for the CO₂-induced decrease in oxygen affinity of crocodile haemoglobin. Most likely the formation of oxygen-linked carbamate compounds is responsible for the observed effects, qualitatively similar to what is found in human haemoglobin, where more carbamate is present at the terminal α -amino groups in the deoxy- than in the oxy-structure. If the same residues mediate the carbamate formation in crocodile haemoglobin, our data suggest that the carbamate equilibrium constant as well as the change of the carbamate equilibrium constant when the haemoglobin binds or releases oxygen must be higher than even in the β -subunits of human haemoglobin, where most oxygen-linked carbamate is being formed^{9,15}. The negatively-charged carbamate itself could stabilise the deoxy-structure and de-stabilise the oxy-structure of crocodile haemoglobin by secondary interactions with positively-charged residues. If such interactions lead to structural transitions of the quaternary structure it may be possible to observe changes in the absorption spectrum as, for example, in certain fish haemoglobins at different pH values¹⁶. Difference spectra in the Soret range obtained on crocodile haemoglobin in the absence and presence of CO₂ ($p\text{CO}_2 = 100$ Torr) gave no indication of such transitions, however.

Whatever the exact molecular mechanism of the strong antagonism between CO₂ and oxygen binding turns out to be, it is significant that this particular interaction, which must have appeared quite early in the evolution of haemoglobin function in vertebrates^{14,17}, has been explored very efficiently in a member of the order *Crocodylia* which possesses haemoglobins with a high intrinsic oxygen affinity and only little intra-erythrocytic phosphates. In situations where the animal moves about very quickly and therefore liberates CO₂ from the tissues, the antagonism between CO₂ and oxygen binding should be very efficient in aiding oxygen release from the blood.

We thank Dr Wiesner from the Tierpark Hellabrunn,

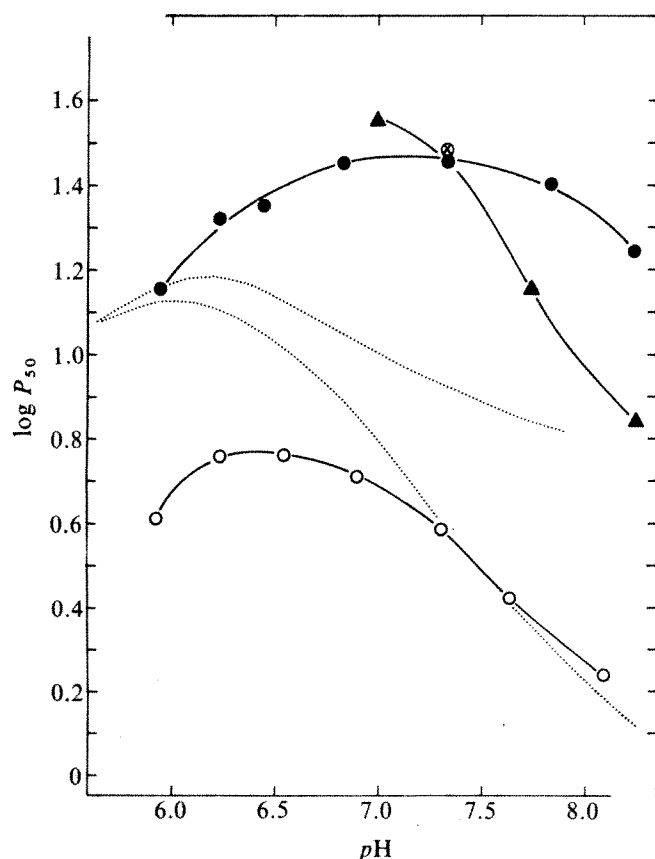


Fig. 1 Plot of $\log P_{50}$ against pH of crocodile haemoglobin in the absence (○) of CO₂ and at a $p\text{CO}_2$ of 40 Torr (●) at 20 °C. Hb concentration 120 μM haem; buffers: 50 mM *bis*-Tris-100 mM NaCl in the absence of CO₂ (total ionic strength 0.150) and bicarbonate-NaCl buffer in the presence of CO₂, 150 mEq l⁻¹. pH was also varied by changing $p\text{CO}_2$ at constant bicarbonate concentration (▲). Blood P_{50} was determined by means of the Lex-O₂-Con apparatus at 20 °C, $p\text{CO}_2$ 40 Torr and extracellular pH 7.47 (⊗). The dotted lines were obtained from similar experiments on human haemoglobin in the absence (lower) and presence (upper) of CO₂⁹.

München, for blood samples, Mrs U. Engels for technical assistance and to Dr G. Gros for helpful comments.

Note added in proof: Oxygen binding to haemoglobin from another crocodile species, *Caiman crocodylus*, exhibits the same strong interaction with molecular CO_2 , but none with ATP.

CHRISTIAN BAUER
WOLFGANG JELKMANN

Physiologisches Institut der Universität
8400 Regensburg, West Germany

Received 4 July; accepted 22 August 1977.

1. Benesch, R. E. & Benesch, R. *Adv. Prot. Chem.* **28**, 211–237 (1974).
2. Weber, R. E., Lykkeboe, G. & Johansen, K. *J. exp. Biol.* **64**, 75–88 (1976).
3. Rapoport, S. & Guest, G. *J. biol. Chem.* **138**, 269–282 (1941).
4. Johnson, L. F. & Tate, M. E. *Can. J. Biochem.* **47**, 63–73 (1969).
5. Isaacs, R. E. *et al. Comp. Biochem. Physiol.* **55A**, 29–33 (1976).
6. Sullivan, B. in *Chemical Zoology* **9**, (eds Florkin, M. & Scheer, B. T.) 377–398 (Academic, New York, 1974).
7. Bartlett, G. R. *Comp. Biochem. Physiol.* **55A**, 211–214 (1976).
8. Coates, M. J. *mol. Evol.* **6**, 285–307 (1975).
9. Bauer, C., Baumann, R., Engels, U. & Pacyna, B. *J. biol. Chem.* **250**, 2173–2176 (1975).
10. Bergmeyer, H. U. (ed.) *Enzymatische Methoden* **2** (Verlag Chemie, Weinheim, 1974).
11. Bartlett, G. R. & Borgese, T. A. *Comp. Biochem. Physiol.* **55A**, 207–210 (1976).
12. Hill, A. V. J. *Physiol., Lond.* **40**, 4–7 (1910).
13. Sullivan, B. & Riggs, A. *Biochim. biophys. Acta* **140**, 274–283 (1967).
14. Bauer, C. & Kurtz, A. *J. biol. Chem.* **252**, 2952–2955 (1977).
15. Morrow, J. S., Matthew, J. B., Wittebort, R. T. & Gurd, F. R. *J. biol. Chem.* **251**, 477–484 (1976).
16. Giardana, B., Ascoli, F. & Brunori, M. *Nature* **256**, 761–762 (1975).
17. Bauer, C., Engels, U. & Paleus, S. *Nature* **256**, 66–68 (1975).

Hapten-induced allosteric transition in the light chain dimer of an immunoglobulin

THE dimer of immunoglobulin light chains (L_2) was shown by X-ray crystallography to be structurally homologous to the native immunoglobulin Fab fragment (HL) (refs 1–3). This led to the suggestion that L_2 is a model for a primitive antibody¹ and for the T-cell receptor^{2,23}. In L_2 315 (from the mouse myeloma protein MOPC 215) functional homology was also implicated in that both L_2 and HL bind the same nitroaromatic haptens with similar fine specificity^{4,5}. We have considered the possibility that this homology extends also to hapten-induced conformational changes and that studies with L_2 will yield information on the nature of such processes in immunoglobulins. L_2 315 constitutes a particularly good model system for such studies as it binds two hapten molecules per dimer at symmetry-related sites^{4,5}. It is thus possible to monitor the conformational effects of the first binding hapten through the mode of interaction with the second, as revealed by the shape of the saturation curve⁶. We report here that dinitrophenyl-lysine (DNPL) and its fluorescent analogue nitrobenzoxadiazole-alanine (NBDA) (ref. 7) both display sigmoidal binding curves, implying positive cooperativity in their interaction with L_2 315. This observed cooperativity is shown to arise from a hapten-induced conformational transition which may be described by the allosteric model⁶. We propose that this allosteric transition involves changes in the relative position of the chains in L_2 , lending support to suggestions^{8,9} that a similar process occurs in the structurally homologous intact immunoglobulin. These findings are, therefore, relevant to the question of antigen-triggered effector functions of antibodies through an allosteric mechanism^{8,10–12}.

The difference spectra observed on addition of hapten to L_2 315 are shown in Fig. 1a and b. Each hapten displays its characteristic difference spectrum which varies little throughout the titration as is particularly emphasised in the well defined isosbestic points for NBDA. This strongly suggests that the two sites on L_2 315 are identical with respect to hapten binding. Accordingly, any model used to account for the observed cooperativity should be symmetric, in line with the previously suggested local symmetry around the binding site of L_2 315 (ref. 5).

The hapten occupancies (calculated from the absorption changes) were plotted against hapten concentration (Fig. 2). For both haptens the binding curve is sigmoidal, the effect being more pronounced with NBDA. For this hapten equi-

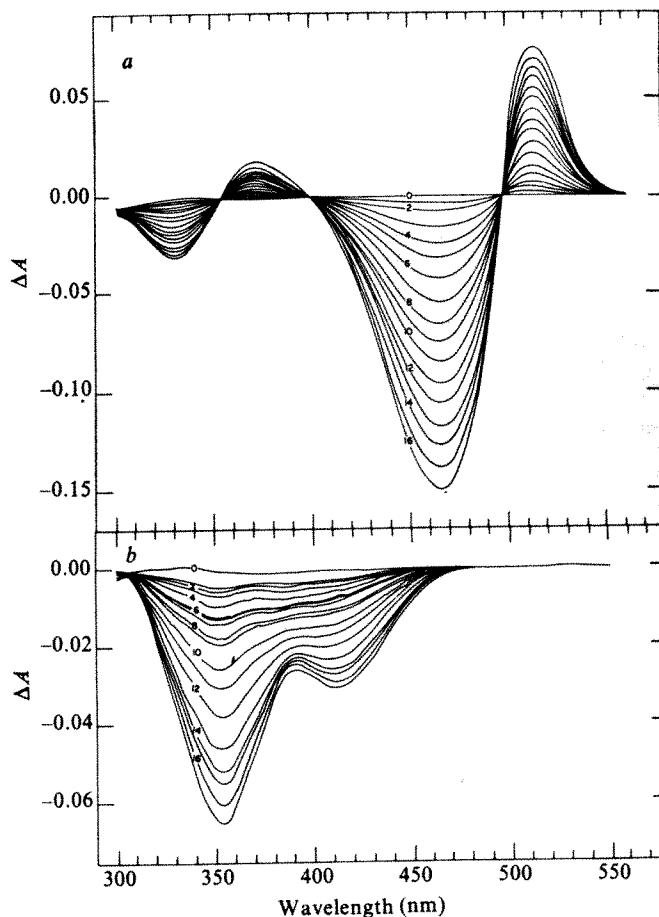


Fig. 1 Difference spectroscopic titrations in which *a*, 4-(α -N-L-alanine)-7-nitro-benz-2-oxa-1,3-diazole (NBDA)⁷, and *b*, ϵ -N-2,4-dinitrophenyl-L-lysine (DNPL), are added to mildly reduced and alkylated L_2 315⁴ in 0.01 M sodium phosphate buffer pH 7.4 with 0.15 M NaCl (PBS). ΔA is the change in hapten absorption on binding to L_2 ($\Delta A = A_{\text{bound}} - A_{\text{free}}$). Spectra were taken on a double beam Cary 118 spectrophotometer in rectangular quartz cuvettes of 0.437 cm pathlength at 4 °C. Equal aliquots of hapten solution were added to a cuvette containing the protein solution and to a reference cuvette containing an exactly equal volume of buffer. The minor contribution of protein absorption below 320 nm was corrected electronically. Initial L_2 concentration was 6.0×10^{-5} M sites (assuming 2 sites per protein molecule, and $\epsilon_{280} = 60,000 \text{ M}^{-1} \text{ cm}^{-1}$ for the dimer).

brum dialysis measurements were also performed and the points were found to lie on the same binding curve (Fig. 2). The origin of the observed sigmoidity is therefore thermodynamic and not spectroscopic—that positive cooperativity occurs in the binding of hapten. The data were analysed according to the Adair¹³ formalism as described in Table 1.

The underlying mechanisms of the observed positive cooperativity may be one of the following: (1) the first bound hapten exerts a direct attractive interaction on the second; (2) hapten binding is coupled to a chain-dissociation equilibrium of the form $L_2 \rightleftharpoons 2L$ (refs 14, 15); (3) hapten binding is linked to an allosteric conformational transition in L_2 . Since the sites on L_2 315 are probably no more than 12 Å apart⁸, direct contact interaction between haptens is possible. But there are strong arguments against it, which are: first, from the chemical nature of the ligands no mutual attraction is expected, particularly not for the negatively charged NBDA; second, attraction between identical ligands would be fortuitous and is unlikely to occur in two different cases; and third, direct hapten interaction is expected to lead to spectral asymmetry because the first hapten interacts only with protein residues while the second hapten also interacts with the first. The possibility of chain dissociation at the L_2 concentration studied (3×10^{-5} M) was ruled out by a series of sedimentation

Table 1 The best fit parameters for the curves in Fig. 2 according to different models

Hapten	Intrinsic Adair constants (M^{-1})	n_H	λ (nm)	$\Delta\epsilon_\lambda$ ($cm^{-1}M^{-1}$)	L	Intrinsic MWC constants (M^{-1})	$c = K_T/K_R$
NBDA	$K_1 = 5.0 \times 10^2$ $K_2 = 5.6 \times 10^4$	1.83	464 515	-12400 +6800	110	$K_T = 0$ $K_R = 5.6 \times 10^4$	0
DNPL	$K_1 = 3.5 \times 10^3$ $K_2 = 1.4 \times 10^4$	1.33	354	-4500	110	$K_T = 2.9 \times 10^3$ $K_R = 6.7 \times 10^4$	0.043

The Adair formalism¹³ makes no mechanistic assumptions other than that L_2 has two initially identical sites, and it enables us to calculate their apparent consecutive binding constants, K_1 , K_2 (see ref. 14). The binding curves are fitted according to this formalism assuming a constant value of $\Delta\epsilon$ throughout the titration (see text). λ is the wavelength at which $\Delta\epsilon$ is measured. n_H is the slope of the Hill plot (insert of Fig. 2) at half saturation, where $\log [R/(N-R)] = 0$. It may be calculated as $n_H = 2/[1 + (K_1/K_2)^{1/2}]$, and for positive cooperativity $1 < n_H \leq 2$ (ref. 14). The MWC model⁶ assumes an equilibrium between two conformations of the hapten-free protein $T_0 \rightleftharpoons R_0$ ($L = T_0/R_0$) which bind the hapten with different association constants K_T , K_R ($c = K_T/K_R < 1$). The MWC parameters were calculated from the Adair constants according to ref. 14. Both exclusive binding ($K_T = 0$) and non-exclusive binding ($K_T > 0$) are mathematically equivalent to the Adair scheme and are indistinguishable by parameter fit. Exclusive binding is the simplest as it makes possible calculation of unique values for L and K_R . As L is an attribute of the protein, it should be equal for both haptens. It was found impossible to fulfill this unless non-exclusive binding is assumed either for DNPL or for both haptens. We chose the first possibility thus getting a lower-limit estimate of L . Hapten binding has an appreciable effect on the conformational equilibrium: in the absence of hapten only about 1% is in the R form [$1/(1+L)$] while in the fully saturated protein R amounts to 100% and 83% for NBDA and DNPL respectively [$1/(1+Lc^2)$]. This is in line with the fact that the free energy change in the T to R transition is $\Delta G = -RT \ln(1/L) = 2.8 \text{ kcal mol}^{-1}$, which is comparable to $-RT \ln K_R \sim -6.5 \text{ kcal mol}^{-1}$ for the binding of both DNPL and NBDA.

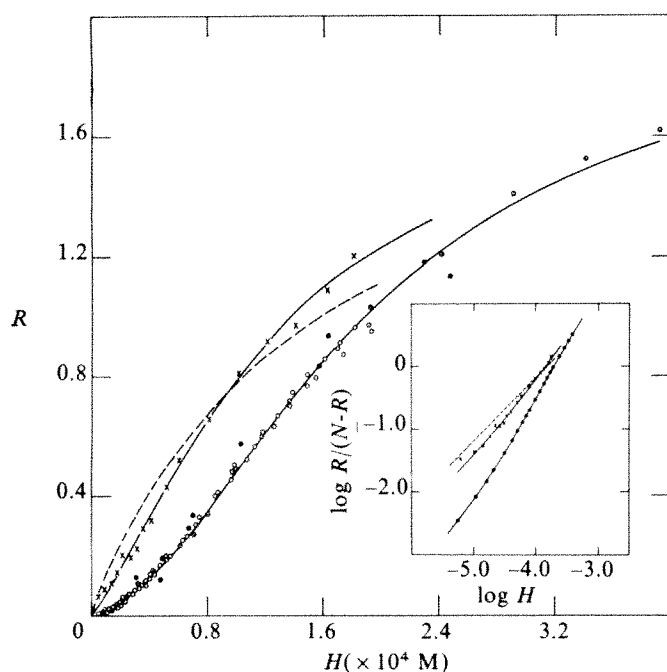


Fig. 2 Saturation curves of L_2 315 with NBDA: \circ , ΔA at 464 nm; \odot , ΔA at 515 nm and with DNPL; \times , ΔA at 354 nm. R is the average site occupancy by the hapten, calculated as $R = \Delta A/(\Delta\epsilon A_0)$ where A_0 is the molar concentration of L_2 and $\Delta\epsilon = \epsilon_{bound} - \epsilon_{free}$ is the change in the molar extinction of the hapten on binding ($R < 2$). H is free hapten concentration. For NBDA equilibrium dialysis points (\bullet) are also shown. These were measured as described⁶ and concentrations were monitored spectrophotometrically. A parameter fitting nonlinear least square procedure²¹ was used to obtain the values of K_1 , K_2 and $\Delta\epsilon$, as given in Table 1. The solid lines are drawn using these values of the parameters. The broken line is calculated assuming a single association constant $K = 6.3 \times 10^3 M^{-1}$ as previously reported⁴ for the binding of DNPL to L_2 315. It is seen that the difference between the solid and broken lines for DNPL is relatively small which may explain why in a previous (equilibrium dialysis) study with this hapten the cooperativity remained undetected. It should be stressed that in view of the observed cooperativity any previous measurements in which the binding curve was not extensively characterised should be treated with caution. Insert: the saturation points replotted in the form of a Hill plot. N , the number of binding sites per L_2 is 2.0. For NBDA only one titration (ΔA at 515 nm) is reproduced for clarity. The lines are drawn with the same calculated parameters as in the main figure. The slope at $\log [R/(N-R)] = 0$ is denoted n_H and its value is given in Table 1.

measurements in which a value of $S_{20,w} = 3.3 \pm 0.15$ (corresponding to L_2) was found in the concentration range of $6.0 \times 10^{-6} M$ to $2.0 \times 10^{-4} M$ in the presence or in the absence of hapten.

A conformational change induced in L_2 on hapten binding seems therefore to be the most plausible explanation for the observed positive cooperativity. The constancy of spectral effects through the titration strongly supports the notion of a symmetry-conserving process which may be adequately described by the allosteric model of Monod, Wyman and Changeux⁶ (MWC) as detailed in Table 1. The data thus fit a model in which the binding of the first hapten leads to a conversion of both sites from a lower affinity conformation T into a higher affinity conformation R . It is hard to conceive such a transition in terms of local changes limited to a few contact residues only. Rather, the data suggest that hapten binding causes a change in the overall configuration of the dimer which is related to inherent structural and energetic properties of the L_2 molecule as a whole. In view of the evidence that in L_2 haptens interact with both L chains simultaneously^{1,5,16}, it is possible that the hapten participates in the inter-chain interactions that mediate the concerted transition. More specifically, we suggest that hapten binding to one of the sites between the two L chains brings about a symmetry-conserving change in the relative chain position. This transition is associated with a relatively small net free energy change, so that haptens having association constants of the magnitude found here may shift the equilibrium appreciably (see Table 1).

In analogy to the L_2 case, haptens that bind to HL also interact with both chains^{9,17,18}, sometimes enhancing their mutual cohesion¹⁹. Antigen or hapten-induced conformational transitions detected in HL by spectroscopic^{8,10} or kinetic^{12,20} methods, were actually proposed (on the basis of indirect inference) to involve changes of relative chain position^{8,19}. Our results strongly support these suggestions in showing that such changes are energetically feasible in a structural and functional homologue of an immunoglobulin. The inherent structural properties of L_2 which lead to the observed concerted conformational transition probably exist also in HL, since conformation and chain interactions in both were shown to be very similar^{1-3,17}. Direct hapten binding measurements cannot detect conformational changes in HL since it binds only one hapten per two chains (see ref. 12). Kinetic measurements, however, give information on such processes as shown by the chemical relaxation study of hapten-induced conformational changes in another mouse myeloma protein, MOPC 460 (ref. 12). In order to relate the conformational transitions in L_2 and HL, L_2 315 is now also being characterised by chemical relaxation. Preliminary results are in good agreement with

the binding data, suggesting two slowly interconverting conformations which bind the hapten differently.

The changes in relative chain position may also be expressed in a change of the domain interactions, hypothesised to be the primary event in antigen triggering of antibody effector functions through an allosteric transition¹¹. The mechanism proposed here may imply that this transition arises chiefly from mere simultaneous interaction with H and L chains, stemming from inherent structural and energetic properties of HL. It would therefore be relatively antigen-independent, which explains how different antigens, interacting with diverse sites, are able to trigger the effector functions common to all antibody molecules. In any antibody-hapten system, however, the conformational effect will depend on the detailed interactions and will not always be expected to occur.

DORON LANCET

ARIEH LICHT

ISRAEL SCHECHTER

ISRAEL PECHT

Department of Chemical Immunology,
The Weizmann Institute of Science,
Rehovot, Israel

Received 13 July; accepted 19 August 1977.

- Edmundson, A. B. *et al. Biochemistry* **13**, 3816–3827 (1974).
- Edmundson, A. B., Ely, K. R., Abola, E. E., Schiffer, M. & Panagiotopoulos, N. *Biochemistry* **14**, 3953–3961 (1976).
- Edmundson, A. B. *et al. in Antibodies in Human Diagnosis and Therapy* (eds Haber, E. & Krause, R. M.) (Raven, New York 1977).
- Schechter, I., Ziv, E. & Licht, A. *Biochemistry* **15**, 2785–2790 (1976).
- Licht, A., Lancet, D., Schechter, I. & Pecht, I. *FEBS Lett.* **78**, 211–215 (1977).
- Monod, J., Wyman, J. & Changeux, J. T. *J. molec. Biol.* **12**, 88–118 (1965).
- Lancet, D. & Pecht, I. *Biochemistry* (in the press).
- Schlessinger, J., Steinberg, I. Z., Givol, D., Hochman, J. & Pecht, I. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2775–2779 (1975).
- Poljak, R. J., Amzel, L. M. & Phizackerley, R. P. *Prog. Biophys. molec. Biol.* **31**, 67–93 (1976).
- Metzger, H. *Adv. Immun.* **18**, 169–207 (1974).
- Huber, R., Deisenhofer, J., Colman, P., Matsushima, M. & Palm, W. *Nature* **264**, 415–420 (1976).
- Lancet, D. & Pecht, I. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3549–3553 (1976).
- Adair, G. S. *J. biol. Chem.* **63**, 529 (1925).
- Levitzki, A. in *Subunit Enzymes: Biochemistry and Function* (ed. Ebner, K. E.) (Marcel Dekker, New York, 1975).
- Levitzki, A. & Schlessinger, J. *Biochemistry* **13**, 5214–5219 (1974).
- Stevenson, G. T. *Biochem. J.* **133**, 827–836 (1973).
- Cathou, R. E., Dorrington, K. J. in *Subunits in Biological Systems*, part C (eds Timasheff, S. N. & Fasman, G. D.) (Marcel Dekker, New York, 1975).
- Padlan, E. A., Davies, D. R., Pecht, I., Givol, D. & Wright, C. *Cold Spring Harb. Symp. quant. Biol.* **XLI**, 627–637 (1977).
- Cathou, R. E. & Werner, T. C. *Biochemistry* **9**, 3149–3155 (1970).
- Pecht, I. & Lancet, D. in *Chemical Relaxation in Molecular Biology* (eds Pecht, I. & Rigler, R. (Springer, Heidelberg, 1977).
- Powell, M. J. D. in *Harwell Subroutine Library* (ed. Hopper, K. J.) (Subroutine VA04A Atomic energy Research Establishment, Harwell).
- Janeway, C. A., Jr, Wigzell, H. & Binz, H. *Scand. J. Immun.* **5**, 993–1001 (1976).
- Krawinkel, U. *et al. Cold Spring Harb. Symp. quant. Biol.* **41**, 285–294 (1977).

Two enzymes are required for strand incision in repair of alkylated DNA

THE excision-repair model for DNA repair involves the incision by an endonuclease of the phosphodiester chain adjacent or close to the damage and subsequent excision by an exonuclease of the defective nucleotide(s) (for review see ref. 1). Specific endonuclease for ultraviolet-induced damage¹ and for apurinic sites have been isolated associated with^{2,3}, or without an exonuclease activity (refs 4–6 and J.L. and L. Grossman, in preparation). Another class of enzymes does not incise DNA, but recognises and excises defective bases as uracil in the DNA. Belonging to this class of DNA N-glycosidases, is the enzyme described here which excises 3-methyladenine (3-MeAde) from alkylated DNA. This base excision repair is a preparatory step for the nucleotide excision repair described

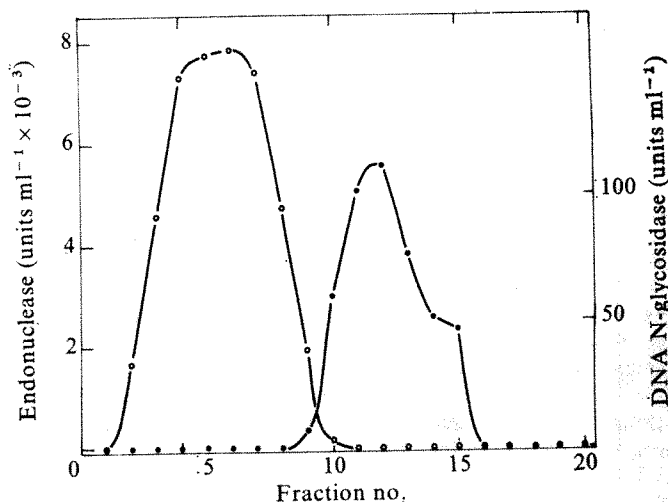


Fig. 1 Separation of DNA N-glycosidase from endonuclease for apurinic sites on DNA-Agarose. ●, DNA N-glycosidase; ○, endonuclease for apurinic sites. *M. luteus* ATCC 4698 was grown to late exponential phase¹³ washed in 1 mM EDTA 5 mM mercaptoethanol, 10% glycerol (solution A) containing 10 mM K₂PO₄ (final pH 6.5) and stored at -20 °C. Freshly grown cells (14 g), dispersed in 30 ml of washing buffer were lysed with 50 mg of lysozyme, cooled and sonicated to reduce the viscosity. The resulting solution was slowly titrated to pH 7.7 with 0.1 M KOH and centrifuged for 60 min at 30,000g. The supernatant was loaded on a DEAE-cellulose column (DE 52 Whatman) equilibrated with solution A containing 0.05 M K₂PO₄ (final pH 7.7). Elution was achieved by a linear gradient, the initial concentration was the equilibrating buffer and the limiting one, solution A, containing 0.35 M K₂PO₄ (final pH 7.7). The two activities were not separated. Active fractions were pooled, dialysed against solution A containing K₂PO₄ 0.05 M, final pH 6.7 and loaded on a P-cellulose column (P₁₁ Whatman) equilibrated with the same buffer. Elution was by a KCl linear gradient, 0–0.3 M in the loading buffer. The two enzymes were not separated. Active fractions are pooled and at this step could be stored at -20 °C with a loss of activity less than 10% in 10 months. The last step was chromatography on DNA-Agarose. P-cellulose fractions (2 ml) were diluted with 4 ml of solution A and applied to a DNA-Agarose¹⁴ column (0.4 × 8 cm) equilibrated with solution A containing 0.02 M K₂PO₄ buffer (final pH 7.2). The column was washed with 5 ml of the loading buffer, and eluted with a gradient of KCl 0–1 M in the same buffer (20 ml). Endonuclease for apurinic sites was measured by a modification of the method of Verly and Rassart³ using *E. coli* ³H DNA (3.2 × 10⁷ c.p.m. μmol⁻¹) heavily alkylated with MMS and subsequently heat depurinated. Incubation mixtures (100 μl) contained 10 μmol of K₂PO₄ buffer (pH 7.5), 4 pmol of apurinic DNA and enzyme. After incubation at 37 °C the mixture was chilled at 0 °C and 50 μl of cold calf thymus DNA (0.5 mg ml⁻¹) was added, after 2 min, 200 μl of cold 0.5 M HClO₄ was added¹⁵. After 5 min at 0 °C the precipitate was removed by a 12-min centrifugation at 6,500g and radioactivity measured in Scintigel (Roth). One unit of enzyme liberates 1 nmol of acid-soluble oligonucleotides in 30 min in the above conditions. DNA N-glycosidase was assayed by measuring the release of ethanol soluble 3-MeAde from a calf thymus DNA (4 mg in 2 ml) alkylated¹⁶ with ³H-dimethylsulphate (5 mCi, Amersham). Assay mixtures (100 μl) contained 10 μmol of K₂PO₄ buffer (pH 7.5), 4.58 nmol dimethylsulphate-alkylated DNA (1.5 × 10⁶ c.p.m. μmol⁻¹) and enzyme. After incubation at 37 °C, 50 μl of a solution containing 12.5 μg of calf thymus DNA and 100 μg of bovine serum albumin were added, chilled to 0 °C for 2 min and 300 μl of pre-cooled (-20 °C) ethanol are added. After an additional 5 min at 0 °C the precipitate is removed by a 12-min centrifugation (6,500g, 0 °C) and radioactivity determined. One unit of enzyme liberates 1 pmol of ethanol-soluble alkylated base in 30 min at 37 °C in the above conditions. Ethanol-soluble products are identified as 3 MeAde by chromatography on Dowex-50 (NH₄⁺ form) at pH 6.65 (ref. 17) using authentic samples of 3-MeAde (Fluka) 7-MeGua, thymine (Sigma) and 7-methyladenine (Vega-Fox) as internal standards. The chromatographic identity of ³H-3-MeAde with an authentic sample was independently confirmed using cellulose thin-layer chromatography (Eastman chromatogram)⁸.

above. It has been claimed that endonuclease II of *Escherichia coli*⁸ both excises the alkylated bases 3-MeAde and O⁶-methylguanine (O⁶-MeGua) and incises DNA at

apurinic sites. This enzyme also recognises a number of additional substrates⁹⁻¹¹. To establish the precise mechanism of incision of alkylated DNA we have purified two enzymes from *Micrococcus luteus*, a DNA *N*-glycosidase, which excises 3-MeAde, and an endonuclease for apurinic sites. We report here a reconstruction experiment showing that incision of DNA treated with carcinogenic and mutagenic compound methyl methanesulphonate (MMS) is a two-step mechanism involving the sequential action of these two enzymes.

Endonuclease for apurinic sites was assayed by the release of acid-soluble oligonucleotides¹ from heavily depurinated *E. coli* ³H-DNA. DNA *N*-glycosidase activity is measured by the liberation of ethanol soluble ³H-3-MeAde (Fig. 1) from calf thymus DNA alkylated with ³H-dimethylsulphate. Supertwisted PM2-DNA was used in additional assays to detect endonuclease activity at the level of one nick per molecule. The assay measures the conversion of supertwisted molecules to nicked ones. When using superhelical DNA in the assay of the endonuclease for apurinic sites, a limited number of apurinic sites was introduced by heating at low pH (ref. 19). For DNA *N*-glycosidase few bases were alkylated by MMS²⁰.

The two enzyme activities showed, in our conditions, similar chromatographic properties on DEAE- and P-cellulose columns, but were resolved by a column of DNA-Agarose (Fig. 1). But slight contaminating traces of endonuclease acting at apurinic sites in DNA *N*-glycosidase could be detected up to fraction 12 when using a large excess of DNA *N*-glycosidase (1.1 units) for the PM2-DNA assay. No DNA *N*-glycosidase can be detected in the endonuclease for apurinic sites peak before fraction 7 using 160 units of this enzyme. Neither of the two enzymes were contaminated by nonspecific endonuclease as they did not nick supercoiled PM2-DNA (Table 1). Endonuclease for apurinic sites acts at or near an apurinic site and has no exonuclease associated (J.L. and L. Grossman, in preparation). DNA *N*-glycosidase liberates 3-MeAde from dimethylsulphate-alkylated DNA and very little, if any, of 7-methylguanine (7-MeGua) (less than 5% of the total bases released).

A reconstruction experiment involving the sequential action of the two enzymes described above should be a strong argument for a two-step mechanism for incision of alkylated DNA. Therefore, we devised the following experiment: ³H-labelled PM2-DNA was treated with MMS²⁰ to alkylate about one base per molecule as 3-MeAde²¹, this latter base being a substrate for DNA *N*-glycosidase. Identification of DNA superhelical and nicked forms was achieved by zone sedimentation or by filter assay and average number of nicks per molecule calculated. Untreated PM2-DNA carried an average of 0.06 nicks per molecule (Table 1). Enzymes used had no activity on native DNA as after incubation the average number of nicks (0.10) was not significantly increased (Table 1). This rules out a possible contamination by non-specific endonuclease. Methylation slightly nicks the DNA (Table 1). On incubation of MMS-treated DNA alone or with apurinic endonuclease or with DNA *N*-glycosidase, there was no significant change in the average number of nicks introduced per molecule of DNA, this number being respectively 0.15, 0.17, 0.16 for the three samples studied (Table 1 and Fig. 2 *a, b, c*). In sharp contrast sequential incubation of the two enzymes induced an average of 1.05 breaks per molecule (Fig. 2*d*).

It is the first time that the DNA *N*-glycosidase for alkylated DNA has been obtained devoid of endonucleolytic activity either on native DNA or on DNA-carrying apurinic sites (Fig. 1) and devoid of nicking activity on MMS-treated DNA. Similar specificity has been observed for endonuclease II (ref. 8). The specificity of the DNA *N*-glycosidase for 3-MeAde but not for 7-MeGua correlates

with the *in vivo* studies of Lawley and Warren²², showing that in *E. coli* 3-MeAde is lost much more rapidly than 7-MeGua.

Strauss *et al.*²⁰ showed that crude extracts of *M. luteus* as well as of *Bacillus subtilis* are able to introduce single-strand breaks in alkylated DNA, and that unidentified

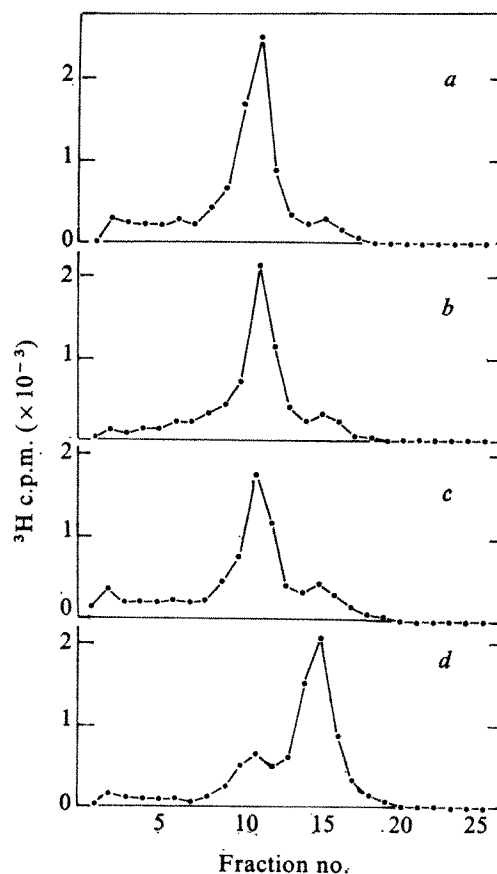


Fig. 2 Zone sedimentation profiles of PM2-DNA after incubation without enzyme (*a*) with DNA *N*-glycosidase (*b*), with endonuclease acting at apurinic sites (*c*) and with DNA *N*-glycosidase then endonuclease for apurinic sites (*d*). ³H-labelled PM2-DNA (17 nmol, 3.2×10^7 c.p.m. μmol^{-1}) was alkylated with MMS as described by Strauss *et al.*²⁰ (0.0125 M for 31 min at 37 °C), cooled to 0 °C and dialysed against 5 mM K_2PO_4 buffer (pH 7.5). This substrate is referred as MMS-DNA. The DNA *N*-glycosidase incubation mixture (150 μl) contained 26 pmol of MMS-DNA in K_2PO_4 buffer 0.05 M (pH 7.5) and 0.066 units of DNA *N*-glycosidase (fraction no. 16) incubation is for 20 min at 37 °C. To show the action of apurase the incubation mixture is as above, DNA *N*-glycosidase being replaced by 0.012 units of endonuclease acting for 10 min at 37 °C. Sequential action of the two enzymes is performed by incubating 0.066 units of DNA *N*-glycosidase for 10 min at 37 °C, then adding 0.012 units of endonuclease acting at apurinic sites and incubating the mixture for an extra 10 min. For the action of the enzyme on native PM2-DNA conditions were the same, MMS-DNA being replaced by the same amount of native ³H-labelled PM2-DNA. At the end of the incubation time the samples were cooled to 0 °C and layered on the top of a 4.8-ml sucrose gradient¹⁸ (5–20% in 0.02 M Tris-HCl, 0.002 M EDTA, 0.04 M NaCl (pH 7.08) over a 0.2 ml cushion of saturated CsCl . The samples were then centrifuged (rotor SW 50-1) for 180 min at 4 °C at 43,000 r.p.m. (Spinco L2-75-B). Gradients were collected using an ISCO UA5 fraction collector and counted in Scintigel (Roth). Separation was achieved at low salt concentration which allows the best separation at neutral pH¹⁸. In these conditions, sedimentation coefficients were respectively 29S and 21S for superhelical and nicked forms¹⁸. Centrifugation in alkaline conditions although allowing even better separations¹² cannot be used as they nick DNA at apurinic sites¹⁹. But alkaline denaturation can be performed in the filter assay as time of contact is short (30 s) and without adverse effect (Table 1).

Table 1 Average no. of nicks introduced by various treatments of PM2-DNA

DNA treatment	Average no. of nicks
None (untreated DNA)	0.06
DNA+MMS+dialysis (MMS-DNA)	0.10
MMS-DNA+incubation	0.15
MMS-DNA+DNA <i>N</i> -glycosidase	0.17
MMS-DNA+endonuclease for apurinic sites	0.16
MMS-DNA+DNA <i>N</i> -glycosidase +endonuclease for apurinic sites	1.05
Untreated DNA+DNA <i>N</i> -glycosidase +endonuclease for apurinic sites	0.10

PM2-DNA was alkylated by MMS, dialysed and treated with the various enzymes as duplicates of experiments described in Fig. 2. After incubation, samples were cooled to 0 °C. The DNA was immediately denatured by alkaline treatment, renatured and filtered through nitrate cellulose filter as described previously¹². Single-stranded DNA arising from nicked molecules was retained on the filter, allowing calculation of the amount of supercoiled form in the incubation mixture. The number of breaks (n) per molecule was derived from Poisson's law, $n = -\ln e$, e being the fraction of remaining supercoiled molecules²³.

methylated sites are not substrate for the enzyme(s). These early experiments had not been devised to ascertain if incision of DNA occurred through a single or a two-step mechanism, however. Goldthwait's group showed that a purified although non-homogeneous preparation of *E. coli* endonuclease II, is active on a wide range of substrates⁸⁻¹¹—it nicks depurinated DNA, releases 3-MeAde and O⁶-methylguanine from DNA reacted with *N*-methyl-*N*-nitrosourea⁸, and also nicks unmodified DNA at high concentrations⁹. Verly and Rassart³ isolated an endonuclease in *E. coli* acting at an apurinic site which was obtained homogeneous and shown to be inactive on alkylated DNA. This endonuclease was found to be exonuclease III (ref. 24), a single molecule carrying the two activities assayed independently with different substrates. Kirtikar *et al.*²⁵ isolated from *E. coli* an apurinic acid endonuclease apparently the same as that isolated by Verly and Rassart³ but they consider it not to be exonuclease III. In *E. coli*, another endonuclease acting at apurinic sites and lacking exonuclease activity (endonuclease IV) was obtained homogeneous and shown to be inactive on alkylated DNA⁵.

The model of excision repair of alkylated DNA best supported by our data and the published data on the *M. luteus* system is shown in Fig. 3; part of this scheme was proposed as a hypothetical model by Lindahl⁷. On action of a variety of alkylating agents on DNA (Fig. 3a) at least *N*-7-guanine and *N*-3-adenine became alkylated²¹ (Fig. 3b). 3-MeAde but not 7-MeGua is recognised by the DNA *N*-glycosidase. It cleaves the N₃ C'1 bond between the base and the deoxyribose, yielding a free 3-MeAde and an apurinic site (Fig. 3c). In turn, the apurinic site is recognised by the endonuclease for apurinic site which nicks the DNA (Fig. 3d). It should be recalled that *M. luteus* endonuclease for apurinic site has no exonuclease activity. This could be a disadvantage as DNA ligase could act before the removal of the damaged nucleotide and thus produce an abortive repair. It is possible, however, that DNA binding proteins could prevent this abortive repair (Fig. 3e) as this has been already suggested in the repair of ultraviolet-induced damage¹. The correxonuclease described by Kushner *et al.*²⁶ which is able to excise thymine dimers would be a good candidate to excise apurinic sites yielding a gap (Fig. 3e). The gap thus formed could be filled by a DNA polymerase²⁷ (Fig. 3f) and the continuity of the phosphodiester chain restored by a polynuclease ligase (Fig. 3g). This model has recently received experimental support in the repair

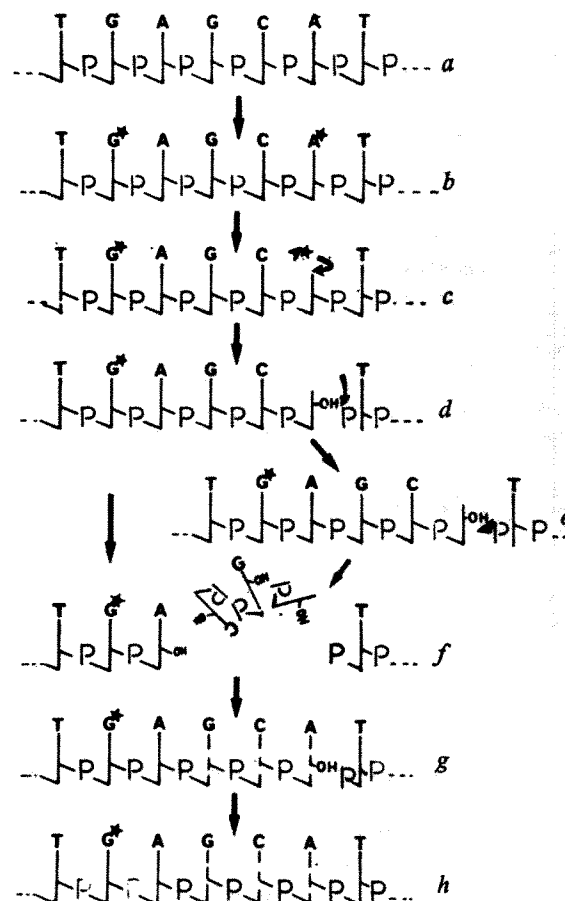


Fig. 3 Model for excision repair of alkylated DNA or uracil-containing DNA. *a*, Unmodified DNA; *b*, alkylation (MMS) giving 3 MeAde (A*) and 7-MeGua (G*); *c*, specific release of free 3-MeAde yielding an apurinic site (DNA *N*-glycosidase); *d*, incision endonuclease for apurinic sites; *e*, a binding protein prevents abortive rejoining; *f*, excision (correxonuclease)—this step could also occur directly after (*d*); *g*, repair replication (DNA polymerase); *h*, resealing (DNA ligase). This model holds true for repair of uracil-containing DNA²⁸ (A* being replaced by U).

of DNA containing uracil where nicking is achieved by sequential action of uracil DNA *N*-glycosidase and endonuclease for apurinic sites²⁸. Endonucleases acting at apurinic sites have been described in calf thymus⁴, HeLa cells²³ and human placenta²⁹. A human binding protein for ultraviolet irradiated DNA³⁰ and a correxonuclease³¹ are described in human placenta. Therefore, our model could also be true for repair in mammalian cells of alkylated DNA, even if so far no mammalian DNA *N*-glycosidase has been described.

In animals treated with alkylating carcinogens (see review 32), certain organs such as the liver seem to be much more resistant to tumourigenesis than the brain or the kidneys. Appearance of tumours is correlated with the persistence of alkylated bases. This suggests that chemical cancerogenesis could be related to defective repair mechanisms such as inefficient cellular DNA *N*-glycosidase.

We thank Drs S. Ljungquist and T. Lindahl for preprints. These studies were supported by INSERM and CNRS and Pierre Philippe Foundation.

JACQUES LAVAL

Laboratoire Associé n 147
CNRS et Unité 147 INSERM,
Institut Gustave-Roussy, 94800 Villejuif, France

Received 4 July; accepted 9 September 1977.

1. Grossman, L., Braun, A., Feldberg, R. & Mahler, I. *A. Rev. Biochem.* **44**, 19–43 (1975).
2. Hadi, S. M. & Goldthwait, D. A. *Biochemistry* **10**, 4986–4994 (1971).
3. Verly, W. G. & Rassart, E. *J. biol. Chem.* **250**, 8214–8219 (1975).
4. Ljungquist, S., Nyberg, B. & Lindahl, T. *FEBS Lett.* **57**, 169–171 (1975).
5. Ljungquist, S. *J. biol. Chem.* **252**, 2808–2814 (1977).
6. Thibodeau, L. & Verly, W. G. *FEBS Lett.* **69**, 183–185 (1976).
7. Lindahl, T. *Nature* **259**, 64–66 (1976).
8. Kirtikar, D. M. & Goldthwait, D. A. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2022–2026 (1974).
9. Hadi, S. M., Kirtikar, D. & Goldthwait, D. A. *Biochemistry* **12**, 2747–2754 (1973).
10. Kirtikar, D. M., Slaughter, J. & Goldthwait, D. A. *Biochemistry* **14**, 1235–1243 (1975).
11. Kirtikar, D. M., Dipple, A. & Goldthwait, D. A. *Biochemistry* **14**, 5548–5553 (1975).
12. Laval, F. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4965–4969 (1974).
13. Kaplan, J. C., Kushner, S. R. & Grossman, L. *Proc. natn. Acad. Sci. U.S.A.* **63**, 144–151 (1969).
14. Arndt-Jovin, D. J., Jovin, T. M., Bähr, W., Frischauf, A. M. & Marquardt, M. *Eur. J. Biochem.* **54**, 411–418 (1975).
15. Laval, J. & Paoletti, C. *Biochemistry* **11**, 3596–3603 (1972).
16. Uhlenhopp, E. L. & Krasna, A. I. *Biochemistry* **10**, 3290–3295 (1971).
17. Lawley, P. D. & Shah, S. A. *Biochem. J.* **128**, 117–132 (1972).
18. Bottger, M. & Kuhn, W. *Biochim. biophys. Acta* **254**, 407–411 (1971).
19. Lindahl, T. & Anderson, A. *Biochemistry* **11**, 3618–3623 (1972).
20. Strauss, B., Coyle, M. & Robbins, M. *Cold Spring Harb. Symp. quant. Biol.* **33**, 277–287 (1968).
21. Lawley, P. D. & Shah, S. A. *Chem.-Biol. Interact.* **5**, 286–288 (1972).
22. Lawley, P. D. & Warren, W. *Chem.-Biol. Interact.* **12**, 211–220 (1976).
23. Teebor, G. W. & Duker, N. J. *Nature* **258**, 544–547 (1975).
24. Weiss, B. *J. biol. Chem.* **251**, 1896–1901 (1976).
25. Kirtikar, D. M., Cathcart, G. R. & Goldthwait, D. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4324–4328 (1976).
26. Kushner, S. R., Kaplan, J. C., Ono, H. & Grossman, L. *Biochemistry* **10**, 3325–3334 (1971).
27. Hamilton, L., Mahler, I. & Grossman, L. *Biochemistry* **13**, 1886–1896 (1974).
28. Lindahl, T., Ljungquist, S., Siegert, W., Nyberg, B. & Sperens, B. *J. biol. Chem.* (in press).
29. Linsley, W. S., Penhoet, E. E. & Linn, S. *J. biol. Chem.* **252**, 1235–1242 (1977).
30. Feldberg, R. S. & Grossman, L. *Biochemistry* **15**, 2402–2408 (1976).
31. Doniger, J. & Grossman, L. *J. biol. Chem.* **251**, 4579–4587 (1976).
32. Pegg, A. E. *Adv. Cancer Res.* **25**, 195–269 (1977).

Mass spectrometric determination of molecular formulas for membrane-modifying antibiotics

THE peptide antibiotics alamethicin and suzukacillin, together with gramicidin A are of considerable biological interest as they facilitate ion transport across membranes by a mechanism involving pore or channel formation in the membranes^{1–3}. In discussing their mode of action it is, of course, necessary to consider the antibiotics' structures and such discussions have been hampered, especially for alamethicin^{1,4–7}, by incorrect structures originally

proposed for the antibiotics. Thus, the earlier structures proposed for valine-gramicidin A and isoleucine-gramicidin A^{8,9} were revised to **1** and **2** (Fig. 1) following the detection of N-terminal formyl and C-terminal ethanolamine groups¹⁰, and earlier structures^{11,12} proposed for alamethicins I and II have recently been revised to **3** and **4** (ref. 13) following the discovery of N-terminal acetyl and C-terminal phenylalaninol groups^{14,15}. (The partial structure proposed for suzukacillin¹⁶ will probably also be revised in view of the newly reported structure for alamethicin¹³.) Much of the difficulty in assigning structures to these antibiotics has derived from an inability to determine accurate molecular formulas. We have until recently been hampered by the same problem in our studies of the structures of antiamoebein I (**5**) (ref. 17) and emerimicins III and IV (**6** and **7**) (ref. 18), new peptaibophol antibiotics [peptide antibiotics containing several moles of α -aminoisobutyric acid (Aib) and a C-terminal phenylalaninol (Phol) unit] which have been shown to have similar membrane-modifying properties (P. Mueller, personal communication). We have developed, and describe here, a new technique, a modification of field desorption mass spectrometry^{19,20} involving cation exchange, which has allowed us to assign molecular formulas directly to these peptide antibiotics and their derivatives in the 1,500–2,100 molecular weight range.

The cation exchange technique involves adding, systematically, a series of alkali metal salts to solutions of the compounds whose FD mass spectra are being determined, and observing the resulting shifts in the molecular ion region due to cationation. The technique was developed during our extensive studies on the membrane-modifying peptide antibiotic antiamoebein I, whose structure has now been shown²¹ to be **5**. We found it difficult to assign the antibiotic a definitive molecular formula, either from its constituent amino acids or from the highest mass ion in the FD mass spectrum of its triacetate, at m/e 1,818 (peak matched against the m/e 1,790 peak ($C_{35}H_{15}F_{60}N_3O_5P_3$) from hexakis (2,2,3,3,4,4,5,5,6,6,7,7, - dodecafluoro - 1 - heptoxy)cyclotriphosphazene²²), which shifted to m/e 1,860 and 1,902 in the FD mass spectra of the tripropionate and tributryate, respectively.

The masses for the triesters would correspond to a molecular weight of 1,692 for antiamoebein I, which did not agree with any of the possibilities under consideration¹⁷. But, m/e 1,692 would fit for a sodium adduct of one of the considered potential molecular weights, m/e 1,669 ($C_{82}H_{127}N_{17}O_{20}$). To test this hypothesis

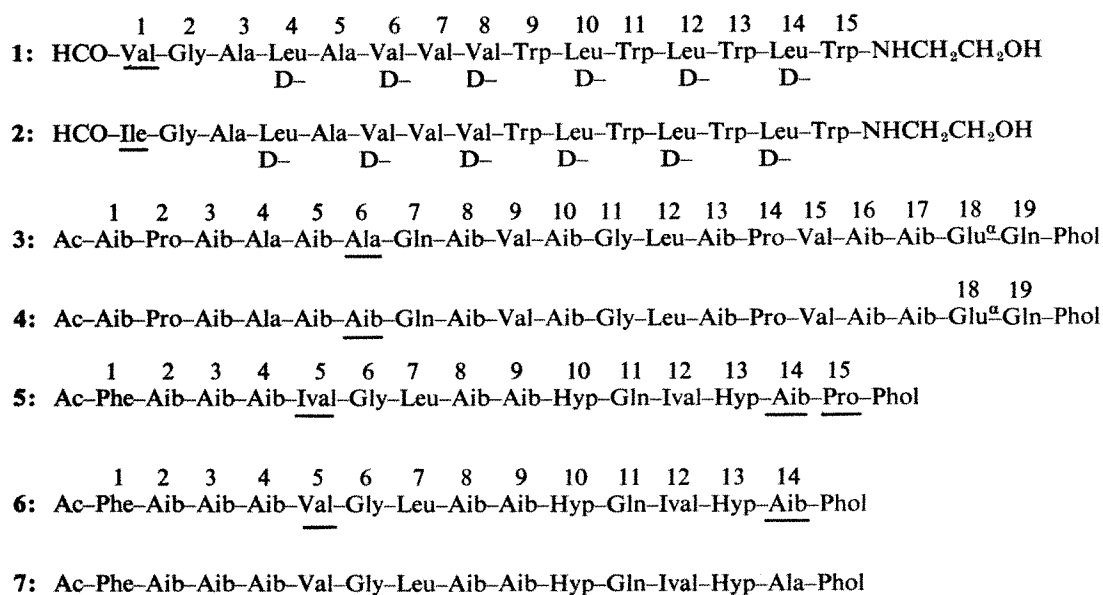


Fig. 1 1, Valine-gramicidin A; 2, isoleucine-gramicidin A; 3, alamethicin I; 4, alamethicin II; 5, antiamoebein I; 6, emerimicin III; 7, emerimicin IV. All optically active amino acids and phenylalaninol units shown have the L configuration except those marked D. Aib, α -aminoisobutyric acid; Phol, phenylalaninol; Ac, acetate.

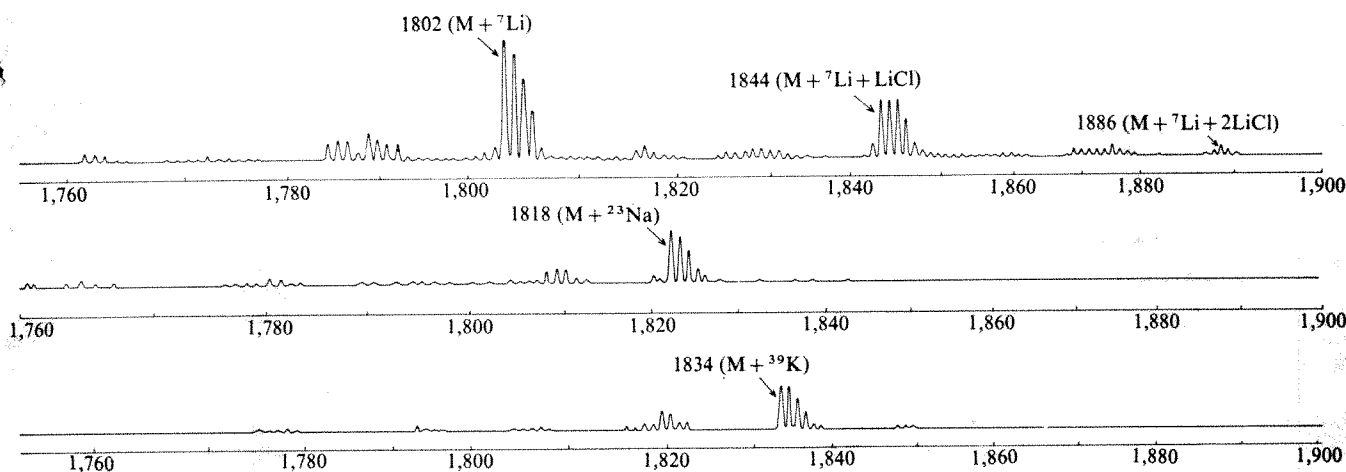


Fig. 2 Field desorption mass spectra of antiameobin I triacetate in the molecular ion region: a, with added lithium chloride (26 mA); b, with added sodium chloride (23 mA); c, with added potassium chloride (21 mA). Peaks due to a small amount of a homologue, antiameobin III, can be observed 14 AMU below the major peaks.

antiameobin triacetate was treated with a saturated methanolic solution of potassium chloride and a 50% saturated solution of lithium chloride. The triacetate's m/e 1,818 peak ($M + Na$; Fig. 2, b) was displaced to m/e 1,834 ($M + K$; Fig. 2, c) and m/e 1,802 ($M + Li$; Fig. 2, a), respectively. In the case of lithium chloride, peaks at higher masses corresponding to ($M + Li + LiCl$) and ($M + Li + 2LiCl$) were also observed (Fig. 2, a), at 1,844 and 1,886. (Addition of rubidium chloride can be even more helpful, since there are two rubidium isotopes (^{85}Rb and ^{87}Rb ; isotopic abundance 2.51:1). In the case of antiameobin I triacetate these gave $M + Rb$ ions at m/e 1,880 and 1,882.)

The intensities of molecular ion peaks were vastly enhanced by this treatment and enormously more durable. (Lithium salts have been added previously, using the FD technique at low resolution with known compounds, to adenosine (molecular weight (MW) 267)²³ and to sucrose (MW 342) and loroglossin (MW 742) (ref. 24). The triacetate's m/e 1,818 peak was matched against the peak at m/e 1,720.9514 ($C_{34}H_{18}F_{56}N_3O_6P_3$) in the FD spectrum of bis (dodecafluorooctoxy) tetrakis (octafluoropentoxo)-cyclotriphosphazene²² at high resolution to give the value 1,818.9596, in good agreement with that calculated for the expected $C_{88}H_{133}N_{17}O_{23}Na$.

The technique works equally well on antiameobin itself. Adding sodium, potassium and lithium chloride solutions to antiameobin I gave strong FD peaks at m/e 1,692 ($M + Na$), 1,708 ($M + K$) and 1,676 ($M + Li$), while addition of sodium chloride solutions to the tripropionate and tributrylate greatly enhanced the peaks at m/e 1,860 and 1,902 ($M + Na$).

The cation exchange technique shows the molecular formula of the membrane-modifying emerimicin IV (6) (ref. 25) to be $C_{77}H_{120}N_{16}O_{19}$ (MW 1,572) by an ($M + Na$) ion at m/e 1,595.8930 and an ($M + K$) ion at m/e 1,611, while its triacetate gave an ($M + Na$) ion at m/e 1,721 and an ($M + K$) ion at m/e 1,737. Emerimicin III (7) (ref. 25), also a membrane-modifier, gave an ($M + Na$) peak at m/e 1,581.8655.

Alamethicin is a mixture of two antibiotics, alamethicins I and II (3 and 4) (ref. 13), each containing a free carboxyl group, and its cation exchange FD mass spectra are more complex. The mixture gave peaks for alamethicin I ($C_{92}H_{150}N_{22}O_{25}$) at m/e 1,984 ($M + Na - H$) and 2,007 ($M + 2Na - H$) and corresponding peaks for alamethicin II ($C_{93}H_{152}N_{22}O_{25}$) at m/e 1,998 and 2,021 in the presence of sodium chloride. Similar peaks were found at m/e 2,000 ($M + K - H$), 2,014 ($M + K - H$), 2,039 ($M + 2K - H$) and 2,053 ($M + 2K - H$) in the presence of potassium chloride, while alamethicin acetate gave peaks at m/e 2,026 ($M + Na - H$), 2,040 ($M + Na - H$), 2,049 ($M + 2Na - H$) and 2,063 ($M + 2Na - H$) in the presence of sodium chloride.

Finally, sodium chloride-treated gramicidin A gave FD peaks at m/e 1,903 ($M + Na$ for valine-gramicidin A, $C_{99}H_{140}N_{20}O_{17}$, 1)

and at 1,917 (the same peak for isoleucine-gramicidin A, $C_{100}H_{142}N_{20}O_{17}$, 2), while treatment with potassium chloride shifted these peaks to m/e 1,919 and 1,933 ($M + K$ for valine- and isoleucine-gramicidins A, respectively).

We are now extending our studies to establish the scope of the cation exchange procedure in assigning molecular weights to additional antibiotics and to compounds of other structural classes of high molecular weight or low volatility.

This work was supported by the US NCI and the National Institute of Allergy and Infectious Diseases.

K. L. RINEHART, JR
J. C. COOK, JR
H. MENG
K. L. OLSON
R. C. PANDEY

School of Chemical Sciences,
University of Illinois,
Urbana, Illinois 61801

Received 4 April; accepted 11 July 1977.

- Haydon, D. A. & Hladky, S. B. *Q. Rev. Biophys.* **5**, 187-282 (1972).
- Mueller, P. & Rudin, D. O. *Nature* **217**, 713-719 (1968).
- Boheim, G. *et al. Biochim. biophys. Acta* **433**, 182-199 (1976).
- Boheim, G. *J. Membr. Biol.* **19**, 277-305 (1974).
- Cherry, R. J., Chapman, D. & Graham, D. E. *J. Membr. Biol.* **7**, 325-344 (1972).
- McMullen, A. I., Marlborough, D. I. & Bayley, P. M. *FEBS Lett.* **16**, 278-283 (1971).
- Jung, G., Dubischar, N. & Leibfritz, D. *Eur. J. Biochem.* **54**, 395-409 (1975).
- Syngé, R. L. M. *Biochem. J.* **39**, 355-362 (1945).
- Syngé, R. L. M. *Cold Spring Harb. Symp. quant. Biol.* **14**, 191-198 (1950).
- Sarges, R. & Witkop, B. *J. Am. chem. Soc.* **86**, 1861-1862 (1964); **86**, 1862-1863 (1964); **87**, 2011-2020 (1965).
- Payne, J. W., Jakes, R. & Hartley, B. S. *Biochem. J.* **117**, 767-776 (1970).
- Ovchinnikov, Y. A., Kiryushkin, A. A. & Kozhevnikova, I. V. *Gen. Chem. U.S.S.R.* **41**, 2105-2116 (1971).
- Pandey, R. C., Cook, J. C., Jr & Rinehart, K. L., Jr *J. Am. chem. Soc.* **99**, (in the press).
- Pandey, R. C., Cook, J. C., Jr & Rinehart, K. L., Jr *23rd Annual Conference on Mass Spectrometry and Allied Topics*, Houston, Texas, 25-30 May 1975.
- Martin, D. R. & Williams, R. J. P. *Biochem. J.* **153**, 181-190 (1976).
- Jung, G. *et al. Biochim. biophys. Acta* **433**, 164-181 (1976).
- Pandey, R. C., Rinehart, K. L., Jr, Meng, H., Deshmukh, P. V. & Millington, D. S. *9th International Symposium on the Chemistry of Natural Products*, IUPAC, Ottawa, Canada, 24-28 June, 1974, Abstr. 1B.
- Pandey, R. C., Rinehart, K. L., Jr & Argoudelis, A. D. *14th Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, California, 11-13 September 1974, Abstr. 302.
- Beckey, H. D. in *Biomedical Applications of Mass Spectrometry* (ed. Waller, G. R.) 795-816 (Wiley-Interscience, New York, 1973).
- Rinehart, K. L., Jr, Cook, J. C., Jr, Maurer, K. L. & Rapp, U. J. *Antibiot.* **27**, 1-13 (1974).
- Pandey, R. C., Meng, H., Cook, J. C., Jr & Rinehart, K. L., Jr *J. Am. chem. Soc.* **99**, 5203-5205 (1977).
- Olson, K. L., Rinehart, K. L., Jr & Cook, J. C., Jr *Biomed. Mass Spectrom.* **4**, (in the press).
- Röllgen, F. W. & Schulten, H. R. *Org. Mass Spectrom.* **10**, 660-668 (1975).
- Veith, H. J. *Angew. Chem. Int. Ed. Engl.* **15**, 696 (1976).
- Pandey, R. C., Cook, J. C., Jr & Rinehart, K. L., Jr *J. Am. chem. Soc.* **99**, 5205-5206 (1977).

New rapid gel sequencing method for RNA

METHODS for sequencing nucleic acids have improved significantly in the last few years due to the development of rapid 'read-off' gel methods. The first method to be developed was the 'plus and minus' copying method for DNA¹ and an analogous copying

method for RNA now exists². A different and more direct method for sequencing DNA has been developed³, but no direct method is available for RNA. We have developed such a method and here we describe its use in establishing the sequence of tyrosine tRNA of *Bacillus stearothermophilus*. This sequence is especially interesting as the tyrosine tRNA and its specific synthetase in *B. stearothermophilus* offer an attractive experimental system for studying the specific interaction of tRNA and synthetases, which is now possible because of progress in studying the three-dimensional structure of the protein by X-ray crystallography⁴.

Classical radioactive sequencing methods⁵ applied to uniformly ³²P-labelled tRNA were used initially to sequence the products of complete T₁ or pancreatic RNase digestion (Table 1). Only brief reference to this work is made here as an extensive report will be published elsewhere (R.S. B. in preparation).

Overlapping of the products in Table 1 was achieved by the development of the new sequencing procedure, which is similar in principle to that used by Maxam and Gilbert for DNA³ except that enzymatic rather than chemical methods were used to degrade the RNA and to define the base-specific point of cleavage. Thus the design of the experiment was to label the RNA at its 5' terminus with ³²P-phosphate and partially digest separate aliquots with either T₁ RNase (G-specific), U₂ RNase (A-specific in partial conditions), pancreatic RNase (C + U-specific), or RNase I from *Physarum polycephalum*⁶. Phy I RNase is less well known than the other enzymes and it emerged^{7,13} that its base specificity was such that it could cleave at all residues except C. Hence we refer to its activity as minus C (–C). The digests were then fractionated in adjacent slots of a 20% denaturing acrylamide gel. The base specificity of these four enzymes should provide enough information to allow the sequence to be 'read-off' from the resultant autoradiograph.

Figure 1 shows the purification of ³²P-5' end-labelled tRNA using γ-³²P-ATP and T₄ polynucleotide kinase by acrylamide gel electrophoresis. In addition to intact tRNA^{Tyr} (band 1), many degradation products were present in lower yield. Bands 1 and 2 were selected for sequence analysis. Figure 2 shows the sequencing method applied to the 5' end-labelled fragments of tRNA^{Tyr} (Fig. 1, band 2) which was identified as deriving from residues 38–85 in the finally deduced sequence (Fig. 4). The enzymatic degradations are identified as G, A, C + U and –C (performed in duplicate and applied at separate times so as to maximise resolution of both fast and slow moving bands). A fifth slot (hot formamide degradation) giving a ladder (L) of every possible intermediate was also included. Hot formamide degradation (P. H. Hamlyn, personal communication) is preferable to partial alkaline hydrolysis for this purpose as it gives oligonucleotides with the same 2', 3'-cyclic phosphate end group as in the enzymatic degradations. Partial alkaline hydrolysis with strong base, on the other hand, gave both

2',3'-cyclic phosphate and a mixture of 2' and 3' phosphate end groups which separated from one another and confused the ladder in the size range of 1–10 on the acrylamide gels.

The sequence may be read from residues 38–83 with the single ambiguity X63, due to the presence of a minor base (see below). G residues are identified without difficulty from the highly specific G slot. 'A' residues are usually present in the A slot as strong bands, although also present are some G and pyrimidine bands. The latter are clearly distinguishable by their low yield and by the presence in the G or C + U slots. The pyrimidine residues are more difficult to identify than the purines, although the C + U and –C slots can be 'read' together to give the correct sequence. C residues are identified by their presence (usually as strong bands) in the C + U slot and by their absence (or low yield) in the –C slot. U residues are identified by the presence of a band in both C + U and –C slots. Usually U residues are stronger in the –C slot than the C + U slot. In this particular experiment the presence of a band, C65, in the –C slot (as well as in the G and A slots) is misleading as it was also present in a control line (not shown) and derived by nonspecific degradation of the RNA during its purification and storage. The sequence overlapped and was in complete agreement with the data in Table 1, apart from the identification of modified bases and the last two residues at the 3'-end. X63 was clearly the T residue of the oligonucleotide t9 (Table 1) and was presumably resistant to digestions in the C + U and –C slots in the partial conditions used. Both Ψ residues (residues 40 and 64) behaved like U residues, and the hyper-modified ms²i⁶A (residue 38) was only a weak band in the A slot.

Figure 3 shows the sequence analysis of intact tyrosine tRNA (Fig. 1, band 1). In this case the sequencing was more difficult than with the fragment as some degradation products were absent or in low yield. This presumably reflected the inability of the enzymes to attack helical regions in the structure. Preliminary experiments with brewers yeast tRNA^{Phe} had shown that a more even intensity of the bands was present in the G and C + U line if 70% dimethyl sulphoxide (DMSO)⁹ at 37 °C was used to denature the tRNA. Unfortunately DMSO could not be used for studies on tRNA^{Tyr} because of a 'band doubling' phenomenon on the gels, which seemed to be caused by oxidation of the s⁴U residue not present in tRNA^{Phe}. Further studies showed that 70% formamide could be used as an alternative but was not quite as effective as the DMSO. Thus Fig. 3 includes several variations in the conditions of enzymatic degradation: 'C + U, condition 3' was a reaction in formamide and was a slight improvement over the other two aqueous digestions; '–C, conditions 1 and 2' and 'C + U, conditions 1 and 2' were variations in the amount of enzyme or time of digestion in a further attempt to obtain as even a spectrum of products as possible. The sequence can, nevertheless, be read from Fig. 3 from residues 2 to 34 with only four ambiguities—

Table 1 End products of T₁ and pancreatic RNase digestions of tRNA^{Tyr}

T ₁ end products	Pancreatic end products
t1 pG	p1 C
t2 G + G >	p2 U + ψ
t3 C – G	p3 A – C
t4 A – G	p4 Q – U
t5 U – Gm – G	p5 G – C
t6 A – A – G	p6 G – U
t7 s ⁴ U – A – G	p7 A – C – G
t8 U – U – C – G	p8 Gm – G – C
t9 T – ψ – C – G	p9 G – G – C
t10 A – A – U – C – C – G	p10 G – G – T
t11 C – U – A – A – m ¹ A – C – G	p11 A – A – m ¹ A – C
t12 C – U – C – C – C – U – U – U – G	p12 G – G – A – C
t13 A – C – U – Q – U – A – ms ² i ⁶ A – A – ψ – C – C – G	p13 G – A – A – U
t14 U – C – C – C – C – C – U – C – C – A – C – C – A _{OH}	p14 G – G – G – U
	p15 A – ms ² i ⁶ A – A – ψ
	p16 G – A – A – G – U
	p17 pG – G – A – G – G – G – G – s ⁴ U

Uniformly ³²P-labelled tyrosine tRNA (*B. stearothermophilus*, NCIB 8924) was purified by acrylamide gel electrophoresis (R.S.B., in preparation) and fingerprints of T₁ and pancreatic RNase digestions were prepared using two-dimensional electrophoresis (cellulose acetate at pH 3.5 followed by DEAE-cellulose ionophoresis using 7% formic acid). Oligonucleotides were sequenced by standard methods⁵. Minor bases were identified by their chromatographic properties on thin layers⁸.

partially denaturing conditions used than were T_1 or pancreatic RNases. Nevertheless tRNA is a particularly difficult example with its 'tight' structure, and many RNA molecules to which the method is applicable would have far less secondary structure.

The technique described here compares favourably in its rapidity and sensitivity (less than 0.1 μ Ci RNA per gel is needed) with the plus and minus method for RNA²; furthermore it is independent of any requirement for oligonucleotide primers². Although we do not claim that the method is 100% accurate, it is promising enough to apply to other RNA molecules. There is no doubt that it is the easiest way of establishing a tentative new sequence, which could be confirmed by more classical methods if necessary. The method in principle is also applicable to 3' terminally-labelled RNA. Preliminary results have shown that ³²P 3'-labelled tRNA^{Phc} (labelled using α -³²P-ATP and the enzyme tRNA nucleotidyl transferase¹⁴) is suitable; ³H-borohydride labelled RNA¹² is more generally applicable and we expect this also to be a suitable substrate. The flexibility of using either 5' or 3'

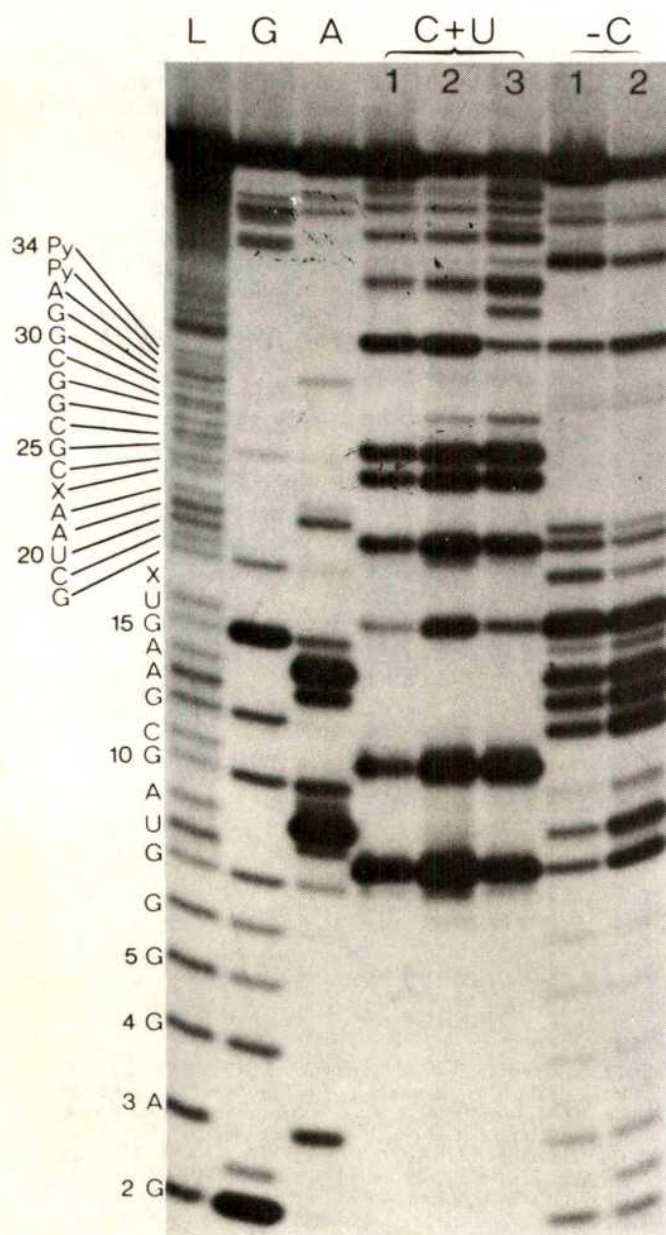


Fig. 3 Sequence analysis of intact tRNA. Letter symbols are as described in Fig. 2. Three separate experiments with pancreatic RNase (C+U, (1) 0.3 ng, (2) 0.5 ng enzyme as in Fig. 2, (3) 0.5 ng enzyme in 70% aqueous buffered formamide, 37 °C, 30 min); two with *Phy* I RNase (1 μ l enzyme solution, for 5 min and for 20 min) were performed.

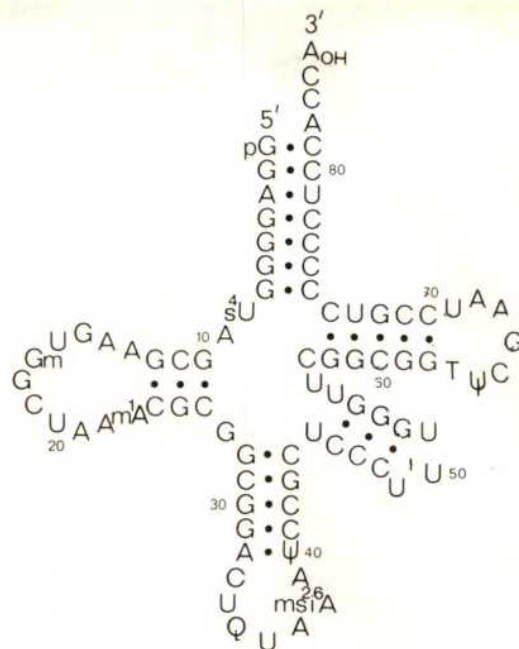


Fig. 4 Sequence of tRNA^{Tyr} of *B. stearothermophilus*.

labelled material should allow the sequencing method to be extended to larger RNA molecules, such as 5S RNA and larger, although there is still a limitation of about 150–200 residues² in the resolution so far obtained in sequencing gels. Finally the method has the potential (not explored here) of probing for conformation of RNA in solution in physiological conditions. Further, the band pattern characteristic of the conformation of the tRNA is likely to be altered when the molecule is complexed with its specific amino acid synthetase. This should give important insight into the specificity of recognition in this tRNA-protein interaction.

While this work was in progress Gupta and Randerath¹² reported a related method using thin-layer chromatography on PEI-cellulose to 'read off' sequences of up to 20 residues in length. As *Phy* I RNase was not used, however, the method was more cumbersome and relied on two-dimensional methods to distinguish the pyrimidine residues. After submission of this report a gel mapping procedure for guanine and adenine residues was reported¹⁵, but a complete sequence could not be established as no method was presented for distinguishing pyrimidine residues.

We thank Professor W. Fiers for suggesting the use of *Phy* I RNase, and Dr J.-P. Bargetzi for providing the enzyme. A.S. is a visiting UNESCO Scholar from the Institute of Biophysics, Hungarian Academy of Sciences, Szeged.

A. SIMONCSITS
G. G. BROWNLEE
R. S. BROWN
J. R. RUBIN
H. GUILLEY

MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge, UK

Received 26 July; accepted 2 September 1977.

1. Sanger, F. & Coulson, A. R. *J. molec. Biol.* **94**, 441–448 (1975).
2. Brownlee, G. G. & Cartwright, E. M. *J. molec. Biol.* **114**, 93–117 (1977).
3. Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
4. Irwin, M. J., Nyborg, J., Reid, B. R. & Blow, D. M. *J. molec. Biol.* **105**, 577–586 (1976).
5. Brownlee, G. G. *Laboratory Techniques in Biochemistry and Molecular Biology* 13 (North-Holland, Amsterdam, 1972).
6. Schmidt, M., Pipy, D., Wolny, M. & Bargetzi, J.-P. *Experientia* **28**, 739–740 (1972).
7. Pilly, D., Niemeyer, A., Schmidt, M. & Bargetzi, J.-P. *J. biol. Chem.* (in the press).
8. Nishimura, S. *Prog. Nucl. Acid Res. molec. Biol.* **12**, 49–85 (1972).
9. Contreras, R. Ph.D. thesis, Univ. Ghent. (1974).
10. Uchida, T., Arima, T. & Egami, F. *J. Biochem. (Tokyo)* **67**, 91–102 (1970).
11. Schmukler, M., Jewett, P. B. & Levy, C. C. *J. biol. Chem.* **250**, 2206–2212 (1975).
12. Gupta, R. C. & Randerath, K. *Nucleic Acids Res.* **4**, 1957–1978 (1977).
13. Contreras, R. & Fiers, W. *Analyt. Biochem.* **67**, 319–326 (1975).
14. Sternbach, H., von d. Haar, F., Schlimme, E., Gaertner, E. & Cramer, F. *Eur. J. Biochem.* **22**, 166–172 (1971).
15. Donis-Keller, H., Maxam, A. M. & Gilbert, W. *Nucleic Acids Res.* **4**, 2527–2538 (1977).

reviews

State of medicine in the US

W. W. Holland

Doing Better and Feeling Worse. Edited by J. H. Knowles. Pp. 287. (W. W. Norton: New York, 1977.) Hardback \$9.95; paperback \$3.95.

This interesting collection of essays written by leading personalities in the fields of medicine, politics, economics and the social sciences presents an analysis of opinion of the state of medicine in the United States. As such, it rarely refers to work in other countries but in discussion of such aspects as health insurance, the place of private medicine and development of training for primary care it will be of interest to all those concerned with development of health policies in a number of countries.

The book was written following an invitation by Nelson Rockefeller in his capacity as Vice-President and Chairman of the Commission on Critical Choices for Americans to John Knowles, President of the Rockefeller Foundation, to organise a group to study the problems of health care. A number of the contributions were first submitted as drafts prepared for study by the Commission.

The book covers a wide range from the apparent increase in "medicalisation" of American society, ethical factors such as the right to die—in this particular chapter, "Therapeutic Choice and Moral Doubt in a Technological Age", a detailed description of the Karen Quinlan situation is given—to the place of science and technology, the responsibility of the individual, the challenge of primary care, a look at political aspects of health policy (including relationships between government), the consumers and the providers, and the role of the physician. The book also includes chapters covering financing, research, the needs of children and of mental health.

The major theme of several of these essays does not vary much from that preponderated by Thomas McKeown in his recent text *The Role of Medicine* or that of Ivan Illich in *Medical Nemesis: The Expropriation of Health*. Aaron Wildavsky, author of the chapter entitled "Doing Better and Feeling Worse: The Political Pathology of Health Policy", states that the great equation Medical Care equals Health is wrong. "More available medical care

does not equal better health. The best estimates are that the medical system (doctors, drugs, hospitals) affects about 10% of the usual indices for measuring health: whether you live at all (infant mortality), how well you live (days lost due to sickness), how long you live (adult mortality)". He continues by saying that since the other 90% are affected by life-style and social and environmental conditions, "most of the bad things that happen to people are at present beyond the reach of medicine".

Walsh McDermott, in his chapter entitled "Evaluating the Physician and His Technology", takes this point a bit further by saying that it is wrong to say that the personal encounter physician has little influence on health but he lacks influence on the indicators of community health largely devised in the eighteenth, nineteenth and twentieth centuries for a different purpose. He continues, however, by saying that we in fact lack measures of the physician's ability to "modulate the deranged physiology of ultimately fatal chronic disease". McDermott's conclusion on the note that doctors play, coincides with that of several other authors.

Another valuable point made by several authors is that a half-way stage has been reached in the development

of medicine. Major advances have been made but the gains in the past have been fairly minimal compared with what might be expected in future. The book contains the underlying messages of the need to change life-style and the false expectations that populations have of medicine. Further points particularly mentioned by David Callahan in his chapter, "Health and Society: Some Ethical Imperatives", are "the breakdown of the ethical distinction between need and desire in our culture" and also "the continuing utopian lure of technology, a lure whose net effect is to thwart any attempt to place limits on medical aspirations".

This book, despite its impressive variety of subject matter, does not really state much that is new. The particular question, however, that comes to mind is can such publications as this and that of McKeown, Cochrane and Illich have any influence on consumers, providers, or governments; and if they can, will this result in greater rationalisation of requirements for health care? □

W. W. Holland is Professor in the Department of Community Medicine at St Thomas's Hospital Medical School, London, UK.

Control of cellular differentiation

The Differentiation of Cells. By N. Maclean. Pp. iii+216. (Edward Arnold: London, 1977.) Hardback £12; paperback £5.95.

THIS volume, the first of a series entitled *Genetics-Principles and Perspectives*, covers one of the central problems in developmental biology—the control of cell differentiation. There are now several recent texts which cover aspects of the control of gene expression in developing systems. The present volume presents a wide ranging view of current problems.

The first chapter discusses multicellularity in relation to the evolutionary significance of differentiation. Thereafter, the chapters cover differential gene expression, control mechanisms in gene expression, the cytoplasm in differentiation, the role of

hormones, episomes and viruses, the cell surface, and cancer and differentiation. The book ends with the author's views of the important unanswered questions in this field and the experimental systems that hold special promise for their elucidation.

The wide range of subject matter reflects the enormous range of controls which may operate in the orderly progression of cells to their terminally differentiated states, and the maintenance of these states once achieved. A major aspect of the control of cell differentiation is the regulation of gene activity. In introducing the problem of differentiation Dr Maclean emphasises most strongly that "differential gene activity may be a result of differentiation, rather than its causal mechanism". I found this introduction confusing because the terminology is not defined. It is unfortunate that the

book does not have a glossary, since, as the author points out in the case of heterochromatin, the implication of particular terms can change with time.

The subject material is laid out in a readily accessible manner and profusely illustrated with diagrams. The significance of some of the illustrations to the text (for example, the fibre autoradiographs p. 94) is, however, not clearly stated, and in the section on histones only the old nomenclatures are used. At various places the clarity of information has been enhanced by tabulation, though this format in some cases (for example, observations explicable by the master-slave hypothesis) has limited discussion of points which

may have alternative explanations.

A wide range of experimental systems and approaches have been used throughout the text. Perhaps as a consequence of this there are places where advanced students will need greater detail. The questioning style in which the text is presented should motivate the undergraduates and graduates for whom it was written to follow up the references to specialist reviews, and stimulate them to deeper consideration of the complex but intriguing problems of cell differentiation.

C. C. Ford

C. C. Ford is Lecturer in Developmental Biology at the University of Sussex, UK.

Lunar science

The Moon Book: Exploring the Mysteries of the Lunar World. By Bevan M. French. Pp. 287. (Penguin: New York and Harmondsworth, UK, 1977.) \$4.95; £1.95.

THIS is a book for the layman, as might be guessed from the name of the publisher rather than from the presumptuous title. A reader who has some general acquaintance with the Earth sciences, however, will find considerably greater profit in it than the 'intelligent layman' who starts from a state of near-total ignorance.

May natural inclination was to turn first to chapter 10, entitled, "The Lunar Interior: Mapping with Echoes". It contains a disappointing discussion of deductions made from geophysical evidence about the lunar interior. The seismic evidence which has been included seems to be a simplified rehash of the appropriate section of Ross Taylor's 1975 book, referenced in the bibliography.

There are some elementary errors, such as the statement that the inner core of the Earth is under a pressure of nearly a million atmospheres—in fact, it is over three million atmospheres—and a confusion between kilometres and miles in the figure showing cross-sections of the Earth and Moon. But the worst error I found was in the discussion about the Moon's ancient magnetic field, and whether it could have been provided by a molten iron core which has since frozen. French suggests this is unlikely because, "A large body like the Moon loses heat only by the slow conduction of heat through its interior." Ignorance of modern ideas about convection in planets, with the consequent ability for planetary interiors to lose heat at rates several orders of magnitude above conduction

rates, is appalling in a work of this kind.

The author also seems to confuse the ideas of a lunar asthenosphere and a possibly molten lunar core. Deep moonquakes occur in a zone between 600 and 1,000 km from the surface, whereas the lunar radius is 1,738 km. Seismic signals penetrating below this depth have rarely been observed. This suggests attenuation in a region where the pressure is similar to that in the Earth's low velocity zone, at a depth of around 100 km; so, by analogy it may be inferred that the lunar mantle at 1,000 km depth must be in a similar state to the material in the Earth's low velocity zone, possibly including some partial melt, in order to cause the seismic attenuation. Best estimates for the Moon's moment of inertia factor, plausibility arguments about planetary heat transfer and another tenuous piece of seismic evidence, not available to Ross Taylor, combine to suggest that if the Moon does have a metallic iron core, it is probably solid and not more than 500 km in radius.

In the rest of the book the author is on more familiar ground. He gives a very readable account of the pre-Apollo ideas of the Moon's origin and history, the Apollo missions themselves, and the knowledge gained from studies of the returned samples of rock and lunar soil. A set of thirty-six black and white photographs illustrate the story well. The final chapter rounds off the book nicely with a discussion of future developments.

I can recommend this book for general reading provided that the more technical reader bears in mind the author's qualifications as a geologist and geochemist. It would benefit substantially from a rewriting of chapter 10, possibly by a co-author.

Neil R. Gouly

Neil R. Gouly is Research Associate at the Department of Geophysics and Planetary Physics, University of Newcastle-upon-Tyne, UK.

Plasma low density lipoproteins

Low Density Lipoproteins. Edited by C. E. Day and R. S. Levy. Pp. xx+445. (Plenum: New York and London, 1977). \$47.40.

THIS book, written by several authors, provides up-to-date information on the structure and metabolism of plasma low-density lipoproteins (LDL), together with an examination of their comparative biology and their relationship to disorders of lipid metabolism and atherosclerosis. The material is grouped in five sections, comprising a total of 15 chapters which provide good critical coverage of the various topics.

The reader is likely to appreciate the efforts of the editors, authors, and publisher to condense a large body of information into a single, well-organised volume. For example, the section devoted to the discussion of "Aberrations of Metabolism" presents a rather detailed description of the pathogenesis and treatment of a few selected genetic disorders, identified as hyper- β and hyperpre- β lipoproteinaemia, type III hyperlipoproteinaemia (also known as "broad β disease" or dysbetalipoproteinaemia), and abetalipoproteinaemia, together with an analysis of the variants of LDL with particular reference to the Lp(a) antigen.

The section on "Comparative Biology", which gives a good overview on the LDL of mammals and non-mammalian vertebrates, is of particular interest. Concerning atherosclerosis, a useful discussion is presented on the interactions *in vitro* of LDL with either small polyanions (heparin and dextran sulphate) or macromolecules (collagen and elastin); these interactions are believed to play a role in the deposition of LDL in the arterial wall and in atherogenesis.

Perhaps the least rewarding section for both authors and readers may prove to be that dealing with LDL structure, since much of the current information, particularly on the LDL protein, is rather uncertain and is still the subject of an active research pursuit.

Overall, this is a good book, especially for investigators in the lipoprotein field who are likely to find both the condensed information and the more than 1,500 citations very valuable for their work.

Angelo M. Scanu

Angelo M. Scanu is Professor in the Departments of Medicine and Biochemistry, University of Chicago, Pritzker School of Medicine, Chicago, Illinois.

Applying physics to chemistry

Modern Physics in Chemistry. Vol. 1. Edited by E. Fluck and V. I. Goldanskii. Pp. xiii+406. (Academic: New York, San Francisco and London, 1977.) £18.50; \$36.10.

THIS is a collection of papers intended to describe the basic principles of some of the experiments of modern physics which have in the past decade been applied with such signal success to the problems of chemistry. It is not, as could easily have happened, primarily a collection of the various new spectroscopies, although inevitably resonance methods feature strongly. Chapters on positronium and mesic chemistry vary the diet.

This is not a unified, critical or comparative text which would serve, say, for the education of physical chemists in newer methods. This is a disappointment for there is always room for well written interdisciplinary teaching books of this kind. It may, however, be interesting for the researcher to have under one cover such a collection of reviews which, though they are now rather dated (the manuscripts were collected in 1973), provide a useful insight into the Russian literature in some of the areas.

Two chapters on X-ray spectroscopy divide into valence shell and band structure and core level shifts, respectively. They are, however, really too brief to provide a useful

survey of this rather slowly but long developing field. Electron paramagnetic and nuclear quadrupole resonance methods have much fuller coverage. Nuclear magnetic resonance is not featured; it is not "modern" any longer, except in the form of chemically induced dynamic nuclear polarisation. Mossbauer spectroscopy is brought up-to-date with Mossbauer double resonance experiments.

Although a different viewpoint on these newer techniques is to be welcomed, it is rather difficult to see much benefit being derived from this first volume by anyone outside the ranks of the historians of science. Unfortunately, in translation, clarity has somehow escaped and even post-graduate students will find quite a lot of up-hill work, sometimes intelligent guesses being necessary to perceive exactly what the authors mean. There is little evidence of editorial effort to attain a unity of style or to secure an error-free translation (Gammett constant?) although stilted or archaic usage can, as the late Gerard Hofnung showed, have charm. More serious slips are to be found, including a blatantly erroneous diagram of nodal surfaces for the benzene π orbitals. It seems as if the chemists' voice has been too weak throughout. It is difficult to see who would be recommended to pay £18.50 for this volume.

D. W. Turner

D. W. Turner is Lecturer in Physical Chemistry at the University of Oxford, UK.

Gas chromatography methodology

Modern Practice of Gas Chromatography. Edited by R. L. Grob. Pp. (Wiley-Interscience: New York and London, 1977.) £16; \$28.

THERE are now so many good books on the theory and practice of gas chromatography that a newcomer to the ranks must be of high quality indeed to stand any chance of acceptance. The book under review has the now almost universal style of a multiplicity of authors, each of whom holds responsibility for his own chapter and the editor for the resultant patchwork. The subjects covered separately are: Introduction (history and nomenclature); Theory and Basics; Columns and Column Selection; Qualitative and Quantitative Analysis; Detectors; Instrumentation; Trace Analysis; Selection of Analytical data (data handling); Analysis of Foods; Clinical Applications; Physico-Chemical Measurements;

and Drug Analysis.

The sections are very variable. The best is by Touchstone and Dobbins on "Clinical Applications", where the descriptions are clear, concise and accurate and the authors have actually read papers appearing before 1968. One of the less good sections is that dealing with instrumentation where the illustrations are frequently commercial black boxes that convey no information at all (except advertising). The editor also needs to note that the same illustration is used on page 355 as on page 173 in a different chapter.

All-in-all, the book is rather like the curates egg (good in parts); and with a well understood and practised technique like gas chromatography, this is not good enough. This is a pity, since a lot of hard work has gone into the chapters; a clearer intent as to what needs to be put across in each section would have produced a shorter and better book.

A. T. James

A. T. James works in the Colworth Laboratory of Unilever Ltd, Sharnbrook, UK.

Organosulphur chemistry

Organic Chemistry of Sulfur. Edited by S. Oae. Pp. ix+713. (Plenum: New York and London, 1977.) \$48.

THE importance of organosulphur chemistry, as demonstrated by its rapid growth over the past decade or so, has meant that a book covering this area in breadth and depth is somewhat overdue. Twelve chapters on various aspects are contained in the book and these and their contents are arranged in a logical sequence. Each of the chapters is written by one or more leading authorities; and it is a compliment to the contributors and to the editor that, although individual styles are apparent, continuity is maintained.

In the first chapter, the basic theoretical aspects of sulphur bonding are described. This is followed by a discussion of elemental sulphur and its reactions, which provides a necessary introduction to the vulcanisation of rubber covered in the third chapter. The remaining chapters deal with the physical and chemical properties of specific sulphur compounds—thiols, thiones, sulphides (mono-, di-, and poly-), sulfoxides and sulphilmines, sulphonium salts, sulphones and sulfoximines, sulphinic acids and esters, and sulphonate and sulphate esters. Some detail is also given to sulphur biochemistry within various chapters.

On the credit side, the book contains many excellent qualities with the diagrams, tables and general layout being attractive and comprehensive. The mechanistic approach used is also a highly commendable feature.

On the debit side, however, it is surprising that the industrially important sulphonic acids are not discussed in some detail. Furthermore, over 2,300 references are quoted, but no references later than 1973 are included and very few 1972 or 1973 references are given. In fact, for the majority of the chapters, the literature is covered adequately only to the end of 1971 and for one chapter only to the end of 1970. This overlong delay between completion of the manuscript and publication must detract in some degree from the overall usefulness of the book.

In spite of these drawbacks, and the presence of a considerable number of trivial and typographical errors, the book is worthy of a place in chemistry libraries and on the bookshelves of the more affluent workers in the field.

A. E. Tipping

A. E. Tipping is Reader in Organic Chemistry at the University of Manchester Institute of Science and Technology, Manchester, UK.

Karst landforms

Morphogenetics of Karst Regions. By L. Jakucs. Pp. 284. (Adam Hilger: Bristol, UK, 1977.) £17.

THIS book is a revised and enlarged version of an original text by the Hun-

garian geographer, Professor Jakucs. The generally good translation is by B. Balkay. In recent years there has been a number of books in different European languages on the landforms of karst (massive limestone) regions, but this book is the first to focus primarily on the processes and origins of karst landforms. It therefore not only fills a gap in our literature on karst but is

also a highly perceptive contribution to the subject.

After discussing the concepts of karst and of karst corrosion Jakucs treats the subject under the main factors which control karstification. These he lists as petrovariance; epirogenic movement (that is, structural variance); climatic variance; erosional and geomorphological variance; and anthropovariance. Each of these controls is discussed in length, and this forms the main theme of the book. The author aims to show that all variations of karst can be discussed in the light of the variants proposed.

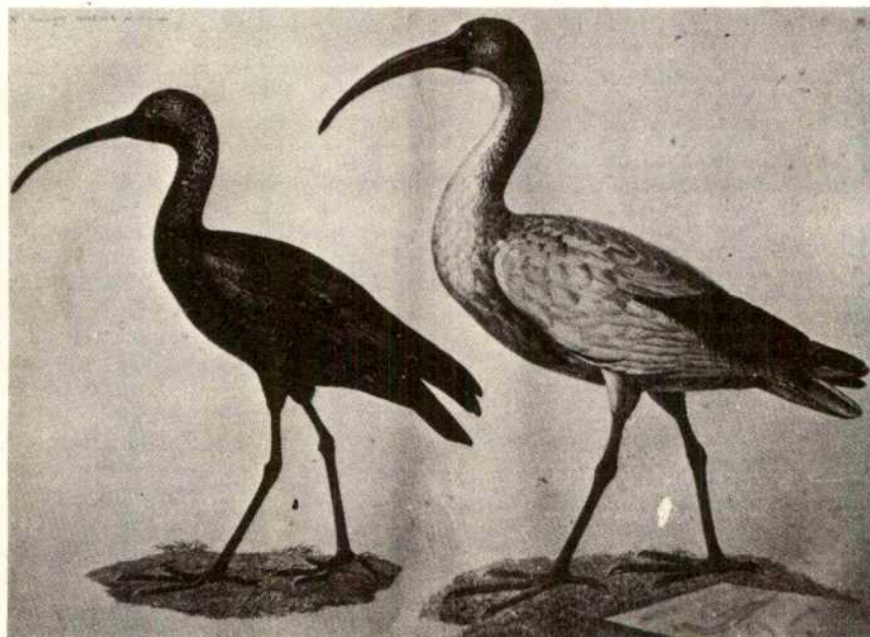
The treatment of each section is comprehensive and the discussion covers some ground familiar to karst geomorphologists. But the text is illuminated by Jakucs' own illustrations and careful experiments, which form the main scientific contribution of the book. There are certain sections which are therefore new and also controversial to the English karst morphologist. Four aspects are of particular interest. The first is that dealing with the effects of limestone crystallinity and lithostructure on karst corrosion. The second discusses the effects of soil microclimates on the development of karst depressions. Thirdly, the author has some interesting observations to make on the role of geomorphological situation and erosional variations on the origin of karst landforms; he usefully differentiates between authigenic and allogenic karst evolution. And fourthly there is an extended discussion on the effects of man on karst evolution; the results of deforestation, of spring development and of dam building are among the topics included. The author is perhaps least at home when dealing with the effects of climatic factors on the development of karst; even here however, he has some shrewd comments to make, though he has had to rely mainly on the published literature.

The book is well illustrated with clear diagrams and photographs. The bibliography is especially good on Central and Eastern European works, but shows a lack of knowledge of the more recent Western European and American work (that is, since 1970).

The Hungarian school of karst morphology has long been distinguished for its contributions to all aspects of karst, both surface and underground. Professor Jakucs' book illustrates the depth and grasp of Hungarian work in this field, and his succinct work should be carefully read by all English workers in karst geomorphology.

M. M. Sweeting

M. M. Sweeting is Lecturer in Geography at the University of Oxford, UK.



Illustrations taken from *Food: The Gift of Osiris* by W. J. Darby, P. Ghalioungui and L. Grivetti (Academic: London, New York and San Francisco, 1977; two volumes £12.50/\$27 and £12.50/\$25). These two volumes detail exactly what is known about ancient Egyptian food and eating habits. The book considers medical as well as nutritional uses, and provides a wealth of archeological and anecdotal information. *Top*, Sacred ibis drawn by scientists accompanying Bonaparte in 1799. The bird was associated with the god Thot, master of knowledge and secret science. Strict measures were taken to protect the bird in ancient Egypt, but it has since become extinct in that region. *Bottom*, Hand fish net (tomb of Ka-Gem-Ni at Saqqara, Old Kingdom, sixth dynasty, reign of King Teti, about 2390 BC). Open mouth V nets were used to collect fish which had been channelled into a fenced barricade, and this type of net is still in use today.